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Assessing the welfare of kennelled dogs—A review of animal-based measures

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ABSTRACT

Hundreds of thousands of dogs are housed in kennels worldwide, yet there are no standard protocols for assessing the welfare of dogs in these environments. Animal science is focusing increasingly on the importance of animal-based measures for determining welfare states, and those measures that have been used with kennelled dogs are reviewed in this paper with particular focus on their validity and practicality. From a physiological standpoint, studies using cortisol, heart rate and heart rate variability, temperature changes, and immune function are discussed. Behavioural measures are also of great relevance when addressing canine welfare, thus studies on fear and anxiety behaviours, abnormal behaviours like stereotypies, as well as responses to strangers and novel objects are reviewed. Finally, a limited number of studies attempting to use cognitive bias and learning ability are also mentioned as cognitive measures. The literature to date provides a strong background for which measures may be useful in determining the welfare of kennelled canines, however more research is needed to further assess the value of using these methods, particularly in regard to the large degree of individual differences that exist between dogs.

1. Introduction

The study of animal welfare is a constantly evolving field. Historically, there have existed two views on the nature of animal welfare: one based on how the animal is doing (i.e. its physical functioning and fitness) and another based on how it is feeling (i.e. its mental state in experiencing the world around it) (Broom, 1986; Veissier and Boissy, 2007; Mills, 2010b). Today, it is often argued that a true measure of welfare incorporates both elements and is not only a measure of the absence of negative states, but also the presence of positive ones (Boissy et al., 2007). It is from this idea that the Five Freedoms were born, detailing five essential areas that must be met for an animal to be considered to have good welfare, and on which UK animal welfare laws are based (FAWC, 2009). Standard protocols based on these principles have been created for a variety of farm animals, including cattle, poultry, and pigs (Welfare Quality®, 2009) however, no such protocols exist for measuring the welfare of dogs in kennels.

Around the world, dogs are kept in kennels for a variety of reasons. Abandoned and stray dogs are kennelled in rehoming centres until they are either adopted or euthanised. In the UK alone, approximately 27,000 dogs go through the three largest rehoming organisations and over 81,000 stray dogs pass through kennels in local authorities each year (RSPCA, 2016; Battersea Dogs and Cats Home, 2016; Dogs Trust, 2017). At any one time, the number of dogs in shelters in the USA is as high as 3.3 million (ASPCA, 2017). Working dogs, such as police dogs, military dogs, and sled dogs, are also generally kennelled when they are not on duty (Gaines et al., 2008). Greyhounds kept for racing are housed in kennels, with over 15,000 in just the UK (Environment Food and Rural Affairs Committee, 2016). Laboratories also breed dogs which are kept in cages or kennels for research purposes (Hubrecht, 1993). Even pet dogs, who may not normally reside in kennels, may be left at kennels for short periods where they are exposed to reduced social contact, limited space, excessive noise, and altered daily routines, while their owners are on holiday, or if the dogs are in quarantine, receiving veterinary care, or have been seized due to breed-specific legislation. Thus, it is clear that a large population of dogs are housed in a kennel environment at some point in their lives, some for extended periods. While the kennels may vary in their sizes, set-ups and features, the welfare implications of keeping dogs in housing conditions that are often so restricted - both spatially and socially - are substantial and deserve consideration (for a detailed review, see Taylor and Mills, 2007).

Although individual organisations that house dogs in kennels tend to have their own welfare assessment procedures, there is no consensus on what measures should be included in these assessments, nor on the reliability and validity of those measures. Indeed, the differences in

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housing environments as well as differences in dogs' past experiences (for example the rearing of kennelled racing greyhounds is likely to be very different to the early life experiences of police dogs, which again would be different to those of pet dogs housed in rehoming centres) mean that certain welfare indicators that might be valuable in one context may not be relevant in other contexts or with different groups of dogs.

Some attempts have been made to create evidence-based welfare assessment tools specifically for rehoming centre dogs. Barnard et al. (2014) developed the Shelter Quality Protocol, which uses 26 indicators of welfare – including management, resource, and animal-based measures, as well as an 'Emotional State Profile,' measured through a visual analogue scale – to identify areas of welfare concern in long term shelters. Kiddie and Collins (2014) developed a quality of life assessment tool based on behavioural indicators of both positive and negative emotional states, where a binary scoring system was used for each indicator; the proportion of negative indicators recorded was then subtracted from the proportion of positive indicators to produce an individual quality of life score for each animal. When discussing each measure within this review, we will mention which are included in each of these tools.

Although encouraging progress has been made, it is timely to also review additional potential measures that may be relevant and practical indicators of welfare in kennelled dogs. Ideally, these measures should be applicable to all kennelled dogs, and not just those in rehoming centres. Most early assessments of animal welfare focused on measuring the animals' conditions and the resources that were provided to it, rather than the animal's perception of those conditions (Whay et al., 2003; Whitham and Wielebnowski, 2009). Admittedly, measuring management practices as potential indicators of welfare is infinitely simpler than attempting to discern well-being from animal-based measures. Unfortunately, management practices only give an indication of an animal's "potential" for good welfare (i.e. good welfare cannot be achieved without appropriate housing, but appropriate housing does not guarantee good welfare) and thus animal-based measures are still needed for a full assessment. This does not mean that management level measures are not valuable however, as meeting an animal's basic needs is still essential. Taylor and Mills (2007) have summarised the effects of different aspects of the kennel environment and management (i.e. space allowance and furniture, visual, olfactory and auditory enrichment, exercise, social contact, predictability of routine, etc.) on canine welfare in their review.

As it has now been established that welfare is not something that is given to an animal but rather a characteristic that it has (Broom, 2007), greater emphasis needs to be placed on animal-based measures that are able to quantify how an individual animal is coping with the conditions that it is in. In this review, the different physiological, behavioural, and cognitive measures that are being used to assess canine welfare at the animal-level will be discussed, with specific emphasis placed on novel procedures that are being developed, as well as the feasibility of applying the methods to a range of kennel environments. This review will discuss whether measures have been validated, however, whilst in the case of laying hens and some other production species there have been true validation studies in which potential welfare indicators are compared to animals' preference/choice (e.g. Nicol et al., 2009), for dogs, no such studies exist. Therefore, we review the extent to which each indicator has been validated against others. The goal of this review is not to identify a single catch-all welfare indicator, but to identify which measures would be most useful in a welfare assessment that incorporates the most practical and reliable measures. Table 1 provides a summary of the measures discussed, as well as their feasibility and potential confounds.

2. Physiological measures

2.1. Cortisol

Cortisol is one of the most commonly used biomarkers of stress as it is indicative of the level of activity within the hypothalamic-pituitary-adrenal (HPA) axis, the body's primary physiological stress-response system. Cortisol levels increase in response to arousal regardless of valence, but when examined together with behavioural responses, this increase can be a useful indicator of distress (i.e physiological stress that occurs in response to negative experiences, as opposed to eustress, which would occur in response to positive experiences (Mills, 2010a)). Cortisol measurements have become a cornerstone in animal welfare research and have been used to measure stress responses across a wide selection of species (for a review, see Mormède et al., 2007).

As can be seen in Table 1, in studies of dogs, a variety of methods have been used to measure cortisol levels, each presenting different confounds and relative feasibility. Early studies tended to rely on plasma cortisol found in blood samples, however, since the process of taking blood is quite stressful in itself, concerns have been raised about the confounding effects of the procedure (Beerda et al., 1996). As a number of studies both in dogs (Vincent and Michell, 1992; Beerda et al., 1996) and other mammals (Fell et al., 1985; Parrott et al., 1989) have now demonstrated that salivary cortisol levels are highly correlated with plasma levels, blood cortisol sampling has largely been abandoned in favour of less invasive measures, although it is still used if blood is being taken for other purposes or measures (Dudley et al., 2015). Urinary and faecal cortisol have also proved to be popular non-invasive measures used to measure stress in dogs as both provide an indication of cortisol production over a slightly longer period of time than either saliva or plasma (Mormède et al., 2007; Accorsi et al., 2008; Rooney et al., 2009; Bryan et al., 2013; Titulaer et al., 2013).

