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Can biomarkers of biological age be used to assess cumulative lifetime experience?

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Abstract

Objective methods for assessing the cumulative lifetime experience of non-human animals would be valuable. We develop the hypothesis that biological age is a common currency that integrates the overall quality of an animal's lifetime experience across a range of types of exposure. Ageing is the result of the accumulation of somatic damage, and its rate is determined by the balance between experiences that cause damage and experiences that mitigate damage or promote repair. Negative affective states are associated with somatic damage via both direct causal and indirect pathways. Based on these premises, we predict that individuals that are biologically old for their chronological age will, on average, have experienced worse lives than individuals that are biologically younger, both in terms of their overall health and affective experience. Biological age is, thus, an attractive measure of cumulative experience because it requires no subjective decisions either about how a given exposure impacts an animal, or about how different dimensions of welfare should be weighted in an overall assessment. Biological age can be measured objectively using biomarkers. We argue that two biomarkers, namely leukocyte telomere length and hippocampal volume, are valid biomarkers of cumulative experience in humans, with potential for use in non-human vertebrates. We discuss how these biomarkers could be used to assess cumulative experience in animals, highlighting some of the limitations. We conclude that biomarkers of biological age offer a viable objective solution to the assessment of cumulative experience and their application in an animal welfare context deserves further exploration.

Keywords: *animal welfare, biological age, exposome, hippocampus, lifetime experience, telomere length*

Introduction

In recent years, there has been an increased emphasis in animal welfare on optimising the cumulative lifetime experience of both laboratory and farm animals. This focus is driven by the recognition that while single experiences can be acutely positive or negative, what matters more from a welfare perspective is the lasting cumulative impact of these experiences; it is the overall balance between positive and negative experiences over an animal's lifetime that determines its average quality of life. A focus on cumulative experience is present both in recommendations for improving the welfare of farm animals, which have introduced the concepts of 'a life worth living' and 'a good life' (Farm Animal Welfare Council [FAWC] 2009), and also in legislation governing the use of animals in scientific research, which has introduced the related concepts of 'cumulative severity' and 'cumulative suffering' (European Parliament & European Council 2010; Honess & Wolfensohn 2010; Pickard 2013). Current European legislation regulating the use of animals in research now requires both prospective and retrospective assessment of the cumulative effects of research on animal welfare (European Parliament & European Council 2010).

What exactly is cumulative experience? In the laboratory animal literature, cumulative experience is used to refer to the sum of all impacts on the health and well-being of an animal over its lifetime from conception to death or the present time (Pickard 2013). An identical definition could be adopted for farm, zoo and companion animals. For a laboratory rat (*Rattus norvegicus*), impacts contributing to cumulative experience might arise from maternal health, litter size, age of weaning, cage type, number and type of scientific procedures and method of euthanasia. For a broiler chicken (*Gallus gallus domesticus*), impacts might arise from flock size, husbandry, feather-pecking, pathogen load, lameness, transport and slaughter. In the Pickard report definition, we interpret well-being to mean affective state, and more specifically the long-term states that are influenced by an animal's history of acute emotional experiences (Mendl *et al* 2010; Nettle & Bateson 2012). Thus, both health and affective state contribute to cumulative experience, making the definition similar to the concept of animal welfare advocated by Dawkins (2006, 2017). Some experiences will have negative impacts on health and well-being, whereas others will have positive impacts; some impacts will be long-lasting, whereas others will be short-lived. Some husbandry decisions may

even have different impacts in the short and long term. For example, rewarding rhesus macaques (*Macaca mulatta*) with sweets may have a positive impact on their affective state in the short-term, but result in negative impacts on health and affective state arising from obesity and tooth decay in the longer term. The problem currently facing animal welfare scientists is how cumulative experience can be assessed in non-human animals.

A variety of methods have been suggested for assessing cumulative experience (for a review, see Pickard 2013). Measuring single welfare indicators has the advantage of being objective, but all current approaches suffer from limitations. Single measures, such as bodyweight and cortisol levels, have the advantage of being practical to measure, but are insensitive and non-specific making them hard to interpret (Ralph & Tilbrook 2016). Single behavioural measures, such as the presence of stereotypies, are also relatively simple to measure and may relate better to some aspects of past exposures (eg Gottlieb *et al* 2013; Greco *et al* 2016), but can also be hard to interpret and need to be validated for each species (Mason & Latham 2004; Poirier & Bateson 2017). Perhaps the most promising single behavioural measures developed, thus far, are so-called 'cognitive biases', that are argued to provide an integrative measure of affective state (Paul *et al* 2005; Mendl *et al* 2009, 2010). A major advantage of cognitive bias measures is that they are considered specifically sensitive to the valence of affective state and their interpretation is likely to generalise across species. However, the behavioural tasks required to measure cognitive biases still need to be developed and validated within each species and typically require extensive training of the animals, making them impractical for applied welfare assessment in their current form.

Due to the difficulties outlined above, measures that integrate several simple welfare indicators are currently the favoured method for practical assessments of cumulative experience. 'Clinical impression', cited in UK law relating to laboratory animals (UK Government 2012), involves a veterinary examination and consideration of all potentially relevant information available. However, this approach is currently poorly specified and lacks standardisation. It is also prone to using subjective or anthropomorphic criteria. To address these criticisms, practical methods have been developed for integrating multiple welfare indicators that are standardised and transparent. These include the Extended Welfare Assessment Grid for laboratory animals (Honess & Wolfensohn 2010) and the Welfare Quality® scheme for farm animals (eg Welfare Quality® 2009). However, a major criticism of all these approaches is that they rely critically on two sets of untested assumptions. The first set of assumptions concerns how specific exposures impact an animal's cumulative experience, and the second set concerns how different dimensions of welfare (eg physical health versus affective state) are weighted in the overall assessment of cumulative experience.

The crux of the problem is therefore that there is currently no objectively measurable common currency for measuring cumulative experience that is sensitive to the impact of the various exposures that an animal has over its lifetime. The Pickard report on the assessment of cumulative severity in non-human primates used in neuroscience research reached the depressing conclusion that, "There is no mathematical way of integrating all positive and negative events in an animal's life" (Pickard 2013). Our aim in this essay is to ask whether measuring biomarkers of biological age might provide a novel solution to this problem that goes some way towards addressing the criticisms that we have made of existing approaches.

