OPEN ACCESS

University of BRISTOL

Peer reviewed version

University of Bristol - Explore Bristol Research

General rights

Minimising fear and anxiety in working dogs: a review

Rooney Nicola J [a], Clark Corinna CA[b], Casey Rachel A[c, d].

[a]Anthrozoology Institute,

Animal Welfare and Behaviour Group, University of Bristol

Department of Clinical Veterinary Science, Langford BS40 5DU

[b] Life Sciences, University of Warwick, Coventry, CV4 7AL

[c]Dogs Trust, 17 Wakley Street, London, EC1V 7RQ

[d]Royal Veterinary College, Hawkshead Lane, Hatfield, Hertfordshire, AL9 7TA

Corresponding author: Nicola Rooney

Tel: 0117 928 9469, Fax: 0117 928 9582

Mobile: 0781 841 3310

Email: Nicola.Rooney@bristol.ac.uk

Abstract

The causes of fear and anxiety in working dogs are multifactorial, including inherited characteristics that differ between individuals (e.g. Goddard and Beilharz, 1982; 1984a, b), influences of the environment (Lefebvre et al., 2007), and learnt experiences during particular sensitive periods (Appleby et al 2002) and throughout life as well as inappropriate initial introduction to new stimuli . Fear-related behaviour compromises performance, leads to significant numbers of dogs failing to complete training (e.g. Murphy, 1995; Batt et al.,2008), early withdrawals from working roles (Caron-Lomier et al 2016) and can jeopardize dog and handler safety. Hence, its amelioration is critical to maintain dogs in working roles, as well as ensuring their wellbeing. Whilst current methods of selection and training are seemingly effective at producing many dogs which work in a remarkable array of environments, some dogs do not make the grade, and longevity of service is not always maximized. Programmes should strive for optimal efficiency and they need to continually analyse the value of each component of their programme, seek evidence for its value and explore potential evidence-based improvements. Here we discuss scientific evidence for methods and strategies which may be of value in reducing the risk of fear behaviours developing in the working dog population and suggest potentially valuable techniques and future research to explore the benefit of these approaches. The importance of environmental influences and learning opportunities as well as underlying temperament on the outward expression of fear and anxiety, should not be underestimated. Identification of characteristics which predict resilience to stress are valuable, both to enable careful breeding for these traits and to develop predictive tests for puppies and procured animals. But vitally important also is rearing animals in optimal environments and introducing them to a range of stimuli in a positive, controlled and gradual way, as these can all help minimize the number of dogs which develop work-inhibiting fears. Future research should explore innovative methods to best measure the relative resilience of dogs to stressful events. This could include developing optimal exposure protocols to minimize the development of fear and anxiety; exploring, the influence of social learning and the most effective elements of stimulus presentation.

Key words

Fear, anxiety, prevention, working dog, selection test, habituation

1. Introduction

Domestic dogs are used for a variety of working roles in which they are required to continue performing trained tasks in environments which may include novel, unexpected or potentially aversive stimuli. Roles include herding, deterring predators, guarding, transportation, hunting, entertainment (e.g. racing), contraband detection (police, military and rescue services e.g. Rooney et al. 2004), emotional therapy and medical alert dogs (Strong et al., 1999; Rooney et al., 2013). This diverse range of activities can expose dogs to potentially stressful stimuli in their surroundings, including: high levels of noise from machinery and gunfire explosions (and subsequent shock waves); transportation; variable terrain with unstable and varied surfaces underfoot; high levels of dust and smoke, and loud machinery and traffic (Brown, 2011). In addition to this huge range of physical factors, dogs may need to cope with a variety of people, some behaving in unpredictable ways, the presence of other animals, and variable routines. Dogs can respond to these types of situations with behavioral signs of fear or anxiety, such as freezing, withdrawing, or showing aggression (Casey, 2010), and such responses are likely to intefere with a dogs' ability to perform its role. In this paper, fear is defined as the behavioral responses shown to actual danger (Boissy, 1998) and anxiety as the emotional state elicited in potentially threatening situations, e.g. novelty, or when some elements of the environment predict a negative outcome (Ennaceur et al., 2006; Massar et al., 2011). Both affective states (fear and anxiety) are particularly concerning in roles for which optimal performance is critical for safety. For example, fear-related behaviors on the part of guide dogs or military search dogs, can be life-threatening to both dog and handler, and is therefore a common reason for failure in these roles (e.g. Murphy, 1995; Rooney et al., 2002; Batt et al., 2008).

The experience of fear is aversive (Grandin and Deesing, 2002), and by analogy with the experience of humans, it is likely that anxiety is similarly aversive since it too functions to promote avoidance of eliciting stimuli. Individual dogs which show fearful behaviors in a rehoming kennel environment appear to have an impaired ability to learn operant tasks (Blackwell et al., 2010), with similar effects demonstrated in a range of species (e.g. Shors 2004). This potentially makes training more time consuming and costly. Research has also suggested that high levels of fear related behaviours can lead to increased disease risk (Terlouw et al., 1997) through modulation of immune responses, and perhaps even ultimately lead to a reduced lifespan (Dreschel, 2010). Particularly relevant to dog programmes with breeding schemes is the finding that decreased sperm quality is associated with anxiety in dogs (Memon, 2007), whilst fear and anxiety can inhibit complete erection and ejaculation (Kutzler, 2012, cited in Mills et al 2014), as well as reducing proceptive and receptive behaviors in bitches resulting in a failure to breed (Grundy, 2002). Thus, in addition to welfare concerns arising from exposing working dogs to fear-provoking situations for which they are poorly prepared, and the issue of handler safety when dogs perform poorly, there are potential economic consequences. Fearfulness and anxiety can lead to the loss of dogs from programmes at assessment, during training, or even fully trained dogs being withdrawn early from active working roles (Caron-Lomier et al 2016), with an associated loss of the potential contribution they could make during their working lives.

Fear responses develop when animals are exposed to events or stimuli that they perceive as negative and salient (i.e. above their individual threshold of tolerance). On first exposure, animals will tend to show a startle response towards a novel stimulus, and also orientate towards it – an adaptive response to a change in their environment, as this may have some survival relevance. On subsequent exposures, this response may either increase or decrease. Habituation is the process whereby a response gradually reduces with repeated presentation of the eliciting stimulus, and the threshold thus increases (Grissom and Bhatnagar, 2009). In contrast, sensitisation is the process

whereby an animal's response increases on presentation of a stimulus (Davis, 1974).. Having

sensitized to a stimulus, and identified it as a threat, the animal will attempt to both identify

predictors for the stimulus, and develop a response to avoid it e.g. to flee. In the working dog, as in

the companion animal world, the aim is to manipulate factors both in the dog and its environment in

order to maximise the chance that habituation occurs, whilst minimising the risk of sensitisation.

Multiple factors influence the development of anxiety and fear behaviors across species and

specifically in dogs. The nature of the stimuli and the manner in which it is first presented, including

the social context, are both important (Section 4).   High intensity stimuli (e.g. explosions), or

situations where aversive events occur consecutively without allowing animals a chance to recover,

are very likely to provoke a response in most animals. Sensitisation is more likely to occur when the

presented stimulus is of high intensity or low predictability to an individual (Gray, 1971). Risk is also

increased where animals have had prior exposure to other negative events in their general

environment or in the specific contexet (Section 3). However, individual differences in response

thresholds mean that even seemingly benign stimuli will provoke a fear response in some

individuals. Thus, in apparently identical situations, some individuals develop signs of fear and

anxiety whilst others do not. This can be attributed partly to inherited characteristics that differ

between individuals (i.e. aspects of personality), but also to previous environmental influences,

learnt experiences throughout life and especially during sensitive periods, and to interactions

between these effects

Studies of personality in a range of species shed light on individual differences in the likelihood of

animals developing fears, and sensitising to stimuli, as opposed to habituating.   For example, the so-

called 'shy-bold' continuum has been demonstrated in a wide range of species (Jones and Gosling

2005). Brown et al. (1978) were able to develop a distinct population of pointer dogs that showed

extreme fear responses by selecting those which reacted most to a range of standardised fear-inducing tests involving a range of stimuli (e.g. loud noises). The relevance of the 'shy-bold' continuum in dogs is further supported by Svartberg and Forkman (2002) who used trained observers to score dogs undertaking a standard set of sub-tests, and identified five underlying factors of which four appeared to form a higher order factor analogous to the shy-bold characteristic.