There has been some research into the use of hair and nail samples to potentially measure chronic distress (see Mesarcova et al., 2017 for a review). One major advantage of these is that they may eliminate the confounding effects that natural fluctuations of cortisol levels throughout the day have on the other sample types (Accorsi et al., 2008; Mesarcova et al., 2017). It is well established in humans and some other mammals that cortisol fluctuates in a diurnal pattern, with levels being the highest shortly after waking (Mormède et al., 2007). This pattern is not as distinct in dogs, with some studies finding evidence of cortisol levels being highest in the morning, others suggesting that the pattern may not exist at all, and one study finding differences in whether diurnal patterns exist depending on the usual routine of the dog (see Kolevská et al., 2003 for a review). Regardless of whether the fluctuations are diurnal or not, there is ample evidence to suggest that fluctuations do occur - in response to normal hormone releases, altered rates of blood flow through the adrenal system (for example due to exercise or other physical exertion), or even cold temperatures (Beerda et al., 1996; Hennessy, 2013; Protopopova, 2016) - the effects of which would be cancelled out when measuring long-term stress through hair or nail samples.

Another significant advantage of using hair samples in particular is the potential to measure chronic stress and baseline cortisol levels over time (Accorsi et al., 2008; Bryan et al., 2013; Grigg et al., 2017; Mesarcova et al., 2017). Other measures of cortisol like plasma, urine, and saliva, assess the levels currently circulating within the animal's body. This makes them potentially useful measures of acute stressors that have affected the animal in the time (minutes to hours) before the measurement was taken but may not be reflective of the level of chronic stress that the animal is experiencing long term (see Table 1). Because the cortisol amounts in hair are bound to it over time, both as the hair follicle grows and as the sweat and sebum excreted from skin glands comes in contact with it, it gives a more precise picture of long-term cortisol elevation (Mesarcova et al., 2017). Indeed, it has been found to give more stable measurements than either faeces or saliva, with four

Table 1
Practicality of Animal-Based Measures for Assessing Welfare of Kennelled Dogs. A summary of the feasibility and confounds of physiological, behavioural, and cognitive measures, including reference to whether they indicate chronic (across multiple days) or acute (within a single day) stress, and whether their valence (distress v eustress) can be determined.

Welfare Measure	Feasibility	Chronic v. Acute	Clear valence?	Potential Confounds	References
PHYSIOLOGICAL MEASURES					
Cortisol – urinary or faecal	Relatively easy to obtain. Expensive to analyse	Acute	No – only indicates arousal	Diurnal patterns, temperature, activity levels may confound results	Beerda et al., 2000; Stephen and Ledger, 2006; Mormède et al., 2007; Rooney et al., 2007; Accorsi et al., 2008; 2009; Bryan et al., 2013; Titulaer et al., 2013; Denham et al., 2014
Cortisol – salivary	Relatively easy to obtain. Expensive to analyse.	Acute	No – only indicates arousal	Sampling procedure as well as diurnal patterns, temperature, activity levels may confound results	Vincent and Michell, 1992; Beerda et al., 1996, 1999b; Hiby et al., 2006; Part et al., 2014; Cobb et al., 2016
Cortisol – plasma	Requires skill to obtain. Expensive to analyse.	Acute	No – only indicates arousal	Sampling procedure as well as diurnal patterns, temperature, activity levels may confound results	Beerda et al., 1996; Clark et al., 1997; Hennessy et al. 1997, 2002a, 2002b, 2006, 2013; Dudley et al., 2015
Cortisol – hair/nail	Relatively easy to obtain. Expensive to analyse.	Chronic	No – only indicates arousal	Some evidence that hair colour and brushing may influence results. Hair processing procedures vary in effectiveness of recovering cortisol.	Bennett and Hayssen, 2010; Bryan et al., 2013; Siniscalchi et al., 2013; Svendsen and Søndergaard, 2014; Nicholson and Meredith, 2015; Rosen, 2016; Grigg et al., 2017; Mesarcova et al., 2017
Heart rate	Requires special equipment	Acute	No – only indicates arousal	Dog's body movement and posture may affect measures. Wearing the devise may distress some dogs.	Maros et al., 2008; Wormald et al., 2016; Craig et al., 2017; Wormald et al., 2017
Heart rate variability	Requires special equipment	Acute	No – likely only indicates arousal	Dog's body movement and posture may affect measures. Wearing the devise may distress some dogs.	Maros et al., 2008; Katayama et al., 2016; Zupan et al., 2016; Wormald et al., 2016; Craig et al., 2017; Wormald et al., 2017
Temperature	Thermography requires special expensive equipment, thermometers require handling/restraint	Acute	No – only indicates arousal	Restraint for thermometers may confound results. Thermographic results may be influenced by external temperature, material, dimensions and heat-reflecting ability of environment. Some dogs may also show aversive behaviour toward camera.	Ogata et al., 2006; Part et al., 2014; Vainionpää, 2014; Nääs et al., 2014; Redaelli et al., 2014; Travain et al., 2015; Proctor and Carder, 2015; Telkänranta et al., 2016; Whitham and Miller, 2016; Riemer et al., 2016
Immune function (IgA/ antibody and WBC levels)	Requires skill to obtain. Expensive to analyse.	Chronic	Potentially – more research needed	Some studies have found significant intra- and inter-individual variations. Some evidence of diurnal patterns. Breed and age need to be taken into account. Handling may confound results depending on sampling method.	Skandakumar et al., 1995; Clark et al., 1997; German et al., 1998; Kikkawa and Uchida, 2003; Olsson et al., 2014; Dudley et al., 2015; Protopopova, 2016
Weight change	Relatively easy to assess	Chronic	Potentially – more research needed	Changes in diet, exercise levels, or medications could confound results. Some physical ailments (e.g. worms) may influence weight without necessarily immediately impacting welfare.	Broom and Johnson, 1993; Rooney et al., 2009
Disease incidence	Relatively easy to assess	Chronic	Yes	Unclear if due to stress or close proximity and poor cleaning procedures in kennels	Edinboro et al., 2004; Erles and Brownlie, 2008; Lavan and Knesl, 2015
Oxytocin – urinary	Relatively easy to obtain. Expensive to analyse	Acute	Yes	More research is needed.	Mitsui et al., 2011; Pekkin et al., 2016
Paw sweating	Requires dry environment to assess	Acute	No	More research is needed. Could also be caused by warm temperatures	Belpedio, 2010
Pupillary dilation	Difficult to assess without confounding	Acute	No – only indicates arousal	More research is needed. Effects of handling could confound results. Lighting and medication need to be taken into account.	Belpedio, 2010; Part et al., 2014
Eye redness	Relatively easy to assess	Acute	Unclear	More research is needed. Could also be caused be allergies or physical irritants. Breed differences in facial morphology may also confound results.	Part et al., 2014
Faecal consistency	Easy to assess	Acute	No – only indicates arousal	More research is needed. Effects of diet/allergies/medications also need to be considered.	Belpedio, 2010; Part et al., 2014
Excessive shedding of skin or hair / skin dryness	Relatively easy to assess	Chronic	Unclear	More research is needed. Effects of diet/allergies/temperature also need to be considered.	Belpedio, 2010; Part et al., 2014
BEHAVIOURAL MEASURES					
Anxiety & fear related behaviours	Easy to assess	Acute	Yes	Individual differences in behaviours exhibited.	Beerda et al., 1997, 1999a; Stephen and Ledger, 2005; Hiby et al., 2006; Rooney et al., 2009, 2009, 2016; Blackwell et al., 2013
Response to strangers	Easy to assess	Acute	Yes	Previous experiences/early socialisation may influence response.	Hubrecht, 1993; Arhant and Troxler, 2014; Conley et al., 2014
Response to novel object	Easy to assess	Acute	Yes	Previous experiences/early socialisation may influence response. Some indication of breed differences.	Plutchik, 1971; King et al., 2003; Forkman et al., 2007; Ley et al., 2007

(*continued on next page*)