We start by introducing two central concepts that we borrow from human epidemiology and biomedical science: the exposome and biological age. We highlight the importance of distinguishing between the totality of the variables to which an animal is exposed over its lifetime — its exposome — and the magnitude and duration of any impacts that these exposures have on the individual animal's experience. We develop the hypothesis that biological age is a common currency, measured in units of time, that integrates the somatic impact (both damaging and restorative) of an animal's exposome, taking into account variation in stress resilience. We argue that exposures that increase biological age usually also have a negative impact on long-term affective state, whereas those that either slow ageing or decrease biological age usually also have a positive impact on long-term affective state. Thus, in addition to being associated with health, biological age is likely to be associated with the cumulative affective impact of experience. It follows that cumulative experience can be assessed via the difference between an animal's biological and chronological ages. We introduce two biomarkers of biological age that we argue have promise for assessing cumulative experience, namely measures of telomere length and hippocampal volume. We end by describing how biomarkers could be used to assess cumulative experience and discuss some limitations of the biomarker approach.

Cumulative experience and the exposome

The 'exposome' is a concept developed in the human epidemiology literature that describes every exposure to which an individual is subjected from conception to death (Wild 2005, 2012). The aim of the exposome is to provide a neutral description of the totality of an individual's non-genetic exposure that can then be used to identify those specific exposures associated with well-being, health and disease. According to Wild's (2012) original conception, the exposome integrates exposures in three domains that reflect the different types of information about an individual that are available to epidemiologists: first, the general external social and ecological context in which the individual lives, second, the specific external events or agents to which the individual is exposed and third, the internal environment of the body. General external exposures are likely to determine specific external exposures, and the internal environment of the body will, at least partially, be a response to the external environment of the animal. Furthermore, internal state will sometimes, via changes in behaviour, bring about changes in external exposures. External exposures can be thought of as operating at the organismal level, whereas internal exposures are at the cellular level (for a use of this distinction, see Bateson 2016). In Figure 1, we have adapted the concept of the exposome for a laboratory rat.

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This formulation of the exposome highlights three points that are relevant to the concept of cumulative experience. First, not all external exposures will necessarily cause changes in internal exposures. This is important because an external exposure can only cause a lasting somatic impact if it produces some change in internal exposure. For example, exposure to environmental ultrasound causes depressive symptoms in laboratory rats and mice (*Mus musculus*) (Morozova *et al* 2016), but is undetectable to adult humans. In a similar vein, exposure to the flicker of low frequency, fluorescent lighting raises corticosterone levels in European starlings (*Sturnus vulgaris*) (Smith *et al* 2005), but is undetectable to humans. Thus, some aspects of the exposome may be completely irrelevant to cumulative experience in some species. The examples given here highlight the dangers of anthropomorphism in deciding which exposures might be relevant to the experience of non-human animals.

Second, not all internal exposures will necessarily produce lasting somatic impacts. Exposures could involve temporary changes in the internal exposome that leave no lasting somatic record necessary for effects to accumulate over time. For example, it is possible that infrequent exposure to minor acute stress, while producing a temporary spike in glucocorticoid stress hormones lasting an hour or two, produces no permanent change in either HPA-axis function or the brain.

Third, even if a given external exposure does produce a lasting impact on somatic state, the magnitude and duration of this impact may not be constant, either between, or within individuals. There are multiple reasons why there is variation in how individuals experience their exposome. Stable individual differences in stress-sensitivity and reactivity will affect the impact of exposure to chronic stressors on an individual. Such individual differences in stress resilience can occur as a result of differences in either genes and/or developmental experience (for a review, see Ebner & Singewald 2017). For example, Zebra finches (*Taeniopygia guttata*) exposed to increased levels of corticosterone as chicks showed exaggerated and prolonged responses to acute stress at 60 days of age (Spencer *et al* 2009). Stress-resiliency may also vary between and within individuals as a consequence of variation in non-stressful components of their adult exposome that moderate the impact of stressful components. For example, the cellular damage caused by exposure to major life stressors, was diminished in women who did more physical activity, had better quality sleep and ate healthier diets (Puterman *et al* 2015). Finally, animals can learn about repeated events, either habituating, meaning that successive exposures to the same event result in less impact, or sensitising, meaning that successive exposures result in an increased impact. There are also unlearnt, age-related changes in responsiveness to the external environment that occur as a result of either maturation or senescence. For example, the glucocorticoid response to acute stress has been found to change with age in many species (eg Lendvai *et al* 2015).

In summary, it is important to distinguish between everything an animal is exposed to during its lifetime — its exposome — and the lasting impact of these exposures on its health and well-being — its cumulative experience. Not all external exposures will produce changes in internal exposures, and not all internal exposures will leave a lasting impact on somatic state. Furthermore, the impact of a given external exposure could vary between or within individuals. If follows that cumulative experience cannot be assessed by simply quantifying an animal's exposome, or even those specific exposures that are known to have impacts on some individuals. Good measures of cumulative experience should involve assessing an individual animal's response to its exposome.

Figure 1

The concept of the 'exposome' for a laboratory animal (adapted from Wild's [2012] original diagram for the human). The three domains of the exposome distinguished by Wild are presented with non-exhaustive examples. The domains are shown as overlapping to indicate that it will not always be possible to allocate an exposure to a single domain. The arrows are our own additions. They indicate that exposures internal to the body will, at least partially, be a response to external exposures and internal exposures will sometimes, via changes in behaviour, bring about changes in external exposures.

Biological age and cumulative experience

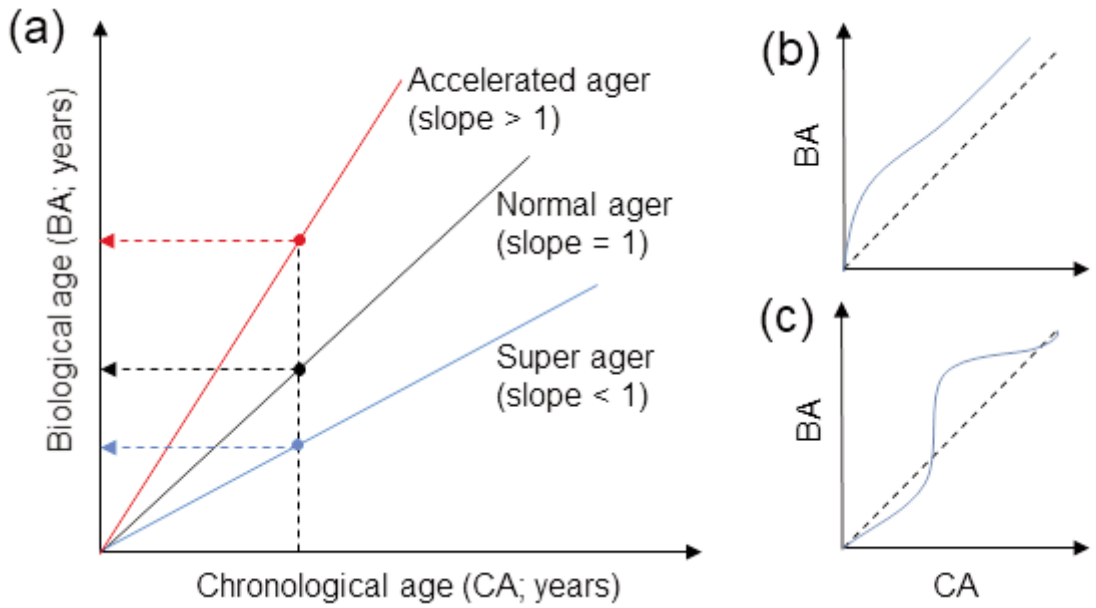
What changes in somatic state reflect an animal's response to its exposome? In 1956, stress biologist Hans Selye made the observation that:

> Every stress leaves an indelible scar, and the organism pays for its survival after a stressful situation by becoming a little older (Selye 1956)

This quote sets up the hypothesis that there are causal links between the exposome, specifically those components of it that are stressful, and the rate of ageing. Henceforth, we define a stressful exposure as one that is experienced with acute negative affective valence (ie a negative emotional response, such as fear, pain or extreme hunger).

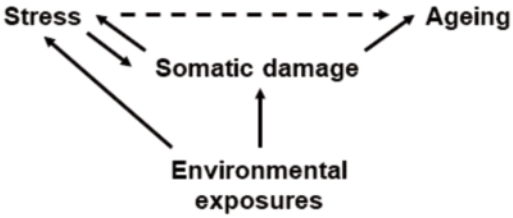
To unpack Selye's observation it is first necessary to understand what ageing is. Ageing is associated with accumulation of molecular damage that has knock-on effects at the cellular,

Figure 2



The concept of biological age (adapted from Khan *et al* 2017). (a) The solid lines show the relationship between chronological age (CA) and biological age (BA) for three different individuals, a super ager (blue), a normal ager (black) and an accelerated ager (red); at a given CA, indicated by the vertical dashed line, these three individuals have different BA, indicated by the arrows. Although the functions relating CA to BA are shown as straight lines, there is no reason to assume that the trajectory for a specific individual should be a straight line: an individual's pace of ageing could change with age: (b) shows the BA trajectory for an individual that ages rapidly early in life, but then slows to the normal rate of ageing in adulthood, and (c) shows a more complex trajectory for an individual with periods of both accelerated and super ageing. In panels (b) and (c) the dashed line indicates normal ageing for the population.

Figure 3



Pathways connecting stress and ageing. The dashed line indicates the association referred to by Selye (1956) and the solid arrows indicate established causal relationships. Selye's association between stress and ageing is explained by a direct causal pathway (stress→somatic damage→ageing) or via an indirect pathway (stress←somatic damage→ageing) through somatic damage. Environmental exposures cause stress either directly or via somatic damage.

tissue and whole-organism levels. In terms of function, ageing manifests itself as a decreased ability to maintain homeostasis under stress resulting in increased morbidity and mortality. Ageing occurs with time in all individuals of most species (Nussey *et al* 2013), but within species there is individual variation in the pace of ageing, resulting in variation in health and mortality for individuals of the same chronological age (eg Lemaître *et al* 2013). This variation has led to the concept of biological age as distinct from chronological age (Figure 2). Super agers have better health and lower mortality than would be expected for their chronological age, whereas accelerated agers have poorer health and higher mortality than would be expected for their chronological age (Khan *et al* 2017). Normal ageing for a given population is determined by measuring the mean rate of ageing in a large sample of individuals. Thus, biological age is always a relative measure, and its interpretation will depend on the sample used to establish it. Variation in biological age within a population can be quite dramatic: for example, Belsky *et al* (2015) have estimated a biological age range of between 28 and 61 years for a cohort of 38-year old New Zealanders.

What leads to variation in biological age? The heritability of human lifespan has recently been estimated at only 16% (Kaplanis *et al* 2018), suggesting that the majority of variation in lifespan is explained by the environment of an individual and hence some components of its exposome. Since biological age predicts life expectancy, the low heritability of lifespan suggests that much of the variation in biological age is also determined by the exposome of an individual. The low heritability of lifespan therefore supports Selye's idea that lifetime exposures can be ageing. Following on from the definition of ageing as accumulation of somatic damage, exposures that result in damage will accelerate ageing, whereas exposures that mitigate the effects of ageing exposures or result in somatic repair will slow or even reverse ageing.

Returning to Selye's observation, why should those exposures that cause somatic damage, and are hence ageing, also be those that are stressful (ie associated with acute negative affective experiences)? There are two pathways that are responsible for the association between stress and ageing, one direct causal pathway and one indirect pathway

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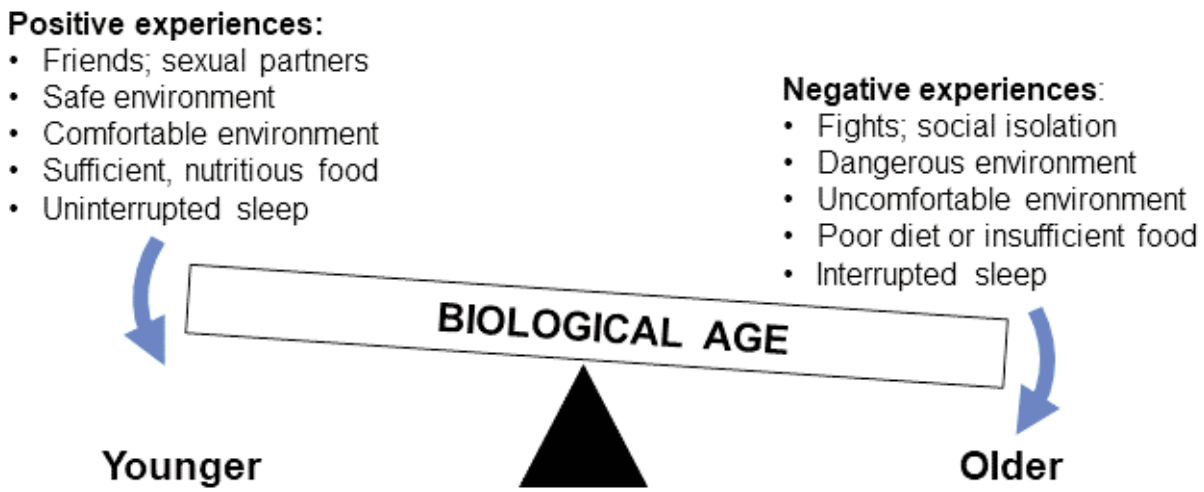
Figure 4