However, if we simply test the appearance of fear behaviours in an individual, we can't be sure of the extent to which observed behaviour is a manifestation of previous experience or underlying personality. What we actually need to assess in working dogs are
those underlying characteristics or traits that influence 'resilience' to withstand stressful circumstances. Measuring these characteristics may be a promising approach in identifying risk factors for the occurrence of fear, independent of the influence of environmental context. Resilience (Yehuda et al., 2006a) is defined as the extent to which an individual is able to adapt in the face of adversity, trauma, or risk of threat Whilst the concept of resilience is relatively new, the idea that individuals have characteristics that confer differing abilities or strategies to cope with stress is not. Coping strategies are well studied , and have been defined in terms of approach (active or proactive coping) or avoidance (passive or reactive coping) styles; Roth and Cohen, 1986). It has been suggested in humans that active coping strategies improve individuals' ability to manage stressful situations and reduce their risk of psychiatric illness (Valentiner et al., 1994). For example, people who showed active coping strategies had lower levels of distress and Post Traumatic Stress Disorder six months after the New York World Trade Center attacks (Silver et al., 2002).

Proactive and reactive types of responding have been well documented in laboratory rodents (de Boer et al., 2003) and in other species (see review, Koolhaas et al., 1999; but see also Forkman et al., 1995, and Jensen et al., 1995). Attempts have been made to characterise 'coping styles' in dogs in

rehoming centres, based upon their physiological and behavioural responses a to kennelling and their ability to learn a operant task, and these suggested either a 'reactive' style of responding, or a more 'proactive' style (Blackwell et al 2010). In contrast, a study of police dogs found three, not two, categories of dogs (Horvath et al., 2007), which illustrates some of the conceptual issues with the approach of applying post-hoc determination of characteristics from behaviour that may have differing motivations (e.g. anxiety, fear, aggression). Coping responses are also not immune to environmental influences or circumstantial changes in emotion, and individuals will often learn to show different behaviours in response to different situations (Roth and Cohen, 1986). They are also particularly important to consider in a working dog context when some coping response, such as avoidance, may not be possible, hence we may be inadvertently be selecting dogs with specific coping styles, or causing stress by not enabling dog to perform their preferred response.

This variation in coping styles also means that not all individuals of a given species will respond to equivalent stressors in the same way. Hence the outward manifestation of the same inner emotional state will differ between animals. Some dogs may appear less overtly fearful than others, since their coping strategy is more passive or reactive rather than proactive. This raises an important challenge both when identifying signs of fear and anxiety when testing individuals (Section 2) or when introducing new stimuli at an appropriate intensity (Section 5), It is also essential when assessing the welfare of working dogs that different types of response to stressful circumstances are recognised: dogs which show a more active response, such as spinning or wall bouncing show more obvious signs, but dogs which are inactive and inhibited may also be in a negative emotional state.

Given that there are both heritable and environmental aspects influencing the development of fear behaviours, there are three potential approaches to reducing the risk of these behaviors developing in working dog populations;

a) select and breed individual dogs with the lowest risk of developing these behaviors (section 2),

b) control the environment in which dogs are kept and where possible reared to minimize the risk of fear behaviors developing (section 3);

c) optimize the method of initial stimulus exposure (section 4) , to minimize the risk of dogs sensitising and fears becoming established.

.

 Most working dog agencies aim to achieve all of these, but they vary in their approaches. In this paper we describe some of the methods used and review evidence for their success. Possible practical solutions, such as improved selection (2), management (3.1.2) and training (3.1.1) of working dogs (are also discussed, and questions raised about the value of some current practices. In addition, since rigorous studies of fear prevention in the working and pet dog environment are generally lacking, we draw on evidence from other species and suggest areas where empirical evidence and future research is still required.

2. Selection of animals most suitable for working environments

Working dogs are selected in a variety of ways. Most large assistance dog agencies have their own established breeding programmes, smaller organisations often procure puppies which may be selected on the basis of a suite of tests (with varying degrees of validation) and are puppy walked in homes, before being reassessed to decide which are best suited to go on to further training. Police, military and security dog programmes around the globe include several breeding schemes, but a majority rely upon purchasing adult dogs from private breeders or vendors. Some agencies also rely on procuring unwanted companion animal from owners or rehoming centres. Whilst the Scandinavian military (Swedish and Norwegian) have well established and validated methods for testing dogs prior to selection (e.g. Dog Mentality assessment DMA: Wilsson and Sundgren 1997), a multitude of less scientifically tested selection tests also exist.   Most working dog agencies have their own criteria for breeding and/ or selecting animals with an assumed reduced risk of developing

problematic fear and anxiety related behaviors, in particular fears of humans, loud noises and novel environments Criteria are implemented differently between programmes and may involve selection of breeding stock, adult dogs, and/or puppies. Those agencies which breed in-house select both breeding animals and suitable puppies or any combination thereof; others procure puppies, adolescent or adult dogs from private breeders, working-dog breeding establishments, vendors or from the rescue population and they are selected at this stage. In all cases, knowledge of the best methods for screening dogs, their efficacy and reliability, as well as knowledge of the heritability of fearfulness is imperative.

2.1 Testing for fearful behavior

Many agencies train working dogs have developed tests aimed at screening out animals predisposed to show fear behaviors. It is clearly beneficial to preserve resources by selecting animals which are least likely to fail during training or operations because of the development of fear responses. When procuring adult dogs, or when puppies progress from controlled breeding populations into training, there will still inevitably be some whose temperament is unsuitable for the working environment. Many details of the commonly used tests are undocumented (e.g. UK military; Rooney et al., 2003), although others are published, such as those used with guide dogs (Serpell and Hsu, 2001; Asher et al., 2013); police dogs (Slabbert and Odendaal, 1999; Svobodová et al., 2008), general service dogs (Wilsson and Sundgren, 1997), IED detection (Sherman et al 2015), and military dogs (Haverbeke et al., 2009; Sinn et al 2010). Predictive testing is fraught with also several other difficulties, and the extent to which this is objectively assessed is variable. There are several potential problems with many tests which need to be addressed. Below we review each of the main problems and suggest possible improvements

2.1.1 Poor validity

Many tests have poor validity (Taylor and Mills, 2006); since conducting single tests in one context is not necessarily indicative of behavioral responses in others. For example, tests of behavioral tendencies in rehoming centres show limited predictability of later owner reports of fearful and anxious behaviors in the home (Mornement et al., 2015). Similarly, tests routinely performed to select US military dogs, including tests for environmental and gun shyness, have been shown to have limited predictive validity when compared to later certification outcomes (Sinn et al 2010). Haverbeke et al. (2009) found that although some test elements seemed to predict later fear behaviors in Belgian military dogs, most tests were poorly predictive of later undesired behaviors. What's more, for many working dog tests the predictive value cannot actually be calculated, as dogs which 'fail' initial testing do not enter training and so no data is available on how these dogs would have performed compared to those that passed, and hence criterion validity cannot be ascertained (Taylor and Mills, 2006). We suggest that more data should be collected on the relationship between such tests and the outcome, including for dogs that would ordinarily be rejected.

2.1.2 Study bias

Often studies which claim to reduce fear behaviors via selection tests concurrently change several procedures making it difficult to determine true causation. For example, whilst Haverbeke et al. (2010) reported increased efficiency in military dogs which were selected specifically not to show fear of people, they simultaneously introduced the Human Familiarisation and Training Programme, which changed other factors, such as increasing levels of training for both dogs and handlers. Hence the impact of selection based on exhibition of fear is difficult to ascertain from outcome measures.

2.1.3 Presentation of high intensity stimuli

Many selection tests involve presentation of sudden unexpected stimuli. For example, the popular Dog Mentality Assessment (Wilsson and Sinn 2012) used in Scandinavia presents a stranger, a dark room, loud metallic noises, an unstable surface, dummies, human "ghosts" and gunfire – all of which

are potentially fear-provoking . This risks causing sensitisation during testing, resulting in increased fear behaviour on subsequent presentation (Davis, 1974).This is a particular concern as tests tend to be conducted consecutively (Wilsson and Sundgren, 1997; Sherman et al 2015), so sensitisation risk is increased because dogs may still be emotionally aroused from the previous stimulus when the next is presented (De Meester et al., 2011).

Rather than high intensity exposure of particular stimuli, which risks sensitisation, we suggest observing the dog's reaction to gradual exposure to specific stimuli which they are likely to encounter during operational activities. Exposure to a gradually increasing intensity not only indicates the situations to which a dog may react, but also the level of exposure at which such a reaction occurs. Identification of this for particular stimuli may be indicative of the potential value of entering animals into treatment programmes for specific fears (Section 5), rather than rejecting animals which may otherwise be suitable for a working life. Similar ideas have been posed by Ogata et al. (2006) who applied a Pavlovian aversive conditioning protocol and measured autonomic and behavioral parameters. They saw increased heart rate and body temperature in response to a conditioned stimulus, and suggested using their fear-conditioning as a method to test for trait fear (by measuring extinction of to the learned CS) in dogs. Behavioral changes did not consistently correlate with physiology and the authors concluded that autonomic reactions can be more reliable and consistent measures than some behavioral measures. Testing may thus benefit from the use of physiological measures, such as heart rate variability, shown to be associated with dogs' behavioral responses to perceived stressors (Vincent and Leahy, 1997), and may prove more valuable than simple heart rate which was not seen to correlate with behavioral signs of fear and anxiety during working dog assessments (Sherman et al 2015).