Table 1 (*continued*)

Welfare Measure	Feasibility	Chronic v. Acute	Clear valence?	Potential Confounds	References
Abnormal repetitive behaviours	Easy to assess	Chronic	Yes	Behaviours can persist even after animal has been removed from stressful environment. Behaviours may persist because they are rewarded. Behaviours may serve as coping mechanism.	Hubrecht et al., 1992; Beerda et al., 1999a; Mason and Latham, 2004; Stephen and Ledger, 2005; Taylor and Mills, 2007; Hewson et al., 2007; Rooney et al., 2009; Belpedio, 2010; Denham et al., 2014; Protopopova et al., 2014, 2016
Coprophagia	Easy to assess	Chronic	Unclear	More research is needed. Possible breed differences.	Beerda et al., 1999a; Gaines et al., 2008 as cited in Rooney et al., 2009; Col et al., 2016
Excessive barking	Easy to assess	Both	Unclear	More research is needed. Possible breed differences. Behaviours may persist because they are rewarded.	Hetts et al., 1992; Clark et al., 1997; Stephen and Ledger, 2005
Excessive drinking	Easy to assess	Both	Unclear	More research is needed. Temperature or previous experiences may confound.	Clark et al., 1997; Stephen and Ledger, 2005; Hiby et al. 2006; Kiddie and Collins, 2014
Activity/Resting	May require specialised equipment	Both	Unclear	Difficult to distinguish between rest and boredom/learned helplessness/depressive states	Haley et al., 2000; Hiby et al., 2006; Rushen et al., 2012; Jones et al., 2014; Part et al., 2014; Owczarczak-Garstecka et al., 2016
Play	Spontaneous play needs video recording. Elicited play relatively easy to assess.	Both	Yes	Previous experiences/early socialisation may influence willingness to play. Possible breed differences. Spontaneous behaviours are more relevant than elicited ones.	Hubrecht, 1993; Rooney et al., 2000; Coppola et al., 2006; Horváth et al., 2008; Belpedio et al., 2010; Bergamasco et al., 2010; Held and Špinka, 2011; Titulaer et al., 2013; Johnson et al., 2013; Shiverdecker et al., 2013; Conley et al., 2014; Bradshaw et al., 2015; Flower, 2016; Ahloy-Dallaire et al., 2017
COGNITIVE MEASURES					
Cognitive bias	Requires training/time	Chronic	Yes	Differences in trainability and motivation may influence results	Harding et al., 2004; Mendl et al., 2009, 2010; Bateson et al., 2011; Burman et al., 2011; Owczarczak-Garstecka et al., 2016
Learning ability	Requires training	Both	Unclear	Individuals may be differently motivated by different rewards. Different training styles may be more/less effective.	Gácsi et al., 2009; Blackwell et al., 2010; Rooney and Cowan, 2011; Hobbs et al., 2018

faecal samples being needed to reach the same level of repeatability as a single hair sample (Bryan et al., 2013). In the as-of-yet only study using hair cortisol to measure the long-term welfare of kennelled dogs, Grigg et al. (2017) found that hair cortisol levels were significantly lower in dogs eight weeks after being switched from solitary housing to social housing than they were prior to the intervention. The same study also found reductions in repetitive behaviours and barking, although no statistical analysis was performed to assess the association between hair cortisol and these behaviours.

When looking at studies of dogs that have analysed relationships between cortisol and behaviour, hair samples have been one of the only measures that have found strong associations between the levels of the steroid and stress behaviours such as running away, hiding, panting, lowering body posture, and attention seeking, following a period of being exposed to stressful stimuli – although it should be noted that these associations were only significant with samples taken in the morning and not samples taken in the afternoon, despite there being no significant difference between the two samples (Siniscalchi et al., 2013). Other studies which have attempted to correlate behavioural indicators of stress with cortisol levels in either saliva or urine, have found few significant associations (Beerda et al., 1998, 1999b; Hennessy et al., 2001; Hiby et al., 2006; Jones et al., 2014; Part et al., 2014).

While it appears that hair samples may be a practical and effective way to quantify basal cortisol levels, more research needs to be done to gain a better understanding of their use. For example, some studies have suggested that there may be differences in cortisol level based on hair colour, with darker hairs having less cortisol than lighter hairs, sometimes even within the same dog (Bennett and Hayssen, 2010;Svendsen and Søndergaard, 2014). However, other studies have not found such differences (Nicholson and Meredith, 2015; Rosen, 2016). It is also unclear what effect external factors, such as sunlight exposure or excessive brushing may have on cortisol concentration in hair (Mesarcova et al., 2017). It has also been found that different methods of preparing the hair for analysis, for example powdering compared to chopping, can cause over a three-fold difference in the amount of cortisol that can be recovered from the sample (Mesarcova et al., 2017). Furthermore, although hair samples seem to be promising potential measures of chronic stress in dogs, only one study as of yet (Grigg et al., 2017) has used cortisol concentrations in hair to assess the welfare of kennelled dogs specifically.

There have however been several studies assessing the welfare of kennelled dogs using the other measures (primarily salivary and urinary) of cortisol concentration. When looking at the impact of moving dogs into kennels, most of the research suggests that dogs tend to have an initial increase in cortisol levels when entering kennel environments that are more challenging than those they are used to, but this is modulated by past experiences. For example, when dogs were transferred to indoor kennels after having been housed in outdoors areas during favourable weather, their cortisol levels increased far more than dogs that had been brought to kennels from areas that typically have stormy, unpleasant weather, despite their levels being comparable previously (Beerda et al., 1999b). Similarly, dogs that were brought into kennels after having been strays or having been previously in kennels showed declining cortisol levels over their first ten days, whereas dogs surrendered from homes showed increasing levels (Hiby et al., 2006). Indeed, Rooney et al. (2007) found that, when housed in kennels, if dogs were previously habituated to the kennel environment, their urinary cortisol levels increased significantly less than if the dogs had not been habituated. In contrast, another study that conducted a direct comparison between urine samples taken in a home environment and on the first days in a kennel found that cortisol was significantly higher in the kennel than at home regardless of past kennelling experience, however these dogs had not gone through a specific habituation programme as in Rooney et al. (2007), but had simply had a history of kennelling (Part et al., 2014).

Some studies suggest that cortisol first increases, then levels out with time (Hennessy, 2013; Protopopova, 2016). One study found that dogs newly admitted to shelters had higher plasma cortisol levels than dogs that were sampled in homes, however, their levels were comparable after just five days (Hennessy et al., 1997). Another found that, on average, urinary cortisol peaked around the seventeenth day, after which levels steadily declined and were not significantly different from baseline levels taken from dogs in homes by Day 31 (although the authors did note large individual variations between the dogs) (Stephen and Ledger, 2006). When looking at plasma cortisol levels, Dudley et al. (2015) found that, on the first day, cortisol levels in newly kennelled dogs were significantly higher than those of control dogs, however these levels significantly decreased over the next ten days (but were still higher than control dogs on Day 10). A study on laboratory beagles looking at cortisol levels immediately after being transferred to small, single steel cages from outdoor, social pens, found that levels were highest on the first day and gradually decreased and stabilised over the next 77 days (Clark et al., 1997).

Other studies however, have not found evidence of such a change over time. Titulaer et al (2013) noted that dogs housed in kennels for six months did not have significantly different urinary cortisol levels from dogs that had been housed in kennels for between one week and three months, and that levels for all groups were "relatively high," although the initial levels upon first entering kennels were not measured. Beerda et al. (2000) found that dogs housed in the most severe living conditions tended to have higher urinary cortisol levels than dogs housed in more enriched environments even after being kept in those conditions for multiple years. A meta-analysis of 31 studies found no difference between the salivary cortisol levels of dogs living in shelters for less than two weeks compared to more than two weeks (Cobb et al., 2016).