Biological age as a common currency that integrates the impact of multiple different experiences. The impact of negative experiences will be moderated by stress-resilience. Biological age is represented by the angle of the bar. A horizontal bar is assumed to represent a normal ager. Exposure to positively valenced experiences, which are either rejuvenating or moderate the damage caused by negative experiences, shifts the balance towards a biologically younger phenotype (super ager), whereas exposure to negatively valenced experiences, which are typically ageing, shifts the balance towards a biologically older phenotype (accelerated ager — shown here). Details of which exposures are important and the direction of their impact will obviously depend critically on the evolutionary ecology of the species under consideration.

(Figure 3). The indirect association is brought about by the evolutionary function of acute affective (emotional) states. Emotions are an integral part of the psychological machinery that motivates adaptive behaviour. Negative emotional reactions to events function to inhibit behaviour that reduces Darwinian fitness (by minimising exposure to punishers), whereas positive emotional reactions function to promote behaviour that increases fitness (by increasing exposure to rewards: Nettle & Bateson 2012; Rolls 2013). Since somatic damage is typically fitness-reducing and regeneration and repair are typically fitness-enhancing, we predict those exposures that accelerate ageing to also typically be those exposures that are negatively valenced in terms of the acute affective experience they invoke (ie punishers), whereas those exposures that retard ageing or are rejuvenating will typically be those that are positively valenced (ie rewards). (We discuss exceptions to this general rule in the following section).

In Figure 4, we list some of the exposures that have been shown to be associated with estimates of biological age in humans (for references, see Table 1). Exposures associated with ageing include: psychosocial stress, an environment that is perceived as dangerous, an environment that is physically uncomfortable, inadequate food or interrupted sleep. In contrast, exposures associated with slower ageing or rejuvenation include: psychosocial support, a safe, physically comfortable environment, adequate food and uninterrupted sleep. The ageing exposures are typically associated with poorer health and long-term negative affective experience, whereas the rejuvenating exposures are typically associated with better health and long-term positive affective experience.

In humans, there is a relationship between the valence and frequency of acute affective responses to specific events or exposures and longer-term affective states. Individuals that experience more acutely negative responses, and/or fewer acutely positive responses are more likely to develop negatively valenced longer-term states, such as anxiety and depression (for reviews, see Mendl *et al* 2010; Nettle & Bateson 2012).

Thus far, we have only made the indirect argument for an association between ageing and stress: both are caused by exposures that are somatically damaging. However, in humans, there is additionally some evidence for a direct causal link between longer-term affective states and ageing. When other potential causes of variation in morbidity and mortality are controlled for, clinical anxiety and depression (which are extreme presentations of chronic negative affective states) are still associated with increased morbidity and mortality, whereas positive affective states are associated with reduced morbidity and mortality (for a review, see Walker *et al* 2012). Potential mechanisms assumed to underlie a causal effect of long-term affective states on morbidity include changes in immunosuppression (an important factor in increased cancer risk and cardiovascular disease). Depression also directly causes increased morbidity and mortality by increasing self-harm and suicide rates (Walker *et al* 2012). Thus, there is evidence for both indirect and direct causal links between stress and ageing.

One of the challenges with which we began this essay was the observation that there can be individual differences in how animals react to the same stressor. Interestingly, many of the genetic mutations that are associated with extended lifespan have been found to affect genes involved in how the organism responds to stressors (Kenyon 2010). If genes that confer stress-resilience also confer longevity, this

Table 1 Example evidence from humans on the associations between telomere length, hippocampal volume and aspects of cumulative experience.

Type of validity	Criteria for validity for biomarker		Telomere length (TL)	Hippocampal volume (HV)†
Construct validity	1. Biomarker reflects subjective experience of stress exposure		• Perceived stress is associated with TL (Mathur *et al* 2015)* • Perceived neighbourhood quality is associated with TL (Park *et al* 2015)	• Perceived stress is associated with smaller HV (Zimmerman *et al* 2016)
	2. Biomarker covaries with positive and negative experience in opposite directions	Positive experiences	• Physical activity associated with longer TL (Mundstock *et al* 2015)* • Healthy lifestyle mitigates stress-induced telomere attrition (Puterman *et al* 2015) • Mindfulness-based cancer recovery and supportive-expressive group therapy associated with reduced telomere attrition in cancer patients (Carlson *et al* 2015)	• Aerobic exercise associated with larger HV (Firth *et al* 2018)* • Healthy diet associated with larger HV (Jacka *et al* 2015) • Mindful meditation associated with larger HV (Luders *et al* 2009; Hölzel *et al* 2011)
		Negative experiences	• Childhood trauma associated with shorter TL (Li *et al* 2017)* • Chronic pain is associated with shorter TL (Hassett *et al* 2012) • Sleep apnoea associated with shorter TL (Huang *et al* 2017)* • All causes of stress and adversity are associated with shorter TL (Pepper *et al* 2018)	• Childhood trauma associated with smaller HV (Paquola *et al* 2016)* • Chronic pain associated with smaller HV (Ezzati *et al* 2014; Niddam *et al* 2017) • Excessive daytime sleepiness associated with smaller HV (Sforza *et al* 2016)
	3. Biomarker integrates experience(s) over time (effects of frequency and/or duration of events)		• Number of years as a care-giver is associated with shorter TL (Epel *et al* 2004)	• Time in institutional care as a child associated with decreased HV (Hodel *et al* 2015) • Number and frequency of traumatic events associated with decreased HV (Dannlowski *et al* 2012)
Criterion validity	4. Biomarker covaries with measures of mood		• Anxiety is associated with shorter TL (Darrow *et al* 2016)* • Post-traumatic stress disorder is associated with shorter TL (Darrow *et al* 2016)* • Depression is associated with shorter TL (Schutte & Malouff 2015; Darrow *et al* 2016; Lin *et al* 2016)*	• Paediatric anxiety is associated with smaller HV (Gold *et al* 2017) • Post-traumatic stress disorder is associated with smaller HV (Woon *et al* 2010; O'Doherty *et al* 2015)* • Depression is associated with smaller HV (Koolschijn *et al* 2009; McKinnon *et al* 2009; Arnone *et al* 2016; Wise *et al* 2017)

† HV refers to either amount (or concentration) of hippocampal grey matter or hippocampal volume.
* Indicates that the paper cited is a meta-analysis of multiple empirical studies.

further supports Selye's observation of a connection between exposure to stressful experiences and ageing.