2.1.4 Lack of attention to fear behaviours during testing

In some organisations, testing focusses on perceived priority working traits such as motivation to work and exhibition of trained attack behaviours, and may neglect to observe or record behavioural signs of anxiety or fear.. Although the selection of confident dogs is a major priority for many agencies, recent research has cast doubt on whether selection tests effectively select against fear. Objective video analysis of Swedish military selection tests, actually revealed higher levels of fear amongst those dogs which went on to be selected than those that did not (Foyer et al 2016). This suggests personnel may have inadvertently been selecting those dogs which showed higher levels of fear-related behaviour . It is well established that people, even owners and carers, are variable in their ability to accurately interpret behavioural responses, and in particular subtle early signs of fear and anxiety (Tami and Gallagher 2009; Marti et al 2012). This is also likely to be the case for working dog trainers and assessors..   However in everyday practice, accurate and early detection of behavioural indicators of fear is critical (Clark et al in prep; Loftus et al 2012). Hence resources to teach observers to recognize the commonly missed signs have been developed (Loftus et al 2012) and will potentially prove valuable when used in conjunction with physiological indicators of behavioral reactivity.

2.1.5. Reliance on testing puppies

For those organisations which breed or purchase puppies there is obvious value in rejecting the puppies least likely to succeed as early as possible. Many agencies employ a suite of "puppy tests" which try to predict fearfulness amongst other traits. Most test fear of loud noises, for example by: using weights dropped in buckets (Murphree and Dykman, 1965) or on tables (Svobodová et al., 2008); whistles (Pfaffenberger and Scott, 1976); metal cans (Fisher and Volhard, 1985); party poppers (Hoffmann et al., 1995); simulated thunderstorms (Seksel et al., 1999); clapping (Champness, 1996); or pistols (Slabbert and Odendaal, 1999). Many tests also examine fear of novel people (e.g. Murphree and Dykman, 1965; Slabbert and Odendaal, 1999); novel objects, including umbrellas (Hoffmann et al., 1995); hair driers (Seksel et al., 1999); novel environments (e.g. open

stairs; (Goddard and Beilharz, 1986); and unstable surfaces (London Metropolitan police, pers. comm). In addition to the problems identified for testing in general (2.1.1-2.1.4), a major flaw of testing at a very young age, is that it does not account for modification by ontogeny and the considerable effect of learning which follows especially during sensitive periods. For example, analysis of the test commonly used by Australian Guide dogs (Goddard and Beilharz, 1982; 1984a; b; 1985; 1986) revealed that although fearfulness as an adult was significantly predicted by a test at eight weeks of age (and was the only predictable trait), the predictive value increased with age of testing. This may be unsurprising, given the reduced period between test and outcome and the fact that past learning during puppyhood will affect test results conducted at a later age. However in a large meta-analysis of 31 "personality" tests in dogs, Fratkin et al (2013) found that puppy behaviors were moderately consistent even with longer inter-test intervals but that fearfulness (along with responsiveness to training) in puppies was one of the least consistent dimensions.

Scott and Bielfelt (1976) saw no increase in the adult performance of guide dogs when breeding stock were selected on the basis of their puppy test scores. This is supported by a study of the development of Arms and Explosives search dogs (Rooney et al., 2003), which saw very little correlation between behaviors shown by eight week old puppies and those seen in equivalent tests in adult dogs, and even noted an inverse correlation between fear of slippery surfaces at 8 weeks and that at 11 months. Similarly, Asher et al. (2013) saw that puppies which scored as more confident on a ramp, (one of five stimuli tested), were less likely to qualify as guide dogs. These discrepancies may be partially due to carers using deficiencies highlighted in the puppy test to direct remedial training, as puppy tests detect predispositions that can subsequently be by operant conditioning (e.g puppies finding their existing behavior 'unsuccessful' and changing to an alternative strategy). The variable and general low degree of success in such tests suggests that, at least for individuals not at the extremes of population variance, trying to predict adult behavior from puppy tests is problematic, and as failures at procurement tests are rarely trained, the true test

reliability is not assessed. The value of puppy testing is this questionable and programmes need to fully assess predictive validity, before rejecting valuable young dogs on the basis of such tests.

2.1.6 The influence or prior learning as well as temperament

The fear-related behavior which a dog or puppy exhibits is a result of both underlying temperament, previous experience and the interaction between these. Many tests purport to measure 'temperament' but in fact measure dogs' behavioral response to a specific contextual challenge. In many situations this is desirable e.g. to detect if dogs being rehomed show aggression when handled. It is potentially more problematic where tests are screening breeding animals as some dogs may show fear responses to specific stimuli as a result of a particular aversive experience in their history rather than any underlying predisposition. Whilst it is unlikely that the 'temperament' and 'learnt' component of a behavior in an individual dog can be teased apart, it is important that the different contributions of these elements are understood to enable appropriate testing protocols to be developed for different requirements.

Even during recent scientific studies of temperament testing some of these problems remain implicit. For example, when using an open-field model to assess sound-induced fear and anxiety in potential IED (improvized explosives detection) dogs, Gruen et al., (2015) exposed animals to loud noises on consecutive days, but did not explore differences in the dogs' past experiences or exposure to noises. In the same population of dogs, Sherman et al (2015) saw differences between individuals in their "emotional reactivity"; the dogs showed consistency in their individual responses to a large number of stimuli which may be related to underlying personality, but could equally be affected by the dogs' past history. The degree to which these responses predicted working ability in the operational conditions remains to be tested as does the relative importance of genetic and experiential factors. Thus to overcome this and detect dogs prone to developing fear and anxiety (rather than ones which have a preexisting fear), we suggest testing for variance with respect to

underlying decision-making processes that influence resilience to stress exposure (Yehuda et al., 2006a), rather than testing dogs' responses to specific stimuli. Understanding whether dogs exhibit 'proactive' or 'reactive' styles of responding (Koolhaas et al., 1999; see section 1) may be valuable to identify the nature of their response to fear evoking stimuli. Other approaches might include measuring the relative degree of 'optimism' and 'pessimism' (or cognitive bias). Animals that have a more 'pessimistic' cognitive bias (and hence tend to judge novel or ambiguous stimuli as 'more likely to be negative') may be more likely to develop fear responses. For example, a more 'pessimistic' cognitive bias has been associated with a tendency to show separation-related behavior (Mendl et al., 2010), and is reduced by treatment with a selective serotonin reuptake inhibitor (SSRI), fluoxetine (Reconcile (TM), combined with behavior modification (Karagiannis et al., 2015). Since cognitive bias appears to be a better measure of underlying mood state rather than more transient emotional responses (Mendl et al., 2010) it may be a better predictor of behavioral responses across contexts and hence a valuable tool in predicting success in working environments; we suggest this is a promising area which requires further research

Research into methods by which to better predict behavioural tendencies and predictors of resilience is required, in particular better understanding the extent to which the underlying temperament characteristics themselves become plastic with environmental changes and stress exposure (Yehuda et al., 2006a). For this reason, we suggest it is beneficial to also investigate whether variation in underlying characteristics of temperament, map onto patterns of allelic variation in genome wide association studies (GWAS), or the expression in targeted areas of the genome. Several novel measures have been associated with increased resilience to stressors in laboratory species and humans (Yehuda et al., 2006a). For example, Neuropeptide Y (NPY) may have an important role in reducing risk through modulatory effects on important regulatory systems in the brain such as the HPA axis (Heilig, 2004), and noradrenalin release (Pich et al., 1993). NPY has been found to be higher in human military service veterans who have been exposed to extreme

stress but not developed PTSD, as compared to those with PTSD. NPY also appears to correlate with the extent of symptom improvement in those previously affected (Yehuda et al., 2006b). Hence this may be an interesting biological measure of either resilience to, and / or ability to recover from, exposure to stressors. The ratio of plasma dehydroepiandrosterone sulphate (DHEA) to cortisol has also been suggested as a possible marker of resilience to stress, for example high DHEA-S- cortisol ratios were positively correlated with peoples' ability to perform well in stressful military situations (Morgan et al., 2004). Other potential markers of resilience associated with differences in HPA axis activity include, 24 hour urinary cortisol secretion, circadian rhythm of cortisol release, lymphocyte glucocorticoid receptor number, and lysozyme IC50 as an indicator of enhanced glucocorticoid responsiveness (Yehuda et al., 2006a). Investigation of some of these new measures, either in isolation or through examination of metabolomics profiles may shed valuable light on the profile of animals that are most likely to withstand the stress of working dog life.