To complicate matters further, there are also some studies which have found that cortisol levels decrease to significantly lower than baseline in kennels. The same meta-analysis of over 30 studies found that dogs that have been living in rescue shelters for over two weeks had significantly lower salivary cortisol levels than dogs in working/training kennels or private homes (Cobb et al., 2016). Gaines and Rooney (unpublished data 2007; as cited in Hewson et al., 2007) also found that dogs that had been kennelled for more than one year had urinary cortisol levels that were below the levels of dogs in homes. Although Denham et al (2014) found that the majority of kennelled working dogs still had normally functioning HPA systems even after years of living in kennels, they also noted that the small group of dogs that showed the most atypical behavioural responses (engaging in repetitive behaviours under minimal stimulation) had opposite responses to novel stressors, where their cortisol levels decreased after the challenge. These results provide some evidence that dysregulation of the HPA axis may occur in dogs in response to chronic stress of kennelling.

Indeed, there is other physiological evidence that dysregulation occurs in some kennelled dogs, namely the dissociation of ACTH and cortisol levels over time (Hennessy et al., 2002a, 2002b, 2006, 2013). Dysregulation may also provide a reasonable explanation for why many studies have not found direct associations between cortisol levels and the majority of behavioural measures considered indicative of distress in canines, although individual differences in behavioural responses is likely also a factor (Beerda et al., 1999b; Hennessy et al., 2001; Hiby et al., 2006; Jones et al., 2014; Part et al., 2014). Downregulation and decreased HPA reactivity in response to chronic stress has also been observed in a number of farm animal species (e.g. cows, sheep, horses), wild animals (e.g. European starlings), and even humans (see Pawluski et al., 2017).

Thus, the overall picture of the usefulness of cortisol as a measure of the welfare of kennelled dogs remains unclear and convoluted. Idealistically one may aspire to determine set cut-off levels to identify whether an individual's welfare is unacceptably poor. Unfortunately, due to the myriad of factors that may influence measures (sex, age, neuter status, time of day, etc. - see: Cobb et al., 2016 and Mormède et al., 2007); the limited understanding of when and how dysregulation of the system occurs; the lack of correlation with behavioural measures; and the fact that levels increase in response to arousal regardless of its valence, it is not currently possible to determine such a cut-off value for unacceptable welfare. These factors also suggest that the measures of cortisol that have been used thus far are unlikely to be the most reliable measures of distress in kennelled dogs, particularly for those kennelled long-term. Indeed, no cortisol sampling measures of any kind was used by either Kiddie and Collins (2014) or Barnard et al (2014) in their assessment tools. Nevertheless, there exists compelling evidence that novel measures of cortisol, such as in hair or nail samples, could provide valuable data relating to chronic distress. Future research should aim to assess the usefulness and relevance of such novel measures, and to gain a better understanding of the ambiguity of results when compared to commonly used measures.

2.2. Heart rate and heart rate variability

Heart rate and heart rate variability are also frequently used indicators of potential distress in the study of animal welfare (von Borell et al., 2007). Activation of the sympathetic nervous system causes an increase in heart rate to facilitate any impending ‘fight or flight’ response. While heart rate (HR) simply measures the average number of beats per minute, hear rate variability (HRV) measures the changes in time between heart beats. HRV has been suggested as a particularly useful non-invasive measure of activity in the autonomic nervous system, with HRV tending to decrease in response to stressful events. While HR and HRV have been used extensively to assess welfare in pigs, goats, cattle, poultry, and horses (see von Borell et al., 2007 for a review), a number of recent studies have now also focused on dogs (Craig et al., 2017; Wormald et al., 2016, 2017; Table 1), although none have used HRV to examine the welfare of kennelled dogs specifically, nor has it been used in either of the previously discussed assessment tools (Barnard et al., 2014; Kiddie and Collins, 2014).

A study examining the relationship between HRV and aggression (which is commonly caused by fear/distress, see Rooney et al., 2016) found that aggressive dogs had significantly lower HRV than non-aggressive dogs when measured after the arrival of a stranger into their home, suggesting that they were more distressed at the time of this event (Craig et al., 2017). Wormald et al. (2016) found a similar result when taking HR, HRV, and salivary cortisol measures from greyhounds undergoing a blood donation procedure. Afterwards, the dogs were tested for their reactions to approaching a small, unfamiliar dog. Those dogs which displayed aggression towards the unfamiliar dog had significantly elevated initial HR, significantly reduced HRV, and significantly increased cortisol levels during the blood donation than dogs that did not show aggression. In another study by the same authors (Wormald et al., 2017), dogs diagnosed with anxiety-related behaviour problems had significantly lower HRV after a manual restraint procedure than dogs without such problems. Interestingly however, the restraint procedure in this study did not increase cortisol levels in either group, and reactivity during the procedure was not related to HRV measures, making it unclear whether the dogs in the anxious group had a lower baseline HRV to begin with or if this was influenced by the procedure.

Katayama et al. (2016) found that HRV is reduced in response to separation from a caregiver, but also found reduced HRV in response to being gently petted by their caregiver (which they assumed to be a positive interaction), suggesting that changes in HRV may occur when aroused regardless of the valence of emotional change. In agreement, Zupan et al. (2016) also demonstrated that HRV decreases in response to positive emotional states like being rewarded with high-value food or being petted by a familiar person. These results suggest that HRV should not be used as a measure on its own, but rather should be interpreted together with other behavioural or cognitive information that can determine the emotional valence of the physiological stress the dog is experiencing.

Although HR and HRV are relatively straight-forward, non-invasive measures, there are some considerations that need to be taken into account when using them to assess welfare (Table 1). Firstly, not only does the potential effect of the dog’s body movement need to be taken into account, but there is also evidence to suggest that body posture and even orientation can have an effect, where HR is lowest when lying down, and orienting towards a favourite toy causes a significant increase in HRV (Maros et al., 2008). Secondly, whilst wearing a heart rate monitor does not seem to adversely affect most dogs (Vincent and Leahy, 1997; Essner et al., 2015), individual differences in their sensitivity to this specialised equipment still needs to be taken into consideration and habituation carried out. Furthermore, although HRV seems to be a promising measure of physiological stress, the emotional state of the dog still needs to be determined through other measures for it to be relevant to a welfare assessment.

2.3. Temperature

Body temperature can be a useful physiological measure of stress in animals. In response to acute stress, the activation of the sympathetic nervous system generally causes an increase in core temperature accompanied by a decrease in the temperature of the peripheries, known as stress-induced hyperthermia (Ogata et al., 2006; Travain et al., 2015). In laboratory rodents, stress-induced hyperthermia has been demonstrated as being a direct physiological response to arousal rather than an increase in body temperature due to physical activity or ambient temperature (Long et al., 1990; Oka et al., 2001; Ogata et al., 2006; Part et al., 2014).

Temperature has been widely used in studies of dogs (Table 1). Ogata et al. (2006) studied the responses of dogs to a fear-conditioning protocol. Although the individual behavioural responses were highly variable, all the dogs showed a similar increase in core (rectal) temperature, suggesting that the dogs experienced stress during the protocol. Using infrared thermal imaging, Travain et al. (2015) also found that the internal eye temperature of dogs was significantly higher during a stressful veterinary exam than both before and after the exam. In contrast, Part et al. (2014) found that dogs who were moved to kennels from a home environment had no difference in their core temperatures (measured with a thermometer in the ear canal), but they did have significantly lower surface temperatures, as measured using an infrared camera recording nose temperature. Similarly, Riemer et al. (2016) used an infrared camera to measure surface temperature, specifically the temperature of the ear pinnae from long distances (6–8 m). Although this was a small sample (six dogs), the results showed that peripheral temperature decreased in response to separation and increased again upon reunion with the owner. Thermal imaging has also been studied in dogs in a clinical veterinary context to assess its usefulness in detecting pain (Vainionpää, 2014).

Taking temperature measurements manually with a thermometer is generally a stressful experience for animals, as it is typically involves inserting a thermometer either into the rectum or the ear canal. With the advance of technology, thermal imaging can now be used as a non-invasive measure of temperature, which greatly reduces the stresses involved. This is because thermal imaging requires no restraint and, in some cases, does not even require a person to be present, meaning that the animal is not even necessarily aware that the measurements are being taken (Riemer et al., 2016). Thermal imaging is a useful measure of welfare across a variety of species for both core and peripheral temperatures (for reviews, see: Nääs et al., 2014 and Redaelli et al., 2014), however few studies have used it to assess canine welfare specifically, as a standard methodology has not yet been developed and validated.