In summary, Selye (1956) suggested that stressful experiences cause accelerated ageing. His observation is supported by more recent research on the biology of stress and ageing. Based on this evidence, we propose that the biological age of an animal could be used to assess its cumulative experience. Biological age can be thought of as a common currency, measured in units of time, that integrates multiple different somatic impacts taking into account variation between and within individuals in stress resiliency. We predict that animals that are biologically old for their chronological age are likely to have had relatively worse lives, both in terms of health and affective state, compared with animals that are biologically younger.

Potential criticisms of the biological age hypothesis

While the idea of using biological age in a welfare context is new, longevity has been considered and applied previously (eg Morris *et al* 1998; Clubb *et al* 2008). Given the close connections between biological age and longevity, it makes sense to revisit previous work on longevity and the criticisms that have been raised to it. We have found two papers that explicitly consider the use of longevity as a welfare indicator. Hurnik and Lehmann (1985) discussed the problem of how different dimensions of welfare (needs) should be weighted in welfare assessment. They argued that longevity is a single objective variable that naturally integrates how well an animal's needs have been met over its lifetime. More recently, Walker *et al* (2012) discussed how the past affective states of animals can be assessed. They argued, based on empirical evidence for causal links

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between affective state and morbidity and mortality, that longevity could be used to assess past affective state. Various objections have been raised to the hypothesis that longevity is a potentially useful welfare indicator, both by the authors of these latter papers themselves and by other commentators (eg Fraser 1995). If we think of welfare assessment as a classification problem, then two types of errors are possible. False negative errors occur if using longevity causes us to miss cases of true poor welfare; whereas false positive errors occur if using longevity causes us to infer poor welfare when true welfare is in fact good. A high false negative rate implies that longevity has poor sensitivity as a measure of welfare, whereas a high false positive rate implies that it has poor specificity. Since the various criticisms of longevity all concern the possibility of either false negative or false positive errors, we categorise the objections according to the type of error involved.

Longevity is insensitive

First, assessing cumulative experience based on longevity could miss cases of poor welfare in animals that are killed young rather than being allowed to live out their natural lifespans. This could occur because differences in longevity are often not manifest until animals become old. While this is a limitation of using actual longevity, our suggestion of assessing biological age as opposed to longevity provides a solution. It has become clear that variance in biological age is present even in populations that are currently young and ostensibly healthy (eg Belsky *et al* 2015), meaning that there is no need to wait until animals die to assess the impact of their exposome.

Second, assessing cumulative experience based on longevity could miss cases of poor welfare in animals whose lives are artificially prolonged by modern medical treatments. This could occur because such treatments break the natural link between cumulative experience and longevity. As with the previous objection, this problem is likely to be addressed by using biological age in place of longevity to assess cumulative experience. Unless life-extending medical interventions are actually rejuvenating, reversing the somatic damage that has led to disease, we predict that sick animals kept alive by modern medicine will, on average, have greater biological age than otherwise matched healthy animals.

Third, assessing cumulative experience based on longevity could miss cases of poor welfare in populations in which a substantial source of mortality is unrelated to ageing. In such cases, the non-ageing-related mortality will be a source of noise that will mask ageing-related mortality, leading to false negative errors. Fraser (1995) used the following thought experiment to dismiss the premise that those factors truly important for quality of life are also those that promote longevity:

> If prisoners on average live longer than owners of small aircraft, few would argue that this necessarily indicates greater quality of life

The argument here is presumably that owners of small aircraft will have better welfare than prisoners due to being free and well-off financially, but reduced longevity due to their higher probability of being killed in an accident. In this specific example, we question the assumption that humans forced to live in the sub-optimal environment of a prison would actually, on average, live longer than humans that voluntarily partake in sports with an additional likelihood of accidental death. Prisoners might be less likely to die in plane crashes, but they will be more likely to suffer stress-related morbidity and mortality. Moreover, even if the true difference in longevity was, as Fraser suggested, the problem will again be addressed by using biological age in place of longevity to assess cumulative experience. We predict that, on average, the biological age of prisoners is likely to be greater than that of participants in dangerous sports (we found one paper showing increased biological age in ex-prisoners of war compared to matched controls that had military service but were not held captive (Solomon *et al* 2017), but no one seems to have assessed biological age in owners of small aircraft yet).

In summary, concerns that longevity will be an insensitive measure of cumulative experience, missing true cases of poor welfare, can be addressed by using biological age in place of longevity.

Longevity is non-specific

Possibly the most difficult objection to the hypothesis that longevity or biological age can be used to assess cumulative experience is the apparent existence of situations in which the assumed association between acute negative affective state and accelerated ageing is reversed and acute positive affect appears to be related with accelerated ageing. If such situations exist, then there is a concern that assessing cumulative experience based on either longevity or biological age would result in false positive detection of poor welfare when true welfare is in fact good.

There are at least two categories of situations in which animals are motivated to behave in ways that will result in accelerated ageing. The first arises as the result of evolutionary mismatches between animals' motivational systems and their current environment. Such mismatches arise with substances, such as alcohol, drugs of abuse and high-sugar foods that were either not present, or much rarer, or less concentrated in the environment of evolutionary adaptedness. Consumption of these substances triggers evolved reward pathways leading to excessive consumption and causing long-term somatic damage. For example, excessive alcohol consumption, smoking and drinking of sweetened beverages are all associated with increased biological age (Strandberg *et al* 2012; Astuti *et al* 2017; Rafie *et al* 2017; Wojcicki *et al* 2018).

The second situation arises when an individual's inclusive fitness is maximised by behaviour that increases its own morbidity or mortality. The most obvious examples occur in relation to reproduction (Fraser 1995; Barnard & Hurst 1996). Reproduction is a vital component of Darwinian fitness, but comes at a cost to future morbidity and mortality (for reviews, see Harshman & Zera 2007; Nussey *et al* 2013). Thus, animals should be highly motivated to reproduce and should find exposures related

to reproduction rewarding, but reproduction is associated with accelerated ageing. This cost of reproduction has been documented in several species. For example, in red deer (*Cervus elaphus*) and badgers (*Meles meles*), individuals that invest more in reproduction age more quickly (Nussey *et al* 2006; Beirne *et al* 2015).