Another emerging technology with great potential is measuring emotional valance using skin conductivity. Measures of patterns in sweat production that are linked to emotional responses such as fear; these measures can be obtained non-invasively in humans (Lin et al., 2011) and sheep (Reefmann et al., 2009a;b), although measurement in the dog would require modification due to differences in mechanisms of sweat production . Additionally, recent studies of "laterality" (preferential use of one side of the body) suggest possible links with guide dog success (Batt et al., 2008), and specific aspects of guide performance (Batt et al., 2010) general problem solving ability (Marshall-Pescini et al., 2013) and with measures of fear, although not with agility competition success (Siniscalchi et al., 2014). In humans, an association between hand preference and the propensity to experience anxiety has been described; a finding that Branson and Rogers (2006) also identified in dogs: individuals with weaker paw preferences (a measure of cerebral lateralisation) were more reactive to thunderstorm and firework noise. Interestingly motor lateralisation (along

with other factors), measured by paw preference to remove tape from the nose and the frequency that both paws were used to hold a food enrichment toy (Batt et al., 2007), was indicative of success in potential guide dogs (Batt et al., 2008). There are however some inconsistencies in these studies, with different measures often yielding differing results and the possibility of chance effects resulting from multiple testing, hence the use of laterality measures for other working disciplines requires further testing.

2.2 Breeding against propensity to show fearfulness

Several breeding experiments have suggested some heritability in fearful behavior (Murphree and Dykman, 1965; Goddard and Beilharz, 1982), and have succeeded in changing the behavioral phenotype of subsequent generations by selecting breeding animals of extreme 'types' at either end of a continuum, i.e. the most and least fearful. These studies predominantly concentrate on social fearfulness, although several also look at non-social stressors including reactions to loud noises. Hence, it may be possible to select away from more extreme phenotypes of fearful behaviors where animals are likely to have reduced flexibility of response to environmental events. This approach is relevant for those agencies that or breed in-house but also those that utilize regular suppliers. For example, the breeding programme for UK guide dogs has been effective in reducing the occurrence of fear associated behaviors (Willis, 1995) and many breeding programmes have similarly bred against outward expression of fear.

Studies using working dog populations (e.g. Hsu and Serpell 2003, Arvelius et al 2014a, b) have similarly suggested that the expression of fear behaviors is partly heritable. For example, in the Swedish armed forces the behavioral scores for confidence and environmental sureness as measured during the Dog Mentality Assessment (DMA; a standardized selection test including presentation of several consecutive fear-inducing stimuli) were found to give heritability estimates of 0.23 and 0.15 respectively (Arvelius et al. 2014a), meaning that 23% or 15% of the variation in the

poultations' test scores an be accounted for by the genetic componenet, in comparison human

weight shows a heritability of approximateley 0.78 (Emde and Hewitt 2001). When assessing Rough

Collies Arvelius et al. (2014b) found similar heritability values for test scores and also relatively high

correlations between fearbehavior during testing and that reported by owners using the Canine

Behavioral Assessment and Research Questionnaire (CBARQ, Hsu and Serpell, 2003). Arvelius et al

(2014b) concluded that breeding animals were to be selected on the basis of scores for

curiosity/fearlessness in the Dog Mentality Assessment, then breeders would also report a

concurrent decrease in non-social fear scores. When examining general fearfulness, one of three

fear-reatled factors derived from multivariate analysis of multiple subtests used on potential guide

dogs, Goddard and Beilhraz, (1984) obtained heritability estimates as high as 0.80.

We suggest that such selection based on test scores should be considered with some caution since

little is known about exactly what underlying characteristics might be selected for with breeding

programmes based on behavioral phenotypes. It is unknown, for example, whether dog populations

show the type of structure suggested by Wilson et al., (1994) for shy-bold characteristics where

animals at both population extremes show low environmental flexibility. If this were the case, then

selection against 'innately shy' (or fearful) dogs may not just alter the proportion of dogs which

exhibit fear but also the proportion which are flexible to environmental conditions; clearly a

disadvantage in a working animal needing to adapt to a range of environments. Furthermore, it is

unknown whether such selection acts at the level of the animal's perception of fear-inducing stimuli,

the central evaluation of stimuli and assignment of emotional significance, their expression of fear

behaviors, or even the animal's ability to inhibit these behaviors. By selecting against dogs which

show an active fear response (e.g. for those whose behavior changes little when presented by

stimuli), we maybe selecting against 'proactive' characteristics by favouring more passive or

'reactive' fear responses, (Koolhaas et al., 1999). It is also unknown whether dogs with a 'passive'

style of responding have a different strategy for achieving rewards as well as avoiding aversive

events, with consequent implications for training success. A less obvious behavioral response to a stimulus may not indicate a reduced negative emotional experience and the concern, that selective breeding programmes may result in unreactive "zombies" (D'Eath et al., 2010), has been expressed in the farm animal literature, and is equally, if not more relevant to dogs. Similarly, behavior traits may be genetically linked to other morphological, or physiological characteristics (e.g Mackenzie et al., 1985), which may be undesirable. Hence, although it is theoretically attractive to select animals from which to breed offspring with a reduced risk of showing fearful behaviors, current knowledge about the characteristics which underlie risk of behavioral signs, their patterns of inheritance, and their association with allelic variation in genome-wide studies, is currently insufficient to determine with any confidence the efficacy, or indeed undesired consequences, of such an approach. It may be that a more suitable approach is to investigate the heritability of underlying characteristics (or putative 'endophenotypes' e.g. Gottesman and Gould, 2003) such as stress resilience and optimism (see 2.1.6), rather than selecting directly for phenotypic presentations of fearful behavior.

3. Reducing the risk of fear related behaviors through environmental manipulation and rearing

Preventing problems is ultimately more cost effective than managing them. Therefore, in addition to identifying those animals least likely to develop fear responses, it is important to focus on the aspects of the environment that may influence the development of such responses and are open to manipulation. In this section we discuss aspects of the living environment (3.1) which can be manipulated and which evidence suggests may reduce the risk of fear and anxiety development. For working dog populations with breeding programmes or regular suppliers, we then emphasize the opportunities to control both pre- and post-natal maternal environment (3.2), when there is considerable synaptic plasticity and appropriate actions can ameliorate the chance of later problems developing for example changing the time of weaning and ensuring optimal social environments.

3.1Manipulation of factors affecting susceptibility to stress and hence likelihood of to developing fears

Background levels of arousal and concurrent causes of fear and anxiety during stimulus presentation can increase the risk of sensitisation (Davis, 1974), There is considerable evidence that states of anxiety in rodents increase the magnitude of the startle response (Bijlsma et al., 2010), whilst people with anxiety disorders are more likely to develop a range of conditioned fear responses (Lissek et al., 2005). Therefore adapting living environments, human contact and general training methods to avoid anxiety provoking situations is important, both for home-living and kennelled working dogs .Detailed reviews exploring ways to minimize stress of kennelled dogs are separately available (e.g. Gaines, 2008; Rooney et al., 2009), however factors which may be considered include the following.

3.1.1 General human contact

Interactions between dogs and human handlers are important. Unusual to the domestic dog is the potentially great effect of the interspecific relationship upon numerous aspects of social behavior, including the development of fears. Whilst some studies claim to show evidence of emotional contagion, between human and dog (e.g. Custance and Mayer, 2012), whether this is evidence of true empathy, remains a source of debate (e.g. Edgar et al., 2012). Human interaction can be beneficial to dogs in reducing the stress of confinement (Coppola et al., 2006), as well increasing indicators of positive wellbeing such as decreased blood pressure and increased β-endorphin (Odendaal and Meintjes, 2003). A programme of human interaction in chronically stressed shelter dogs resulted in reduced excitation and cortisol in a novel environment compared to controls (Hennessy et al., 2006). Handlers of military dogs who took their dogs home after work described them as less fearful, more sociable, more accepting of stroking by strangers, and less likely to bite compared to those handlers whose dogs remained in kennels (Lefebvre et al., 2007). Similarly pet

dog owners who shared more activities with their dogs were less likely to categorize them as "nervous" (Bennett and Rohlf, 2007). Although this may be in part due to increased exposure to stimuli and consequent habituation, the effect of human contact is likely profound. Increased human contact is desirable and beneficial for fear reduction in for the majority of dogs, although when individual animals show an established fearful or aggressive response towards people, forced interaction will not be valuable (Rooney et al., 2007a), and a programme of gradual desensitisation and counter-conditioning to handling is required (section 5).

3.1.2 Reduction of stress during routine training

The type of contact is also important, in particular the type of training used to develop the necessary working skills is integral to the risk of development of fear responses. Studies (Hiby et al., 2004; Blackwell et al., 2008 Rooney and Cowan, 2011), strongly suggest that punishment-based methods may increase levels of fear and anxiety, and potentially lead to sensitisation to other environmental stimuli. Punishment-based training of pet dogs is linked to an increased incidence of behavior problems (Hiby et al., 2004), including fear, anxiety and aggression (Blackwell et al., 2008), wariness towards strangers, reduced playfulness (Rooney and Cowan, 2011), and increased anxiety-related aggression and excitability (Arhant et al., 2010). Dogs regularly trained with electric shocks show behavioral evidence of fear and distress in the presence of their owner, even outside the training context (Schilder and van der Borg, 2004). Dogs subjected to physical reprimands have been shown to score significantly higher for aggression (Hsu and Sun, 2010) and dogs whose owners report using a higher proportion of punishment are less likely to interact with a stranger (Rooney and Cowan, 2011), which could be due to fear and anxiety.