One major issue with using thermal cameras is that some studies have reported that the dogs show aversive behaviours towards the camera (Travain et al., 2015), suggesting that camera itself could be a source of distress for the animals. This could clearly influence the measures taken, however other studies have developed methods where the dogs are completely unaware of the camera, with measurements being taken from five to eight metres away (Riemer et al., 2016) or where the animals are habituated to the camera over time (Proctor and Carder, 2015). Even in these cases however, there is still the possible confounding effect of the environment that needs to be taken into consideration, as the temperature, material, dimensions, and heat-reflecting ability of the animal's surroundings could all influence the measurements (Telkänranta et al., 2016; Whitham and Miller, 2016). There is also no standard procedure for using thermographic imaging in dogs, and thus these methods have not been validated for this species, nor have they been used in the mentioned assessment tools (Barnard et al., 2014; Kiddie and Collins, 2014).

2.4. Immune function

Activation of the HPA axis in response to stress requires a great deal of energy from the body, and thus leaves it in a more vulnerable state than normal. Therefore, it is not too surprising that compromised immune function may be another useful physiological measure of poor welfare, particularly of chronic stress (Protopopova, 2016). Immune function is often studied by measuring the level of antibodies such as immunoglobulin-A (IgA) present or by white blood cell counts (WBC). Unfortunately, as with cortisol, studies attempting to assess the immune function response to stress in dogs have found conflicting results.

One of the earliest studies to assess immune response as a measure of stress was by Skandakumar et al. (1995). This study examined kennelled police dogs (German Shepherd Dogs) who underwent stressful training routines (including tracking, agility, retrieving, and searching for and recovering hidden objects marked with specific scents), and kennelled army dogs (German Shepherd Dogs, English Springer Spaniels, and a Labrador Retriever) who were exposed to a novel stressful environment (a veterinary setting where a hip radiography was carried out). Their study found a significant negative correlation between cortisol and salivary IgA and a positive relationship between salivary IgA and behavioural scores (higher scores were associated with more positive states). This is in accordance with the hypothesis that stress reduces immune function, and thus, the authors concluded that IgA could potentially be used as a reliable stress-indicator in dogs. While some authors have argued against this, citing significant intra-individual variations (e.g. German et al., 1998), there have also been studies which have demonstrated similar results: Kikkawa and Uchida (2003) found that salivary IgA levels in laboratory beagles significantly decreased in the 30 min following a stressful noise stimulus and did not return to baseline until 60 min later. Also with laboratory beagles, Clark et al. (1997) found that the number of white blood cells (which produce antibodies like IgA) significantly decreased over the 12 weeks following the dogs' transfer to the laboratory cages.

One study, examining the effects of kennelling specifically however, found contrasting results. Dudley et al. (2015) compared dogs newly admitted to shelters to control dogs in homes, and found that not only were leukocyte, neutrophil, monocyte, and neutrophil-to-lymphocyte ratios higher in shelter dogs, the counts of these continued to increase significantly over the ten days studied. Antibody levels have not been used by either assessment tool (Barnard et al., 2014; Kiddie and Collins, 2014) as practical measures of welfare/quality of life.

While measuring antibodies and white blood cells is a straightforward way of assessing immune function, there are some potential confounds. Firstly, the stress involved with taking blood samples could have an impact on the results, although this could be lessened by opting for saliva samples instead. Secondly, there is some suggestion that certain immunological functions may follow diurnal patterns, meaning that the time of day the measures are taken could have a significant impact on the results (Kikkawa and Uchida (2003)). There is also evidence to suggest that there are significant differences in IgA concentrations based on breed (with eight breeds now identified as being predisposed to low IgA concentrations) and based on age (Olsson et al., 2014). Finally, the level of individual differences and indeed intra-individual differences has been called into question, and thus needs more research to determine the level of variability (German et al., 1998).

To circumvent these potential issues, there are also several measures which can be used as indirect indications of suppressed immune systems. One such possible measure is weight loss/gain which can be calculated either through measuring the weight directly or through body condition scoring. Weight loss is generally considered an indication that an animal is struggling to cope, potentially due to stress causing a decreased appetite, and is a significant welfare issue (Broom and Johnson, 1993; Rooney et al., 2009). Both Barnard et al (2014) and Kiddie and Collins (2014) included body condition score as a measure in their protocols to assess kennelled dog welfare.

Incidence of disease can also give clues about immune function, as animals with compromised immune systems will be more susceptible to diseases than those with normally functioning systems. In one study of dogs in US shelters, the chances of a dog coughing increased by 3% for each day the dog was in the shelter (Edinboro et al., 2004). Indeed, another study found that approximately one half of the shelter dogs measured were infected with some form of canine infectious respiratory disease (Lavan and Knesl, 2015), while another found that after three weeks 100% of dogs tested positive for antibodies for the specific disease canine respiratory coronavirus, compared to 30% on day of entry into the kennels (Erles and Brownlie, 2008). Unfortunately, it is difficult to quantify whether this increased risk of infection is due to suppressed immune functions caused by stress, or due to the greater likelihood of disease spreading due to the close confines and limited cleaning procedures present in most kennels. It is most likely a combination of these factors. Kiddie and Collins (2014) did not measure disease prevalence in their assessment tool, however Barnard et al (2014) did record some symptoms that may be indicative of disease (e.g. diarrhoea, coughing), and also included a measure of the centre's annual expenses for clinical treatment.

2.5. Other physiological measures

There are many other physiological measures that have been suggested as possible welfare indicators in dogs (Table 1), but none have been fully validated and many are difficult to measure. One example is oxytocin, which has been suggested as a non-invasive physiological indicator of positive emotions in dogs (Mitsui et al., 2011). In a study on guide dogs, urinary oxytocin levels were found to increase in response to a variety of stimuli that were associated with positive emotions (eating, stroking, exercising) and this increase may have even inhibited cortisol secretion (Mitsui et al., 2011). More research is however needed to fully understand oxytocin's relationship with various emotional states as well as the effects of individual differences (Pekkin et al., 2016). Some other examples include paw sweating, pupillary dilation, eye redness, skin dryness, faecal consistency and excessive shedding of skin or hair (Belpedio, 2010; Part et al., 2014). Kiddie and Collins (2014) included the "presence of scruff" as well as "eye discharge" as physical measures within their scoring system, while Barnard et al (2014) included a "skin condition" section where the presence of wounds, hair loss, swelling, and ectoparasites was recorded. Future studies should continue to examine these measures to assess their potential as useful indicators of canine welfare.

3. Behavioural measures

3.1. Anxiety & fear related behaviours

Behavioural changes that correspond with fear and anxiety have been extensively documented across a variety of species. Perhaps the most universal of these, which are common among most mammals, including dogs, are a low, cowering body posture, attempts to retreat and avoid the fear-inducing stimulus, freezing, and in severe cases, trembling (Stephen and Ledger, 2005; Rooney et al., 2009, 2016; Blackwell et al., 2013). There are some other behaviours which have been suggested to be more specific to dogs, such as paw lifting, lip licking, gaze aversion, panting, whining, auto-grooming, and body shaking (Beerda et al., 1997, 1999a; Hiby et al., 2006; Rooney et al., 2009). Increases in these behaviours have been shown when dogs enter kennel environments (Beerda et al., 1999a). Aggression is another behaviour that is closely linked to anxiety and fear in dogs and may indicate compromised welfare, however few studies have examined aggression in this context (Taylor and Mills, 2007; Rooney et al., 2016). Kiddie and Collins (2014) included all of these behaviours and additional ones as "behavioural measures assessing negative emotions" in their assessment tool. Barnard et al. (2014) did not list any of these behaviours specifically in their assessment, although the Emotional State Profile section of the protocol does include visual analogue scales for the terms "nervous," "unsure," and "anxious." Panting and shivering are recorded as part of the assessment and are used as indicators of inadequate shelter (i.e. is the dog overheating or too cold) rather than as behavioural indicators of anxiety or fear.