The above situations might seem problematic for the hypothesis that longevity or biological age can be used to assess cumulative experience because these measures lack specificity. However, in the case of both taking drugs and having sex, although the short-term affective consequences may be positive, both behaviours have medium-term consequences that are negative. Thus, the short-term positive affect from taking drugs may be offset by later negative affect arising from comedown, hangovers and withdrawal. Similarly, the short-term positive affect from having sex may be offset by the later negative affect arising from childbearing (nausea, pain) and increased exposure to a variety of stressors associated with child-rearing (post-natal depression, lack of sleep, increased work, financial stresses). Confirming the suggestion that the net effect of reproduction on long-term affective state in humans is negative, many studies have found that overall affective state is more positive in childless couples compared with those with children (eg Glass *et al* 2016).

In summary, while there are undoubtedly situations (specifically in relation to drugs and sex) where animals are motivated to engage in behaviour that accelerates biological ageing, it is an empirical question how these behaviours affect their cumulative experience. There is evidence to suggest that the acute positive affect arising from taking drugs or having sex is offset by later negative affect arising as a direct consequence of these activities. Therefore, we argue that there is currently no strong evidence against our prediction that individuals that are biologically old for their chronological age will, on average, have experienced worse lives overall than individuals that are biologically younger. In general, we believe that exposures that cause somatic damage, and are hence bad for health, will also be those that are associated with more negatively valenced long-term affective states. Thus, biological age should be a relatively specific measure of cumulative experience.

Biomarkers

A biomarker is an objectively quantifiable biological trait that can be used to identify or predict a pathological process. We have criticised previous attempts to use single biomarkers, such as bodyweight or cortisol levels in the assessment of cumulative experience. In the current section we start by considering the criteria that a biomarker of cumulative experience should meet. We then go on to describe biomarkers of biological age and suggest two specific biomarkers that we argue meet these criteria.

Validating biomarkers of cumulative experience

Our discussion in the second and third sections of this essay suggests four criteria that should be met by a biomarker of biological age if it is to serve as a valid measure of cumulative experience. First, the biomarker should reflect an individual's response to their exposome better than mere exposure to it. In humans, one possible strategy for establishing whether this is the case is to ask whether the biomarker covaries with participants' subjective estimates of their experience (eg perceived stress) more strongly than objective measures of what stressors they have been exposed to. Second, the biomarker should covary with good and bad experiences in opposite directions. Unhealthy or negatively valenced experiences should be associated with accelerated ageing, whereas healthy or positively valenced experiences should be associated with slower ageing. Experiences may interact non-additively. For example, a major traumatic experience may increase the ageing impact of smaller subsequent stressors, or healthy/positively valenced experiences may reduce the ageing impact of unhealthy/negatively valenced experiences. Moderating effects of this type would be reflected in interactions between the effects of good and bad experience on ageing. Third, the biomarker should integrate experiences over time and hence reflect the overall balance of unhealthy/negatively valenced experience versus healthy/positively valenced experiences. One strategy to demonstrate temporal integration is to ask whether the biomarker responds in a dose-dependent fashion to the frequency and duration of events experienced. There is no reason to assume that such effects will be linear: an individual might respond more or less to successive exposures to an identical stressor. A second strategy is to ask whether exposures from the distant past have a lasting impact on the biomarker; we would expect traumatic experiences to leave a lasting 'scar'. These first three criteria all follow from the simple theoretical model outlined in Figure 4 and thus comprise what is referred to as 'construct validity' (Cronbach & Meehl 1955; Trochim *et al* 2015).

'Criterion validity' relates to the correlation between the biomarker and an existing measure considered to be the current gold standard (Cronbach & Meehl 1955; Trochim *et al* 2015). As discussed earlier, there is currently no gold standard measure of cumulative experience in any species. However, human moods are relatively enduring affective states that are sensitive to the positive and negative experiences of an individual over time (Mendl *et al* 2010; Nettle & Bateson 2012). Various types of stress including physical and emotional neglect, abuse and trauma, especially when experienced early in life, are associated with subsequent lifelong increased vulnerability to mood disorders, such as anxiety and depression (Heim & Nemeroff 2001; Weich *et al* 2009; Pechtel & Pizzagalli 2011). As discussed earlier, negatively valenced mood can also directly cause somatic damage (Walker *et al* 2012). Mood is therefore closely

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linked to the concept of cumulative experience and we argue that self-reported measures of mood can be used to validate candidate biomarkers of cumulative experience in humans (Bateson 2016; Poirier *et al* 2018). There exists an extensive literature documenting the associations between clinical anxiety and depression and a range of somatic variables including biomarkers of biological age. These extreme presentations of negative affective states can be used for establishing the criterion validity of a candidate biomarker in humans. The fourth criterion for validity is, therefore, that a biomarker of cumulative experience should covary with measures of mood in humans.

From an applied perspective, a practical biomarker of cumulative experience should additionally be one that can be measured cheaply and non-invasively *in vivo* with low measurement error. In practice, there often seem to be trade-offs between these characteristics, as we discuss further below.

Biomarkers of biological age

A biomarker of biological age is a trait that is correlated with chronological age, but predicts morbidity and mortality better than chronological age (Jylhava *et al* 2017; Khan *et al* 2017). Biological age is measured using one or more biomarkers. In humans, a number of different candidate biomarkers of biological age have been identified including 'epigenetic clocks', leukocyte telomere length, 'brain-predicted age' derived from structural neuroimaging, transcriptomic predictors, proteomic predictors and metabolomics-based predictors (Jia *et al* 2017; Cole *et al* 2018). These different biomarkers are typically not perfectly correlated with each other (Jylhava *et al* 2017; Cole *et al* 2018). Low correlation can occur as a result of measurement error in one or more of the biomarkers and may also occur if different biomarkers respond to different types of exposure and are thus sensitive to different causes of ageing. It is possible that some ageing biomarkers may respond to specific types of exposure, whereas others may respond to multiple different types of exposure. These latter problems are sometimes solved by using two or more biomarkers to estimate biological age. If the measurement errors for different biomarkers are uncorrelated, then triangulation with more than one biomarker will provide a more accurate estimate of the underlying latent variable being estimated (Munafò & Smith 2018). In an example of the latter strategy, Belsky *et al* (2015) used a panel of 18 biomarkers (including waist-hip ratio, mean arterial pressure, leukocyte telomere length and white blood cell count) to estimate variation in ageing in a cohort of healthy humans.