Although opinions are gradually changing, with most working dogs being trained primarily using positive reinforcement and negative punishment, there are still some trainers who use coercive based approaches based on the 'dominance' approach to interpreting dog behavior (Bradshaw et al.,

2009), and rough handling remains common in specific disciplines and organisations (Haverbeke et al., 2009). Aversive methods, including pulling the leash and hanging, were associated with low body postures indicative of fear or distress and poor performance in military working dogs (Haverbeke et al., 2008), and those military dogs suspected to have been handled roughly in the past were perceived to be more fearful (Lefebvre et al., 2007). During protection and obedience work, those dogs which had received more punishment, tended to show more fear behaviors (Haverbeke et al 2009) and when examining prison officer's behavior towards their search dogs Rooney et al. (2007b) found that handlers who believed in using high levels of punishment tended to have less confident dogs. These studies to date are all correlational, and cannot prove causation ,since dogs with specific problems may be more likely to be trained using coercive methods. However they do show a consistent pattern between aversive training methods and behavioral problems including fears. Longitudinal cohort studies, and experimental trials are now needed to ascertain any causative links.

Avoiding positive punishment based techniques and interacting with dogs in a calm and consistent way is likely to be very important in reducing the development of fear related behaviors, as demonstrated in numerous species. Horses trained using positive reinforcement interacted more with the handler (Sankey et al., 2010) whilst zoo rhinos (Holden et al., 2006) and primates (Savastano et al., 2003) have been successfully trained to present various body parts in a calm safe manner through operant conditioning using positive reinforcement. This type of approach is also starting to be used e in farm environments for ease of handling, reduction of stress (Kilgour et al., 1991) and even cognitive enrichment (Manteuffel et al., 2009). In a study of military dogs in which positive reward training sessions, as well as altered selection procedures and handler training were used, dogs demonstrated increased confidence and overall performance (Haverbeke et al., 2010), suggesting that training methods are vital in working dog operations

3.1.3 Kennel management and routines

Gaines (2008) stresses the importance of considering husbandry regimes when aiming to decrease stress of kennelled dogs. Specific husbandry processes can lead to distress, for example shutting dogs in small dark inner kennels whilst cleaning their home kennel (Gaines, 2008), using noisy equipment such as cleaning hoses (Mills, 2005) and separating dogs from conspecifics (Walker et al., 2014). Identification and amelioration of these types of procedures is important in reducing overall levels of stress. Increasing the predictability a of dog's routine is likely important, for example walking and feeding at predictable times (Gaines et al., 2008). However we suggest, care also needs to be taken not to make the routine too predictable, as having a highly predictive and regimented daily routine can build strong expectations in the dogs and limit future flexibility (Gaines et al., 2008). This may be problematic when dogs' training experiences and regimes are very different to that in which they will be expected to work. Hence, in order to best prepare dogs for a range of situations and activities, it may be better to gradually adapt from an initially predictable routine to a less predictable and more variable daily schedule over time. In this way, dogs will 'have an expectation of the unexpected' and are likely to cope better with changing circumstances when they become necessary.

3.2 Early life environments

3.2.1 Pre and post-natal effects

Those programmes with breeding and puppy walking schemes have the added potential to affect adult dog behavior by influencing pre- and post-natal environment. Much work on early-life effects has focused on human development (e.g Grant et al., 2009; Hellemans et al., 2010), with comparative studies in rodents (Bosch et al., 2007), and farmed species (e.g. pigs; Jarvis et al., 2006; sheep;;, Dodic et al., 2002) and has shown that pre-natal anxiety adversely affects stress reactivity in

offspring. In comparison, very little is known about the impact of pre-natal experience on the behavior and development of puppies; however, given the compelling evidence in other species, the ability of pups to recognize odours learned in utero (Wells and Hepper, 2006), and similar endocrine profiles and changes in cortisol in pregnant bitches as in other mammalian species (e.g. sheep; Concannon et al., 1978), it is likely that they are similarly influenced by variation in maternal exposure to stressors during pregnancy. It would therefore seem prudent that due attention is given to the range of experiences of breeding bitches during pregnancy, whilst maintaining normal routines of social interaction with people and other dogs and providing enriching experiences when expected (Gaines et al., 2008).

In addition there is some evidence that puppies manipulated in the neonatal period differ in later stress responsiveness. Battaglia (2009) describes a study on a bio-sensor programme for military dogs. Puppies of 3-16 days of age were stimulated daily using a range of handling techniques, including being placed on a cold towel, They identified improved cardiovascular function (HR), stronger heart beats, stronger adrenal gland function, greater tolerance to stress and greater resistance to disease (Battaglia, 2009). These studies are based on principles of promoting resilience via mild stressors applied in early life (Macrì and Würbel, 2006, 2007; Macri et al., 2009; 2010; 2011). It should be therefore assumed that to reduce the likelihood of puppies developing fear responses, the pregnant mother and neonatal puppies should be provided with a stimulating environment but not be exposed to the kinds of severe stressors capable of inducing fears such as moving to new environments (Rooney et al., 2007b), mixing bitches into new social groups, unaccustomed confinement or social isolation, malnutrition (Poore et al., 2010), inconsistent handling, transport or exposure to specific fear-eliciting situations (e.g. loud noises).

3.2.2 Management of weaning

In most 'natural' environments, the process of weaning is a gradual one, with offspring becoming progressively more independent, both emotionally and nutritionally, from maternal care (Latham and Mason, 2008). However, in most companion animals, especially dogs, offspring are removed suddenly from their mothers, and generally concurrently from their familiar social and physical environment. Behavioural (Houpt and Hintz, 1983; McCall et al., 1985), and physiological (McCall et al., 1987; Malinowski et al., 1990) signs of distress at this sudden weaning are observed in many species, Activation of the hypothalamic-pituitary (HPA) axis has also been shown to continue for several weeks after weaning in both rodents and primates, (Levine, 2000), and there is no reason to assume this is not the case for dogs.

Appropriate management of weaning can however reduce the risk of inducing fears developing. The nature and period over which maternal separation occurs, can have positive or detrimental effects on later resilience to stressors. For example, studies on rodents show that early separation of pups from their mother, can induce short and long-term changes in the stress reactivity system as indicated by the potentiated HPA axis response to subsequent stressors (e.g. Knuth and Etgen, 2007, Lippmann et al., 2007). It also enhances manifestations of anxiety and depression-like behaviors (Fabricius et al., 2008, Lambás-Señas et al., 2009) and impairs spatial learning and memory (Aisa et al., 2007 Tata et al., 2015) during adulthood. In contrast, repeated maternal separation, increased resilience to stressful events later in life (Parker et al., 2006; Benetti et al., 2007) and reduced fearfulness (Macrì and Würbel, 2006). Although these separations were of short duration and it has been suggested that the positive effects may have resulted from increased attention from the mother when the pups were returned. Increased maternal stimulation appears to have a beneficial effect on neural development and can reverse the effects of early-life stressors (Imanaka et al., 2008). Slabbert and Rasa (1993) studied South African Police dog puppies which were separated from their mothers at either six or 12 weeks. Both groups received equal human

contact but the late weaned puppies gained weight quicker and were in better condition, the study did not note any differences in fear behavior towards people or other stimuli.

Evidence of optimal weaning protocols for working dogs is limited, but this appears to be critical - for avoiding fear-related and abnormal behaviors in other species. The advantages associated with maternal care, social interaction with littermates, learning social signalling and avoidance of sudden traumatic events, tend to support later and gradual weaning of puppies destined for working environments. In spite of this, some established assistance dogs organisation routinely wean early, in order to ensure socialisation opportunities are maximized. For example, in the UK, Guide Dogs for the Blind routinely remove puppies from their mother at six – eight weeks, and below the age generally permitted for other dogs It it has been shown that the relative quality of breeding and rearing environments of pet dogs affects the ideal weaning time (Appleby et al 2002), and we suggest that research should focus on the optimal time for working dogs if both environments are well controlled.

4. Introduction of potentially fear provoking stimuli in an optimal way

4.1 Habituation

Critical, is the judgement by the animal of the degree of threat posed by a stimulus, since an avoidance response will not develop where the stimulus is not salient and the animal habituates. The relative risk of habituation or sensitisation will vary with both characteristics of the stimulus, the personality of the dog and the state of the individual animal at the time of stimulus presentation. Hence controlling all these factors is important to minimize the chances of a working dog developing fear associated behaviors. Habituation protocols are commonly utilized by working dog programmes aiming to introduce dogs to stimuli to which they will be exposed when working.