While some dogs may frequently exhibit fear and anxiety related behaviours during their time in kennels regardless of any external stimuli, the absence of these behaviours does not necessarily indicate good welfare, particularly in cases of chronic stress, where apathy or learned helplessness may influence behaviour (Mason and Latham, 2004; Gaines et al., 2008). In some instances, increases in fear and anxiety behaviour may become evident only in response to novel stimuli and thus measures looking at these reactive behaviours may be more useful.

One such test which has been used in kennelled dogs is the response to the approach of a stranger. In farm animals, "avoidance distance" is used to quantify this (Welfare Quality®, 2009). In kennel dogs, a combination of approach distance, body postures, and behaviour is generally used. Arhant and Troxler (2014) studied an approach test for dogs in 29 shelters and found that both the test-retest reliability and the between-experimenter repeatability of the procedure was substantial (86.6% and 95% respectively). They also found that the dogs in shelters where staff had more positive attitudes towards dogs and used gentle handling practices were more likely to approach a stranger than dogs at other shelters. This is similar to the results found by Conley et al. (2014) where shelter dogs who received enriched husbandry routines with positive human contact and toys were more likely to approach a stranger than dogs that did not receive the enriched routine. Indeed, Hubrecht (1993) also found that enrichment and human contact reduces avoidance behaviours and increases the willingness to approach strangers. Both the Welfare Assessment Protocol for Shelter Dogs (Barnard et al., 2014) and the quality of life assessment tool for shelter dogs developed by Kiddie and Collins (2014) employ a stranger approach test. Barnard et al. (2014) recorded behavioural responses to a stranger approaching the kennel and standing for 30 s followed by 30 s of crouching down and speaking gently to the dog. Kiddie and Collins' (2014) assessment involved the observer approaching, handling, and initiating play with the dog while its behavioural responses were recorded.

Novel object tests are frequently used in both laboratory and farm animals to assess levels of fear and anxiety (Forkman et al., 2007) and they have also been suggested as useful methods to assess fear in dogs, although it is not included in either of the developed assessment tools (Barnard et al., 2014; Kiddie and Collins, 2014). Ley et al. (2007) found that dogs that were given anxiolytic drugs (which reduce fear) were quicker to approach a novel object (a remote-controlled car) and spent more time in its vicinity, indicating that latency to approach a novel object is influenced by fear. King et al. (2003) also used a remote-controlled car as a novel object and found that dogs' responses were related with a variety of other measures of fear. For example, there was a significant positive correlation between cortisol and the amount of time it took dogs to approach within 100 cm of the object. Interestingly, King et al. (2003) also noted that, compared to other breeds, greyhounds exhibited significantly fewer fear-related behaviours in the novel object test (i.e. quicker to approach and spent more time near) as well as on the other behavioural and physiological tests of fear, suggesting that breed differences or rearing environment could have a large influence on fear and anxiety. Plutchik (1971) also found significant breed differences in the patterns of approach/withdrawal between four different breeds of dogs (beagles, wire-haired fox terriers, basenjis, and Shetland sheepdogs) when presented with novel objects. Thus, it is important to note that responses to novel objects in dogs are not solely determined by their levels of fear and anxiety, but also by their baseline temperaments, which may be influenced by breed type or rearing.

Indeed, one of the main difficulties in using fear and anxiety related behaviours in a specific context is that behavioural signs shown by a dog, for example in an approach test, are affected by a number of factors, including their genetic predisposition and previous life experiences (Stephen and Ledger, 2005). Hence behavioural responses are specific to that context and cannot be generalised to make conclusions about overall welfare. In addition, individual dogs can have different "coping styles" in response to kennelling which influence their behaviour, depending on whether their response style is more proactive or reactive (Blackwell et al., 2010; Rooney et al., 2016). Therefore, the behaviours exhibited when fearful will differ between individual dogs.

3.2. Abnormal behaviours

The emergence of abnormal behaviours is perhaps the most noticeable sign of stress in captive animals (Mason and Latham, 2004). Abnormal behaviours are those that an animal would not generally perform, historically thought to be either maladaptive or non-functional. Apparently functionless behaviours that become repetitive and ritualised are known as stereotypies or abnormal repetitive behaviours (ARBs). The emergence of stereotypies has been documented across a wide range of captive species (for a review, see: Mason and Latham, 2004) and while some behaviours are common among a variety of animals (e.g. pacing) others seem to be species-specific and dependent on the animal's anatomy as well as the environment they are kept in.

In dogs, the most commonly seen abnormal repetitive behaviours are pacing, spinning, bouncing off the walls, circling, and repeatedly walking the perimeter of their kennel, while less common but still notable behaviours include biting at their enclosure (i.e. bars or wall), excessive licking of their environment, and self-mutilating behaviours such as flank-sucking, chewing feet, and excessive auto-grooming (Stephen and Ledger, 2005; Rooney et al., 2009; Belpedio, 2010; Denham et al., 2014; Protopopova, 2016). ARBs were recorded by both Kiddie and Collins (2014) and Barnard et al. (2014) in their protocols.

The incidence of ARBs in dogs has been found to be relatively high in the kennel environment compared to the home environment, although values vary tremendously depending on the definitions used (Protopopova, 2016). Protopopova et al. (2014) found that only between 1.3–4.5% of the pet dogs studied (n = 26) engaged in ARBs in the home, while Denham et al. (2014) found that 93% of 30 kennelled working military dogs showed repetitive behaviours, although this number is reduced to 16.7% in the absence of arousing stimuli such as humans. In Hubrecht et al. (1992) dataset, which was recorded by an observer standing in front of the kennels, between 46–84% of laboratory dogs, and 42–62% of dogs in rehoming centres engaged in such behaviours. The percentage of time engaging is similarly varied (Protopopova, 2016). In an audit of behavioural indicators of poor welfare, Stephen and Ledger (2005) found that dogs in kennels began exhibiting repetitive behaviours such circling and wall bouncing later (on average after 10–13 days in the kennel) than fear-related behaviours such as hiding (which began on average after two days). They also found that the incidence of repetitive behaviours increased the longer the dogs were in kennels, whereas fear behaviours decreased. Beerda et al. (1999a) demonstrated that the incidence of ARBs like circling was influenced by factors such as whether the dogs were housed individually or in groups, and even whether the weather was pleasant or unpleasant, which could partially explain the different incidence levels across studies.

Some studies have found that certain stress-reducing interventions can reduce the incidence of ARBs in kennelled dogs (Taylor and Mills, 2007). However, caution should be taken when extrapolating that the presence of ARBs indicates compromised welfare. Often these behaviours are emancipated from the original cause and can persist after the animal has been removed from the stressful environment, and thus may represent artefacts from their previous poor welfare states, rather than being representative of their current state (Mason and Latham, 2004; Hewson et al., 2007). Furthermore, ARBs may emerge as a coping mechanism for the animal; thus, it is possible that an animal engaging in repetitive behaviour has adapted to its conditions more successfully than an animal not engaging in such behaviour and may have better welfare (Mason and Latham, 2004; Hewson et al., 2007; Denham et al., 2014). It is also possible that the behaviours persist because they are rewarded (e.g. through increased attention and human contact) (Hewson et al., 2007; Denham et al., 2014).