Not all biomarkers of biological age will provide equally good biomarkers of cumulative experience. We need a biomarker that responds to multiple different types of exposure and that meets the four criteria outlined above. Thus far, we have identified two plausible candidates: leukocyte telomere length (henceforth TL) and hippocampal volume or, alternatively, the local amount of grey matter in the anterior hippocampus (henceforth HV). Identification of novel biomarkers of ageing is a rapidly evolving field and we do not want to give the impression that TL and HV are the only two biomarkers worth considering for assessing cumulative experience. However, at the time of writing there is far more data available on TL and HV than for other potential biomarkers.

In the paragraphs that follow we briefly describe TL and HV for readers not familiar with them and provide an overview of the evidence suggesting that they are valid biomarkers of cumulative experience in humans. Since we have reviewed the evidence linking these biomarkers to cumulative experience in non-human animals in detail elsewhere (Poirier *et al* 2018; Bateson 2016 for TL) we do not repeat this information here.

Telomere length (TL)

Telomeres are the DNA-protein complexes at the ends of chromosomes that function to protect coding regions of DNA from damage. TL is a molecular measure that estimates the length in base pairs of the protective telomeric DNA sequence. TL in humans is typically measured in leukocytes obtained from blood samples or occasionally in buccal cells obtained from cheek swabs. Telomere length can be measured via a range of established protocols, the cheapest and most common of which is a qPCR (quantitative polymerase chain reaction)-based method that measures the amount of telomeric sequence in a sample relative to the amount of a chosen single-copy gene (for a review of TL measurement methods, see Nussey *et al* 2014). A major limitation with TL is the magnitude of measurement error; this can be considerable and varies between protocols with qPCR measures often emerging as the least precise (Aviv *et al* 2011; Bateson *et al* 2018).

As required of a biomarker of biological age, TL decreases with chronological age (Müezzinler *et al* 2013) and predicts longevity better than chronological age (eg Kimura *et al* 2008). Table 1 summarises selected evidence from humans suggesting that TL also meets the four criteria for validity as a measure of cumulative experience outlined above. However, some caution is warranted. A recent major meta-analysis showed that while many different forms of stress and adversity (including physical disease, environmental toxins, poor nutrition, poor sleep, less physical activity, psychosocial stressors and low socioeconomic status) are associated with shorter TL in humans, the effect size is small and most studies are underpowered (Pepper *et al* 2018). Thus, large sample sizes are likely to be required to avoid false negative results. A second problem arises from the fact that the majority of the TL studies cited in Table 1 are based on cross-sectional correlational data. In the absence of more longitudinal studies, or better still, randomised controlled trials, it is impossible to infer whether the correlations between exposures and TL are causal. Although it is widely assumed that exposure to stressors of various types accelerates telomere attrition, the evidence that this is true *in vivo* is currently surprisingly weak (Bateson *et al* undated, 2018; Bateson & Nettle 2018). Whilst there is some evidence that telomeres can lengthen, and that health behaviours may moderate telomere attrition or promote repair, the evidence is currently controversial

(Steenstrup *et al* 2013; Bateson & Nettle 2017). Thus, although the evidence presented in Table 1 looks promising, further work is needed to establish the causal links between cumulative experience and telomere attrition in humans.

Telomeres are present in all eukaryotic organisms, and the human telomeric DNA sequence, TTAGGG, is conserved across the vertebrates. While there is variation between vertebrate species in telomere biology that is likely to affect telomere dynamics (Gomes *et al* 2010, 2011), there is mounting evidence that non-human primates and birds share similar dynamics to those in humans (for a review, see Bateson 2016). Supporting the extension of TL to non-human species, a recent meta-analysis found that as in humans, short telomere length is associated with increased risk of mortality in a range of vertebrate species including sheep, several birds and three reptile species (Wilbourn *et al* 2018). In one of the first welfare-focused applications, a recent study of dairy cows showed an association between TL in calves and productive lifespan (Seeker *et al* 2018). Furthermore, there is strong experimental evidence from nestling passerine birds showing that exposure to stressors causes telomere attrition and moreover, that different types of stress are additive in their effects on TL (eg Nettle *et al* 2017). There are a handful of encouraging experimental studies in other vertebrate species showing that stress causes telomere attrition (for a review, see Bateson 2016), but more work is required to validate TL as a measure of cumulative experience in any single non-human species.

Hippocampal volume (HV)

The human hippocampal formation (henceforth hippocampus) is a bilateral, oblong, forebrain structure involved in cognition and emotional regulation. There is regional specialisation within the primate hippocampus with the anterior region implicated specifically in emotional regulation. HV is a macroscopic measure of hippocampal anatomy that can be obtained *in vivo* using structural neuroimaging techniques such as magnetic resonance imaging (MRI) to assess either total hippocampal volume or the local amount of grey matter in the specific regions of the hippocampus. These different biomarkers are likely to be positively correlated, but the latter biomarker should be more sensitive to cumulative experience because it can be localised to the stress-sensitive anterior region (for a review, see Poirier *et al* 2018). The greatest practical limitation to the measurement of HV is access to neuroimaging facilities and the expertise required for data analysis. Measurement error is not discussed as an issue in measurement of HV.

Although HV atrophies with chronological age (Fjell *et al* 2013), it is not referred to as a biomarker of biological age. There is no evidence available on whether HV specifically predicts mortality better than chronological age, but a brain-based biomarker derived from structural neuroimaging data predicts mortality better than chronological age (Cole *et al* 2018), suggesting that this might be the case. The evidence summarised in Table 1 suggests that HV meets the four criteria for validity as a biomarker of cumulative experience in humans. As for TL, the studies cited in Table 1 are largely based on cross-sectional correlational data, but the case for a causal relationship between experience and HV is strong due to the existence of extensive experimental animal data (see below).

The hippocampus is an evolutionarily conserved brain structure, and homologues have been described in all vertebrate lineages (Bingman *et al* 2009). Furthermore, the regional specialisation, whereby it is the anterior region that is specifically sensitive to stress, has been demonstrated in macaques, rats and mice (with the anterior hippocampus of humans and macaques being homologous to the ventral hippocampus of rodents; [for a review, see Poirier *et al* 2018]). Due to the strong conservation of hippocampal structure and function in the mammals, the effects of positively and negatively valenced affective experiences on HV have already been studied experimentally in macaques, rats and mice. We have argued elsewhere that there is already sufficient evidence to show that HV meets our criteria for validity as a measure of cumulative experience in these species (Poirier *et al* 2018).