Research from other species suggests that evolutionarily relevance, suddenness, predictability, intensity, frequency of exposure of the stimulus and along with the ability of the animal to control exposure its current emotional state, will all affect the probability of an animal experiencing sensitisation. In a recent review of stress in pet dogs, (Mills et al., 2014) similarly identified important aspects of a stressor for dogs to be to be physical characteristics including, affective quality, intensity, magnitude, duration, and predictability, as well as the expectation of the animal in relation to this stressor and the preparation given to enable coping and opportunities for control over the stressor.

Initial intensity of stimulus presentation needs to be below the threshold of startle for the animal. In general the principle is to start as low as possible, since it is better to increase the level gradually from well below all dogs' tolerance than risk sensitisation in some individuals. Since each animal will have a unique tolerance to different types of stressors, in developing a programme applicable to populations of dogs it is important to select an initial level of exposure which is below that to which

any in the population will respond aversely. In a diverse population of procured dogs it may be difficult to find a level to which no dogs respond. Hence we suggest a preliminary test to identify those outlying individuals with established fears which require remedial training, desensitisation and counter conditioning (Section 4), and then start controlled exposure and habituation of the remaining animals at an appropriately low level.

.

  The benefits of this type of gradual introduction to novel stimuli, as compared to repeated exposure of a full intensity stimulus (often termed "flooding"), are understood by many animal trainers. Horses for example, given a gradual introduction to a moving white nylon bag showed both behavioral and physiological differences compared to those without such an introduction. Those with gradual exposure protocols needed fewer training sessions to remain calm on stimulus presentation, and all subjects in the gradual exposure group completed training in contrast to the full exposure group (Christensen et al., 2006). Research on noise fears in IED dogs showed that sixteen dogs tested showed an overall reduced response over a 5-day testing period, but as the noises presented in each day were different it is likely that they may have habituated to the testing environment. However, the fact that some dogs did not decrease in responsiveness, suggests that the initial exposure level may have been too high (Gruen et al., 2015).

In rats, habituatio to noise is more likely when there is a longer gap, 24 hrs compared to 60 minute, between stimulus presentations. (Masini et al.2008). Although behavioral and physiological responses r in both groups were similar on the last when re-exposed to the stimulus two days later, the rats which had been presented larger gaps between showed a lower response. . The effect has been suggested to be associated with animals having rapid eye movement (REM) sleep between training sessions (e.g. Levin and Nielsen, 2007) and hence habituation sessions may benefit from being separated by a period of sleep but this requires further stud in dogs.

Further research on laboratory rodents (Jordan and Strasser, 2000), suggests habituation is context - specific, so 'recovery' of the response occurs in new contexts to where the habituation took place);. Should elements of the context alter, a startle response may recur, particularly if the change of context is salient to the animal. Hence a dog may habituate to a noise presented in one situation, but it may react when this is presented at the same intensity in a new context (Leiner and Fendt, 2011). This effect in rats is influenced by the extent to which the 'new' context is familiar or novel; behavioral responses in rats reoccurred when a habituated stimulus was presented in a novel context, but not when the animal was familiar with the context (Nyhuis et al. 2010). Hence, prior habituation to environments in which stimuli will be presented, such as introducing dogs to the environments in which potentially frightening stimuli may occur, may aid with the process of habituation and help prevent fears developing. We suggest that to ensure thorough 'proofing' of working dogs against stimuli likely to be of high intensity and salience, a protocol which involves a gradual presentation in a range of circumstances, and in progressively less predictable, but familiar, circumstances is needed

Despite the evidence and practical expertize relating to gradual and slow habituation of novel stimuli, many industries do not use this gradual approach. For example, initial greyhound schooling generally happens in an unfamiliar location, in the presence of new people, and dogs may have been transported in a new vehicle to the schooling location. Numerous potential stressors are presented simultaneously, which likely leads to sensitisation in some individuals (Rooney 2012; Cobb et al., 2015). Similarly, working dog programmes often employ screening tests which present stimuli at full intensity (see section 2.1). In addition when adult dogs are procured from different sources they may have very different background experiences, meaning that test elements will identify a mixture of reactions to previously encountered stimuli and temperamental characteristics. For some dogs, having a previously learnt fear response may that result in a response at a very low level of stimulus presentation. For others, test scenarios may be entirely novel and potentially lead to the risk of

sensitisation due to exposure to multiple novel stimuli in a novel environment. Hence the importance of pre- testing with gradual exposures to ascertain the levels to which dogs in the population respond for each potential stressor.

4.2 Social context affecting fear responses

4.2.1 Conspecifics

The potential for dogs to be influenced in their development of fear by the presence and behavior of other dogs is important. This has implications both for the context in which dogs should be tested for their propensity to exhibit fear and anxiety, and also for the conditions under which dogs should be introduced and habituated to potentially fear-inducing stimuli. It is commonly reported that if a dog first encounters a new stimulus in the presence of a fearful dog, it is more likely to develop fear itself (e.g. Landsberg et al., 2012) whilst introduction in the presence of a previously conditioned and calm dog may reduce the likelihood of a fearful response. Trainers of a variety of animal species use the presence of calm unreactive conspecifics as a means of reducing fear in "nervous" animals but the potential for using a calm "demonstrator" dog in working scenarios needs to be evaluated scientifically.

4.2.2 Human contact

Human interaction during stressful events can also exert a calming effect. Twenty minutes of gentle stroking inhibited the immediate increase of cortisol during a venipuncture procedure (Hennessy et al., 1998). Humans generally have greater success at inhibiting stress responses to a novel environment in dogs than do familiar dog companions (Tuber et al., 1996).

In contrast certain types of human interaction can be detrimental and increase the likelihood of fears developing. It is commonly asserted that fear "travels down the lead"; that anxious owners and

handlers can increase the likelihood of dogs reacting fearfully. In agility dogs, levels of cortisol increased when their male owners became angry after losing a competition (Jones and Josephs, 2006). Inexperienced owners were more likely to describe their dogs as showing fear of traffic, loud noises and other dogs (Jagoe and Serpell, 1996), suggesting that fear and anxiety may result from owners lacking the understanding or ability to respond an appropriate way. Dogs may also be influenced by the behavior and personality of their owner; tense and emotionally less stable owners for example, are more likely to own aggressive dogs (Podberscek and Serpell, 1997). Since aggression in dogs is often motivated by fear, it is possible that owner personality affects the way the person behaves towards and interacts with their dog, which in turn affects the dog's propensity to be fearful (Podberscek and Gosling, 2000). Dreschel and Granger (2005) noted that owners scoring highly for anger/hostility, depression/dejection and fatigue were less likely to interact with their fearful dog during a simulated thunderstorm and although there were no significant effects of owner behavior on the dogs' behavior. However, this study could not disentangle the extent to which personality per se and past owner behaviour produced this effect. Traditionally interaction with dogs during fearful episodes was not recommended as it may inadvertently reward and thus reinforce fearful responses. In support of this, a survey of dog owners in New Zealand found an association between owners comforting their dogs and increased severity and duration of fear behavior over time (Dale et al., 2010). Whether this is causative cannot be ascertained from this association, and recently the advice against comforting fearful dogs, has been challenged: the reinforcement is argued to be of the coping response rather than the fear itself (Casey, 2013). These studies together highlight the importance of the handler's behavior in the development of fear, and how calm, informed and diligent handlers or trainers are critical to any effective fear-reduction programme.

4.3 Introduction of puppies to potential stressors sensitive period of learning ('socialisation period')

In programmes that breed or rear puppies, appropriate early introduction to potential stressors is important. The period of development and maturation of senses until about 12-14 weeks, when puppies become more independent, is an important time of enhanced synaptic plasticity, where exposure to environmental stimuli has a profound influence on later behavior (Casey and Bradshaw, 2008). Using this period to ensure that puppies have had positive experiences of stimuli and situations they are likely to come across in their adult lives is commonly acknowledged to reduce the risk of fear-related behaviors in companion (Appleby et al., 2002; Howell and Bennett, 2011) and laboratory (Boxall et al., 2004) dogs. In general, it is suggested that dogs must be introduced to the types of situations and stimuli they will encounter in adult life before three months of age (preferably by eight weeks and certainly no later than 12 weeks; (Scott and Fuller, 1965,). As well as being curious of novelty, puppies readily develop fear behaviors from around eight weeks. Studies on research dogs have shown that handling for three minutes per day and exposure to stressors (e.g. changes in ambient temperature, different flooring, different handlers) of gradually increasing intensity and duration, had positive effects on resistance to disease, emotional reactivity and problem solving (Meunier, 2006). Similarly, Gazzano et al. (2008) saw well-handled puppies of various breeds to be calmer. A further study suggested that presenting video images of animate (e.g. people, dogs) and inanimate (e.g. traffic, vacuum cleaner) stimuli to puppies was associated with a reduction in later fear behaviors (Pluijmakers et al., 2010), although whether the audio or visual elements of these presentations were most important, and their potential in a working dog rearing programme is yet to be explored.