As shown in Table 1, other abnormal behaviours that are commonly seen in the kennel environment and have been associated with stress are coprophagia, excessive barking, and excessive drinking. Coprophagia has been found to increase in laboratory beagles when they are socially and spatially restricted (Beerda et al., 1999a). In a study of 120 working dogs housed in kennels, 18% were found to engage in coprophagia (Gaines et al., 2008 as cited in Rooney et al., 2009), compared to non-kennelled dogs, where a study of nearly 8000 dogs found a prevalence of less than 1% (Col et al., 2016). Barking has been found to be more prevalent in dogs housed individually compared to dogs housed in groups and was accompanied by a concurrent increased in the incidence of ARBs (Hetts et al., 1992). In an audit of behavioural welfare indicators, excessive barking was found to be the most commonly observed behaviour in kennelled dogs, seen in nearly a quarter of dogs (Stephen and Ledger, 2005). Exercise has been shown to reduce the frequency of barking in kennelled dogs (Clark et al., 1997). There may be significant breed differences in the frequency with which both coprophagia and barking are exhibited (see Stephen and Ledger, 2005) and this should be taken into account when using them as welfare measures. The incidence of excessive drinking has also been found to increase the longer dogs are in kennels, both in laboratory settings (Clark et al., 1997) and in rehoming centres (Stephen and Ledger, 2005; Hiby et al., 2006; Kiddie and Collins, 2014). Kiddie and Collins (2014) included excessive drinking and coprophagia in their quality of life measures, while Barnard et al. (2014) did not. Conversely, excessive barking was excluded from Kiddie and Collins (2014) but was present as a measure in Barnard et al. (2014).

3.3. Activity level & resting behaviours

Both increased and decreased activity levels have been suggested as potential indicators of stress in animals. As activity level is closely linked with other behavioural measures, such as the presence of ARBs like pacing or spinning, it is unclear how useful it is on its own as a measure of welfare. In farm animals, accelerometers are used to detect reduced activity, which may be indicative of lameness or pain (Rushen et al., 2012). Assessing the frequency and duration of resting behaviours could also shed light on welfare status and housing preferences; dairy cows have been found to engage in resting behaviours more frequently and for longer durations when they are given softer flooring and have more space (Haley et al., 2000). In the existing assessments of kennel dog welfare, Kiddie and Collins (2014) included “high level of activity” and “lying down” as indicators of positive emotional states, and “listlessness” as an indicator of negative emotional states, while Barnard et al. (2014) did not measure activity level or resting specifically but did include the terms “boisterous” and “quiet” in their Emotional State Profile.

In studies on kennelled dogs, Jones et al. (2014) used accelerometers on shelter dogs to measure average activity level and found a significant positive correlation with mean urinary cortisol levels, although the valence of the arousal causing these effects is difficult to determine. Part et al. (2014) also studied kennelled dogs and, while no relationship was found with cortisol levels, they did find that kennelled dogs spent less time lying down, resting, and sleeping (i.e. were more active) than dogs in homes (although this may be due to home dogs being under-stimulated, so care should be taken when interpreting their lower activity as positive welfare). Another study looked specifically at sleep in dogs kennelled at rehoming centres and, although it did not find a relationship between night-time sleep and behavioural measures, it did find that dogs that spent more time resting during the day had a more positive judgement bias and exhibited fewer repetitive behaviours than dogs that did not rest as much in the daytime (Owczarczak-Garstecka et al., 2016). While this may suggest that reduced activity and increased resting during the day, may be a useful indicator of positive welfare in kennelled dogs, another study by Hiby et al. (2006) found that this relationship may be more complex. In their sample of dogs kennelled in rehoming centres, when looking at the data on any specific day, those dogs that were the least active during the day had the most elevated cortisol. However, when looking at longer-term trends over a ten-day period, the cortisol levels of the dogs that had the highest mean activity (trotting, drinking, walking, etc) increased over time, whereas the levels of dogs with lower levels of activity decreased. Some studies have differentiated between truly restful inactivity (i.e. sleeping) and other causes of still but awake inactivity by recording whether the animal’s eyes are open or closed (Fureix et al., 2016), which may provide an important clarifying distinction between inactivity that is indicative of positive welfare compared to inactivity that is negative and may be a sign of depressive states. Thus, activity level seems to have potential as welfare measure, but more research needs to be done to clarify their exact relationship.

3.4. Play behaviour

The modern understanding of animal welfare emphasises that good welfare consists not only of the absence of negative states but also of the presence of positive ones (Lawrence, 1987; Yeates and Main, 2008; Held and Špinka, 2011). Therefore, a thorough assessment of kennel dog welfare should also include measures of positive behaviours, such as the willingness/desire to engage in play. Level of play behaviour has been found a useful measure of welfare in a myriad of other species such as cattle, sheep, deer, cats, wolves, meerkats, seals, rats, chimpanzees and of course humans (for reviews, see: Ahloy-Dallaire et al., 2017, and Held and Špinka, 2011). Kiddie and Collins (2014) included “willingness to play” as an indicator of positive emotional states in their shelter dog quality of life assessment and measured this through recording both unprovoked play behaviours in the kennel (both solitary and with other dogs) as well as initiated play, where the assessor engaged the dog with a “Ragger”. Barnard et al. (2014) included the term “playful” in their Emotional State Profile as an indicator of positive emotional state. It should however be noted that a decrease in negative affect does not necessarily equate with the presence of an absolute positive affective state (Ahloy-Dallaire et al., 2017). It may be that negative states prevent play and therefore, on its own, willingness to play cannot be used a definitive sign that an animal has good welfare.

It has been found that dogs are less likely to play with strangers after having been housed in kennels for more than six months (Titulaer et al., 2013). A study of laboratory beagles found that dogs who were only provided with environmental enrichment played less overall and were less likely to solicit play than dogs that had daily social contact either with humans or larger groups of dogs (Hubrecht, 1993). A number of studies have found that shelter dogs who are given access to play sessions, whether with other dogs (Coppola et al., 2006; Belpedio et al., 2010; Johnson et al., 2013; Shiverdecker et al., 2013; Flower, 2016) or with humans (Bergamasco et al., 2010; Conley et al., 2014;) display fewer stress-related measures than dogs that do not have such access. This suggests that not only are responses to play opportunities a potential measure of welfare, but also that access to these opportunities may affect their welfare states as well.

There are some other difficulties in using play to assess welfare as well. While the majority of studies examining the link between play and welfare have found that increased play is linked to better welfare, there have been some notable counter-examples across a number of species (rats, cats, horses, humans) where those individuals that seemed to have poorer welfare were the ones that engaged in the most play, potentially as a coping mechanism (see Ahloy-Dallaire et al., 2017 for examples). Other potential difficulties include the fact that, although the drive to play may be instinctive in young animals like puppies (Bradshaw et al., 2015), for adult dogs who may not have received proper socialisation when they were young, their readiness to engage in play behaviours may not be as strong. In other words, some dogs may not 'know' how to play (particularly with humans) and thus an assessment of their willingness to play may not reflect their current welfare status, but rather previous learning experiences. There may also be differences between dogs of different breed-types and those reared in different ways which may affect individuals' willingness to engage in play behaviour, or the style of play behaviour exhibited (Rooney et al., 2000). Furthermore, although dogs are naturally more playful throughout their whole lives than other species may be (Bradshaw et al., 2015), there is still a tendency for older dogs to be less playful than young dogs (Rooney et al., 2000), which should be taken into account when using playfulness as a measure. Finally, it should be noted that, when assessing play behaviours as an indicator of welfare, it is important that the behaviours are relatively spontaneous, particularly with working dogs, who may have been trained to 'play' on command (Horváth et al., 2008).

4. Cognitive measures

4.1. Cognitive bias

Recent research in both humans and a variety of species has demonstrated that affective states can have a large impact on an individual's perception of their environment and their consequent decision-making (Mendl et al., 2009). A number of 'cognitive bias' tests have found that animals with negative affective states tend to perceive ambiguous stimuli more pessimistically compared to animals with positive affective states. This is generally tested by teaching an animal to associate one stimulus with a positive experience and another stimulus with a negative experience and then testing whether the presentation of an intermediate stimulus elicits a negative or positive anticipatory response (Mendl et al., 2009). For example, in the first study of this kind in animals, rats learned to associate the pressing of a lever after one tone with the presentation of food (a positive event) and the pressing of a lever after a tone of a different frequency with an electric shock (a negative event). Once they had learned the associations, in addition to the original tones, they were also presented with ambiguous tones of intermediate frequencies. Rats that were thought to have more positive affective states (due to their more stable husbandry routines) tended to press the lever faster and for more of the ambiguous tones, indicating that they anticipated food, whereas rats the were thought to have more negative affective states refrained from pressing the lever more often and were slower at pressing when they did (Harding et al., 2004). Similar results have been found in cows, pigs, horses, sheep, chickens, monkeys (for a review, see Mendl et al., 2009) and even bees (Bateson et al., 2011).