Comparison of TL and HV as biomarkers of cumulative experience

The literature summarised in Table 1 shows remarkable similarities in the types of exposures that are associated with TL and HV in humans. If TL and HV are both sensitive to the same aspects of cumulative experience, a positive correlation between TL and HV is predicted. Supporting this prediction, a study of 1,960 middle-aged Americans found that leukocyte TL and hippocampal grey matter volume were positively correlated after controlling for age, total intracranial volume and a number of other covariates (King *et al* 2014). Furthermore, the pattern of telomere-length-related regional brain volumes overlapped with brain regions associated with stress-related psychopathology more generally (hippocampus, orbitofrontal cortex, insula and anterior cingulate), supporting the hypothesis that TL and HV are both affected by stress. However, a meta-analysis that combined this study with four lower-powered studies only found a non-significant positive association between TL and HV overall (Nilsonne *et al* 2015). Thus far, only a single study has compared changes in TL and HV longitudinally within the same individuals (Staffaroni *et al* 2018). This study of 69 functionally normal older adults showed that greater TL shortening over 2.9 years was associated with greater hippocampal volume loss, even after controlling for global grey matter atrophy. Interestingly, this study found no cross-sectional association between baseline TL and HV, demonstrating the greater power of longitudinal studies in the study of these biomarkers. This latter study provides support for the hypothesis that TL and HV are biomarkers of the same biological processes in humans.

In summary, we have presented evidence that TL and HV both meet our criteria as valid biomarkers of cumulative experience in humans. A major reason for our choosing TL and HV is that these biomarkers depend on biology that is conserved in non-human vertebrates (although this conservation is stronger for HV than for TL). TL and HV thus both have potential for use in the assessment of cumulative expe-

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rience in non-human species. Despite some promising results, further work is required to validate TL as a biomarker of cumulative experience in any non-human species. In the case of HV, sufficient validation has already been performed in macaques, rats and mice to justify the use of this biomarker in the assessment of cumulative experience. We suggest that the minimum validation necessary before TL or HV is applied in a new species is to demonstrate, using a randomised control trial, that exposure to a validated stressor (eg something equivalent to chronic mild stress in mice) causes an increase in biological age as measured by the biomarker.

Uses and limitations of biomarkers

We have argued that it is possible to reduce the complex concept of cumulative affective experience to a single number: the difference between an individual's current biological and chronological age. For many uses of biomarkers in welfare assessment it is not actually necessary to calculate this difference. It is sufficient to compare the value of the biomarker between groups of animals subjected to different exposures (eg Jackowski *et al* 2011; Aydinonat *et al* 2014). Alternatively, the longitudinal change in the value of the biomarker can be measured within animals exposed to different experiences (eg Rahman *et al* 2016; Nettle *et al* 2017). Between individuals of the same chronological age we predict that those individuals with biomarker values indicative of the oldest biological age will have experienced worse lives than those with the youngest. Within individuals, we predict that a greater increase in biological age as indicated by the change in biomarker values, will be associated with a period of life when experience was worse. In general, we expect longitudinal experimental designs, which measure changes in the value of a biomarker within individuals at two or more points in time, to be the most powerful, because such designs control for individual differences in the value of the biomarkers that will add noise to cross-sectional studies. As a consequence, longitudinal designs will require many fewer subjects than cross-sectional designs to observe a given effect. Indeed, it has been estimated that longitudinal studies of TL require five times less subjects than cross-sectional studies (Aviv *et al* 2006).

While it is possible to use TL and HV to make statements about differences in relative experience (either between individuals or between time-periods), it is important to bear in mind that interpretation of absolute TLs or HVs is more problematic. Just because an animal falls in the longest quartile of TL for the sample measured says nothing about the absolute quality of its experience, because the mean of a sample depends on the sample chosen. Even if it was possible to say that, on average, an animal reared under a given husbandry regime is aged by two years compared to an animal reared under a different regime, it is unclear what this means in terms of absolute differences in welfare. This raises the question of how we benchmark or calibrate values of a biomarker. What values are unacceptable from a welfare perspective? In humans, one approach would be to use the magnitude of changes in biomarkers associated with clinical conditions, such as major depressive disorder or generalised anxiety disorder that we know are associated with substantial suffering. This approach could be extended to non-human animals by using the biomarker values observed in validated models of anxiety and depression to benchmark the effect of a given type of exposure (eg Clarkson *et al* 2018).

More fundamental limitations of the biomarker approach are implicit in reducing cumulative experience to a single number. Much detailed information about an individual's experience is inevitably lost. For example, does it matter if several seemingly innocuous, mildly stressful experiences produce the same effect on a biomarker as one very traumatic experience? Does it matter if the 'scar' from a bad experience fades with time, or is overwritten by a subsequent positive experience? If we believe that variance in individual experience over time is important to well-being, then these questions could potentially be addressed by measuring how biomarkers change longitudinally; an individual could be described both by the current difference between their biological and chronological ages and by the variance in this difference over a series of longitudinal measurements. However, if we believe that accumulated damage to the body is all that is important, then a single measure of current biological age is sufficient. Current biological age should, by definition after all, be a good predictor of future morbidity and mortality.

Animal welfare implications and conclusion

We have developed the hypothesis that biological age is a common currency that integrates the quality of an animal's cumulative experience over its lifetime. Ageing is the result of the accumulation of somatic damage, and its rate is determined by the balance between exposure to events that cause damage and events that mitigate damage or promote repair. We have argued that exposures that are ageing tend to be associated with negative affective experience, whereas those that are restorative or that slow ageing tend to be associated with positive affective experience. Thus, we predict that individuals that are biologically old for their chronological age are likely to have experienced worse lives than individuals that are biologically younger, both in terms of their health and affective state. Biological age is an attractive measure of cumulative experience for use in an animal welfare context, because unlike any existing measures, it integrates information about health and affective state in a single measure. Most importantly, using biological age to assess cumulative experience requires no subjective decisions to be made about whether and how a given exposure impacts an animal and how different dimensions of welfare should be weighted in the overall welfare assessment. Therefore, biological age is a natural solution to the mathematical integration problem with which we began this essay. Biological age can be measured using a range of

different biomarkers. We have presented evidence that two biomarkers, namely leukocyte telomere length (TL) and hippocampal volume or local amount of grey matter in the anterior hippocampus (HV) are valid biomarkers of cumulative experience in humans. TL and HV are evolutionarily conserved in other vertebrates and there is already sufficient evidence to warrant using HV to assess cumulative experience in selected non-human species. Our motivation in writing this essay was to inspire animal welfare scientists to explore the application of these and other biomarkers of biological age in the assessment of cumulative experience.

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