 Many working dog breeding establishments (e.g. USA Transport Security Administration TSA; Thomas, 2011) use this sensitive period of development, to successfully introduce dogs to a variety of stimuli including other animals, people, children, noises, slippery surfaces and unstable floors.

When considering socialisation programmes and exposure to potentially frightening stimuli, it is important to control exposure and the level of stimulus, increasing intensity gradually to avoid sensitisation and inadvertently creating anxiety or fear responses. For example, gradual habituation to an indoor crate, followed by an outdoor kennel environment reduced physiological stress levels when search dogs entered military training kennels (Rooney et al., 2007b). As risk of sensitisation is also increased in animals experiencing concurrent stress (see Section 3.1), the experience of puppies with respect to kennelling, handling, and enrichment also needs to be addressed (as with adult dogs).

In the domestic situation, puppy socialisation classes are often used to introduce exposure to stimuli which commonly cause fear reactions in adult dogs, social situations as well as stimuli such as fireworks and thunder (Blackwell et al., 2013) urban noises such as engines (Sherman and Mills, 2008). Population-based studies have had mixed findings as to the benefit of these classes on later behavior. Four, weekly puppy socialisation classes, were successful in training companion puppies but produced no significant change in responses to social stimuli 4-6 months later (Seskel et al 1999). Similarly, five socialisation sessions did not affect the success rate of guide dog puppies (Batt et al., 2008). However large scale population surveys have, identified positive effects; dogs that attended puppy socialisation classes were less likely to be reported to show undesirable reactions to other dogs (Blackwell et al., 2008). Attending puppy classes also produced a protective effect against owner reported aggression (Casey et al., 2014) but was associated with an increased risk of human-directed aggression in a clinical population (Lord et al.in press). The methods used in these classes however are variable and whether they are conducive to reducing fear will depend on stimuli presentation method, for example whether less confident puppies are overwhelmed or able to control their own exposure.

In working dogs there have been several studies on early life experience, for example Goddard and Beilharz (1984) examined fearfulness and activity/distraction in 102 potential Guide. When the

quality of puppy-walking experiences were rated and compared to later behavior, the most significant correlations were between high quality puppy walking and decreased fear of unusual objects (Goddard and Beilharz, 1985). In support of this, Rooney et al. (2003) saw that compliance of puppy-walkers to a programme of introduction to new experiences, was a major factor in determining the later success of specialist search dogs. Programmes that provide gradual, early introduction, to stimuli for working dogs, combined with optimal weaning may therefore be very beneficial and should be investigated further. Play and relaxed social interactions provide ideal contexts in which for puppies to encounter stimuli and learn that they are not threatening. Slabbert and Rasa (1997) used observational learning to demonstrate that pups, allowed to stay with their mothers and observe them in a search and retrieve task, were better at completing the same task (without reinforcement) than non-exposed pups at 6 months old. It seems, therefore, that the behavior of the mother could potentially be very powerful in influencing the behavior of her offspring and may be a potential area for research in the reduction of fear responses (provided that mothers are previously well habituated to stimuli).

In working environments, the number of potential stressors is often great. For example, search and rescue or military search dogs may be exposed to explosions in which loud noise, shock waves and dust are experienced simultaneously. Little evidence exists as to the optimal timing of stimulus exposure in early life, whether composite stimuli need to be initially broken down and presented alone, or whether puppies will tolerate combined stimuli at low intensity. However, because of the importance of this period in influencing adult life across species (Casey and Bradshaw, 2008), we can be confident that controlled exposure will be beneficial and hence would suggest that agencies which breed puppies for working roles carefully consider policy in this area, and those which use regular suppliers, encourage gradual controlled exposure.

5. Treatment

Whilst following evidence-based selection criteria, manipulating the living environment, and providing opportunities for learning will help to minimize the number of working dogs which develop fears and anxiety, some fear responses remain inevitable when animals are working and training in an unpredictable and potentially dangerous environments. Most of the fear-related behaviors in USA military working dogs were reported to be acquired as adult dogs (Burghardt, 2003). There is value to developing effective treatment protocols for dogs showing fear responses both animals which demonstrate pre-existing fear responses at procurement, or before training that may then interfere with ability in training, and for dogs already in training, in service, or returning from operations, which have developed fear responses. Any treatment should include a full medical and behavioural work up of the animal to ensure there is no underlying medical condition. This should be followed investigation of eliciting and maintaining stimuli and deciding on basis of this information what to do in order to restore the animal's well-being   Such investigations may lead to some dogs ending their working career, but many dogs may be able to continue working much longer than if they were untreated. Behavior modification programmes based on desensitisation and counter-conditioning (e.g. Levine et al., 2007)); a combination of presenting an animal with the fear-eliciting stimulus at an initially low level, and also positively rewarding the animal for showing an alternative (calm) response to that shown previously, are vital and are described elsewhere (Clark et al in prep).

6. Conclusions

Propensity to show anxiety and specific fear responses results from an interplay of individual differences in animals and learning throughout life. Since significant failures amongst working dogs are a result of the development of fearful behavior; attention to methods likely to minimize fear in working populations is critical. Whilst current methods are seemingly effective and allow dogs to work in a remarkable array of environments, programmes should strive for optimal and improved

efficiency to reduce the wastage of dogs. As such, programme managers should continually analyse the value of each component of their programme, for puppies and adult dogs alike, seek evidence for its value and explore potential evidence-based improvements. In this paper, we have suggested potentially valuable techniques already utilized, discussed evidence from dogs and other species, and suggested important areas for future research effort.

Whilst the development of fear behavior has been seen to have some heritable component, the environment and past opportunities for learning are also vitally important. Identification of characteristics which reliably predict resilience to stress, and careful breeding for these traits, the derivation of predictive tests for puppies and procured animals, rearing animals in optimal environments and introducing them to a range of stimuli in a positive, controlled and gradual way, can help minimize the number of dogs which develop work-inhibiting fears. Future research should explore innovative methods of best measuring the relative resilience of dogs to stressful events and develop optimal protocols to 'proof' dogs for exposure to potentially fear provoking stimuli, such as the influence of social learning and the most effective elements of stimuli and appropriate exposure protocols.

Conflict of interest statement

We declare that there are no conflicts of interest involving any of the authors and this work. Rachel Casey has started working at the UK dog charity Dogs Trust since the submission of this paper.

Authorship

The manuscript was initially prepared by N Rooney with significant input into academic content, writing and editing form both C Clark and R Casey.

References

Aisa, B., Tordera, R., Lasheras, B., Del Río, J. Ramírez, M.J. 2007. Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats Psychoneuroendocrinology, 32 (2007), pp. 256–266 .Appleby, D.L., Bradshaw, J.W.S., Casey, R.A., 2002. Relationship between aggressive and avoidance behaviour by dogs and their experience in the first six months of life. Vet.Rec. 150, 434-438.

Arhant, C., Bubna-Littitz, H., Bartels, A., Futschik, A., Troxler, J., 2010. Behaviour of smaller and larger dogs: Effects of training methods, inconsistency of owner behaviour and level of engagement in activities with the dog. Appl. Anim. Behav. Sci.123, 131-142.

Arvelius, P; Strandberg, E; Fikse, W. F. 2014a The Swedish Armed Forces temperament test gives information on genetic differences among dogs J. Vet. Behav. 9(6), 281-289.

Arvelius, P.; Asp, H. Eken; Fikse, W. F.; et al. 2014b Genetic analysis of a temperament test as a tool to select against everyday life fearfulness in Rough Collie J.Anim. Sci. 92 (11), 4843-4855.

Asher, L., Blythe, S., Roberts, R., Toothill, L., Craigon, P.J., Evans, K.M., Green, M.J., England, G.C.W., 2013. A standardized behavior test for potential guide dog puppies: Methods and association with subsequent success in guide dog training. J. Vet. Behav. 8, 431-438.

Batt, L., Batt, M., Baguley, J., McGreevy, P., 2010. Relationships between puppy management practices and reported measures of success in guide dog training. J. Vet. Behav. 5, 240-246.

Batt, L., Batt, M., McGreevy, P., 2007. Two tests for motor laterality in dogs. J. Vet. Behav. 2, 47-51.

Batt, L.S., Batt, M.S., Baguley, J.A., McGreevy, P.D., 2008. Factors associated with success in guide dog training. J. Vet. Behav. 3, 143-151.

Battaglia, C.L., 2009. Periods of Early Development and the Effects of Stimulation and Social Experiences in the Canine. J. Vet. Behav. 4, 203-210.