Studies using dogs have found contrasting results. Burman et al. (2011) used light and dark shades of cards to predict either the presence or absence of food and expected that dogs that had just been rewarded would have a more positive cognitive bias towards intermediately shaded cards than dogs that had not. Surprisingly, they found that dogs that had been rewarded were slower to approach the ambiguous cards than the unrewarded dogs. The authors propose several explanations for this, including possible satiation, differences in motivation, and a greater anticipation in the unrewarded dogs eliciting an unexpected positive affective state. Other studies have used setups where dogs were taught that bowls presented in one location contained food while bowls in another location did not (Mendl et al., 2010; Owczarczak-Garstecka et al., 2016). Once the dogs had reached criterion, they were then tested on their speeds to reach bowls that were in different locations between the two original places. Their results matched with those from other species, where the dogs assumed to have more negative affective states (expressing more separation-related behaviours or having less sleep) were slower to approach ambiguous stimuli than dogs with positive affective states. While cognitive bias tests are certainly a promising method for assessing the affective states of dogs in kennels, the current amount of time required to train the dogs to reach the discrimination threshold necessary for testing makes then impractical for most situations (Table 1). Indeed, neither Barnard et al. (2014) nor Kiddie and Collins (2014) incorporated these into their assessments.

4.2. Learning ability

In addition to causing a negative cognitive bias, distress and poor welfare have also been suggested to impact an animal's ability to learn (Blackwell et al., 2010). Using learning ability to assess welfare is particularly relevant in dogs, as they have been selected to be particularly receptive to training through domestication (Gácsi et al., 2009). Few studies have used learning speed to specifically measure canine welfare (neither Kiddie and Collins (2014) nor Barnard et al. (2014) used it in their tools), however relationships have been found inadvertently. In a study assessing training methods and dog-owner interactions, dogs with owners who used high levels of punishment and aversive training techniques (and thus can be tentatively assumed to have poorer welfare) performed significantly worse at a novel training task than dogs whose owners tended to use positive, reward-based training methods (Rooney and Cowan, 2011). Of course, as the techniques used to train the novel task were different for each dog, it cannot be determined whether the impaired learning ability was due to the dogs' welfare states or the inefficiency of the training method, and thus more research needs to be done to validate this measure.

A study examining whether the performance of abnormal repetitive behaviours (ARBs) is associated with learning ability found interesting results when comparing dogs living in rehoming centres to those living in homes. While home dogs performing ARBs did not differ in their ability to learn a spatial discrimination task compared to home dogs not performing ARBs, ARB dogs at rehoming centres took longer to reach criterion compared to their matched non-ARB pairs. Furthermore, centre dogs took longer overall to learn the task compared to home dogs, regardless of whether they engaged in ARBs or not (Hobbs et al., 2018).

Another study that aimed specifically to examine the relationship between canine welfare and associative learning found mixed results (Blackwell et al., 2010). While dogs that displayed the most behavioural signs of fear generally either failed to learn a novel task entirely or learned it more slowly than less behaviourally fearful dogs, it was actually those dogs that had the highest physiological measures of stress (measured through cortisol:creatinine ratios) that learned the task the fastest. More research is needed to assess the usefulness of learning ability in dogs as a welfare measure.

5. Conclusion

Developing optimal methods to assess the welfare of dogs in kennels is clearly a difficult, though worthwhile, endeavour. Hundreds of thousands of dogs are living in kennels, many of them long-term. As there is ample evidence to suggest that this can have a negative impact on their welfare, the need to measure this impact becomes evident. Assessing the various management and environmental elements of kennels can give some insight into whether basic needs are being met. These measurements however, give no information on the dogs' emotional and psychological wellbeing, which the modern understanding of animal welfare suggests is as equally important as physical wellbeing. Animal-based measures, be they physiological, behavioural, or cognitive, need to be included in welfare assessments to provide a complete picture.

The discussion of each measure has mentioned whether or not it is included within the welfare protocols created by Barnard et al (2014) and Kiddie and Collins (2014), however the true validity, practicality, and usefulness or these protocols is still being determined. For the Shelter Quality Protocol, the authors tested the inter-observer reliability, test-retest reliability, and feasibility of the protocol at 29 shelters across six countries and found promising results (Barnard et al., 2015). They also aimed to assess validity, however this was defined as the similarity between observations taken through the fence and observations while handling the dogs up-close. Thus, the data only provides evidence that observations from a short distance have substantial agreement with up-close observations, not that their measures are valid indicators of overall welfare. To date, there has only been one published application of the Shelter Quality Protocol, where the authors noted several concerns, namely the long duration of the audit, the difficulty in scoring, and lack of ability to use the protocol to identify problems and provide advice (Osella and Ferraris, 2014).

In Kiddie and Collins' protocol, dogs were scored on a binary scale for provoked and unprovoked indicators of both positive emotional states (e.g. playing, grooming, exploring, spending time at the front of the kennel) and negative emotional states (e.g. pacing, wall-bouncing, hiding, chewing bars, aggression). They used this system to assess the quality of life of 202 dogs across 13 rehoming centres and found good inter-rater reliability and evidence of content validity (the assessment measured what it was designed to measure), response process validity (those conducting the assessments understood the construct in the same way), and convergent validity (similar constructs hypothesised to agree were shown to agree). In a later study, the authors used the tool to monitor the quality of life of kennelled dogs over time, as well as to assess the impact of different handling and husbandry changes (Kiddie and Collins, 2015). This study was able to identify a number of management practices (i.e. provision of bunk beds, 30+ minutes of staff interaction, daily training, quiet environments) that significantly increased the quality of life scores of the dogs, showing that the tool can be valuable when determining which factors are important for the wellbeing of dogs in kennels (Kiddie and Collins, 2015).

Both of these assessment tools provide useful information about the states of kennelled dogs and represent important progress, but they both focus primarily on behaviour and health and hence may not give a full picture of canine welfare. For example, apart from general body condition and skin condition, neither tool includes any other physiological measures, such as heart rate or cortisol, which would indicate the level of physiological stress the dogs are experiencing. Kiddie and Collins (2014) chose their indices based on a literature search for the most validated behaviours that were either associated with positive or negative emotional states in dogs, and then used a binary scale to produce welfare scores. Their indices therefore do not include any novel behavioural or cognitive measures, nor are they able to take into account differences in welfare states between dogs that perform a behaviour only once during the observation and dogs that perform them continuously. Barnard et al. (2014) chose their indices by developing measures that covered the 12 welfare criteria used by the Welfare Quality consortium in their welfare protocols for farm animals. Because of this, their measures have a larger emphasis on health and management over behaviour and include fewer measures that are specific to dogs. Our review of plausible measures suggests the inclusion of additional measures may be justified, but that their validation is also a priority.

Overall, it is clear that considerable progress has been made in assessing indicators of welfare in kennelled dogs, and indeed in combining these indicators into applicable protocols. However, although a great number have now been identified as being potential indicators of welfare, without a gold standard against which these measures can be compared, very few can be considered truly "validated." When considering the indicators reviewed here, although many measures have been used in multiple dog studies, very few seem to be feasible, indicative of valence, and devoid of confounds (Table 1), Those that are (e.g. oxytocin) have generally only been examined in small studies and require additional research.

The direction of future research should consider following the approach of farm animal welfare studies, and aim to further validate measures using animal-centric designs such as preference and motivation testing. Assuming that animals are motivated to choose the best options for their own interests and overall welfare, assessing the relationships of their choices relative to other supposed welfare indicators is a robust method for validating welfare measures (Nicol et al., 2009). These methods could be used across different kennelling environments, breeds, age groups, and dogs with varying previous life histories to further assess the usefulness of each of the measures discussed in this review, creating a greater understanding of the use of animal-based measures to assess welfare in kennelled dogs.

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