Benetti, F., de Araujo, P.A., Sanvitto, G.L., Lucion, A.B., 2007. Effects of neonatal novelty exposure on sexual behavior, fear, and stress-response in adult rats. Devel.Psychobio. 49, 258-264.

Bennett, P.C., Rohlf, V.I., 2007. Owner-companion dog interactions: Relationships between demographic variables, potentially problematic behaviours, training engagement and shared activities. Appl. Anim. Behav. Sci.102, 65-84.

Bijlsma, E.Y., de Jongh, R., Olivier, B., Groenink, L., 2010. Fear-potentiated startle, but not light-enhanced startle, is enhanced by anxiogenic drugs. Pharmacology Biochemistry and Behavior 96, 24-31.

Blackwell, E., Bradshaw, J., Casey, R., 2013. Fear responses to noises in domestic dogs: Prevalence, risk factors and co-occurrence with other fear related behaviour. Appl. Anim. Behav. Sci. 145, 15-25.

Blackwell, E.J., Twells, C., Seawright, A., Casey, R.A., 2008. The relationship between training methods and the occurrence of behavior problems, as reported by owners, in a population of domestic dogs. J. Vet. Behav. 3, 207-217.

Blackwell, E.J., Bodnariu, A., Tyson, J., Bradshaw, J.W.S., Casey, R.A., 2010. Rapid shaping of behaviour associated with high urinary cortisol in domestic dogs. Appl. Anim. Behav. Sci.124, 113-120.

Boissy, A., 1998. Fear and fearfulness in determining behavior, In: Grandin, T. (Ed.), Genetics and the behavior of domestic animals, Academic Press, New York, pp. 67-111.

Bosch, O.J., Müsch, W., Bredewold, R., Slattery, D.A., Neumann, I.D., 2007. Prenatal stress increases HPA axis activity and impairs maternal care in lactating female offspring: Implications for postpartum mood disorder. Psychoneuroendocrino 32, 267-278.

Boxall, J., Heath, S., Bate, S., Brautigam, J., 2004. Modern concepts of socialisation for dogs: Implications for their behaviour, welfare and use in scientific procedures. Atla-Altern. Lab. Anim. 32, 81-93.

Bradshaw, J.W.S., Blackwell, E.J., Casey, R.A., 2009. Dominance in domestic dogs-useful construct or bad habit? J. Vet. Behav. 4, 135-144.

Branson, N.J., Rogers, L.J., 2006. Relationship between paw preference strength and noise phobia in Canis familiaris. J. Comp. Psycho. 120, 176-183.

Brown, C.J., Murphree, O.D., Newton, J.E.O., 1978. The effect of inbreeding on human aversion in pointer dogs. Journal of Heredity 69, 362-365.

Brown, J., 2011. Developing the Disaster Search Dog, Defining, Developing and Documenting Success in Working Dogs, Pearl River, New York.

Burghardt, W.F., 2003. Behavioral considerations in the management of working dogs. Vet. Clin. N. Am.-Small 33, 417-446.

Caron-Lormier, Geoffrey; Harvey, Naomi D.; England, Gary C. W.; et al 2016 Using the incidence and impact of behavioural conditions in guide dogs to investigate patterns in undesirable behaviour in dogs Scientific Reports 6: 23860

Casey, R., 2010. in: Lindley, S., Watson, P. (Eds.), BSAVA Manual of Canine and Feline Rehabilitation, Supportive and Palliative Care: Case Studies in Patient Management, British Small Animal Veterinary Association.Casey, R., 2013. Should fearful dogs be comforted on fireworks night?, Reigning Cats and Dogs: A scientific perspective on companion animal behaviour and welfare, by veterinary behaviourist Dr Rachel Casey, https://behaviourvet.wordpress.com/2013/11/04/should-fearful-dogs-be-comforted-on-fireworks-night/. Accessed 16 Aug 2015.

Casey, R.A., Bradshaw, J.W.S., 2008. The effects of additional socialisation for kittens in a rescue centre on their behaviour and suitability as a pet. Appl. Anim. Behav. Sci. 114, 196-205.

Casey, R., Loftus, B., Bolster, C., Richards, G., Blackwell, E., 2014. Human directed aggression in domestic dogs (Canis familiaris): Occurrence in different contexts and risk factors. Appl.Animal Behav. Sci. 152, 52-63.

Champness, K.A., 1996. Development of a breeding program for drug detector dogs - based on studies of a breeding population of guide dogs, PhD Thesis University of Melbourne.

Christensen, J.W., Rundgren, M., Olsson, K., 2006. Training methods for horses: habituation to a frightening stimulus. Equine Vet. J. 38, 439-443.

Clark, C.C.A., Casey, R., Rooney, N., In prep. Treatment of fear and anxiety in dogs; a review.

Cobb, M., Branson, N., McGreevy, P., Bennett, P., Rooney, N., Magdalinski, T., Howell, T., Dawson, K., 2015. Review & Assessment of Best Practice Rearing, Socialisation, Education and Training Methods for Greyhounds in a Racing Context Report To Greyhound Racing New South Wales

Concannon, P.W., Butler, W.R., Hansel, W., Knight, P.J., Hamilton, J.M., 1978. Parturition and Lactation in the Bitch: Serum Progesterone, Cortisol and Prolactin. Biol.Reprod. 19, 1113-1118.

Coppola, C.L., Grandin, T., Enns, R.M., 2006. Human interaction and cortisol: Can human contact reduce stress for shelter dogs? Physiol. Behav. 87, 537-541.

Custance, D., Mayer, J., 2012. Empathic-like responding by domestic dogs (Canis familiaris) to distress in humans: an exploratory study. Anim. Cogn. 15, 851-859.

D'Eath, R.B., Conington, J., Lawrence, A.B., Olsson, I.A.S., Sandoe, P., 2010. Breeding for behavioural change in farm animals: practical, economic and ethical considerations. Anim. Welf. 19, 17-27.

Dale, A.R., Walker, J.K., Farnworth, M.J., Morrissey, S.V., Waran, N.K., 2010. A survey of owners' perceptions of fear of fireworks in a sample of cats and dogs in New Zealand. New Zeal. Vet. J. 58, 286-291.

Davis, M., 1974. Sensitization of rat startle response by noise. J.Comp. Physio, Psychol. 87, 571-581.

de Boer, S.F., van der Vegt, B.J. & Koolhaas, J.M.2003 Individual Variation in Aggression of Feral Rodent Strains: A Standard for the Genetics of Aggression and Violence? Behav Genet 33: 485

De Meester, R.H., Pluijmakers, J., Vermeire, S., Laevens, H., 2011. The use of the socially acceptable behavior test in the study of temperament of dogs. J. Vet. Behav. 6, 211-224.

Dodic, M., Hantzis, V., Duncan, J., Rees, S., Koukoulas, I., Johnson, K., Wintour, E.M., Moritz, K., 2002. Programming effects of short prenatal exposure to cortisol. FASEB J. 16, 1017-1026.

Dreschel, N.A., 2010. The effects of fear and anxiety on health and lifespan in pet dogs. Appl. Anim. Behav. Sci. 125, 157-162.

Dreschel, N.A., Granger, D.A., 2005. Physiological and behavioral reactivity to stress in thunderstorm-phobic dogs and their caregivers. Appl. Anim. Behav. Scie. 95, 153-168.

Edgar, J.L., Nicol, C.J., Clark, C.C.A., Paul, E.S., 2012. Measuring empathic responses in animals. Appl. Anim. Behav. Sci. 138, 182-193.

Emde, RN, Hewitt JK. 2001. Infancy to Early Childhood: Genetic and Environmental Influences on Developomenet Change. Oxford University Press. Oxford

Ennaceur, A., Michalikova, S., Chazot, P.L., 2006. Models of anxiety: Responses of rats to novelty in an open space and an enclosed space. Behav. Brain Res. 171, 26-49.Fabricius K ,. Wörtwein G, Pakkenberg B. 2008.The impact of maternal separation on adult mouse behaviour and on the total neuron number in the mouse hippocampus Brain Struct. Funct., 212: . 403–41.

Forkman, B., Furuhaug, I.L., Jensen, P., 1995. Personality, coping patterns and aggression in piglets. Applied Animal Behaviour Science 45, 31-42.Fisher, G.T., Volhard, W., 1985. Puppy personality profile: the specifics of testing procedures, scoring, and interpretation of the results of PAT, Purebred Dog American Kennel Club Gazette, pp. 36-42.

Foyer, P Svedberg, A-M. ; Nilsson, E et al. 2016 Behavior and cortisol responses of dogs evaluated in a standardized temperament test for military working dogs

: 4th Canine Science Forum and 1st Feline Science Forum, Lincoln, UK Journal of Veterinary Behvaior-Clinical Applications and Research   SI11   SI   7-12 Fratkin, J.L., Sinn, D.L., Patall, E.A., Gosling, S.D., 2013. Personality Consistency in Dogs: A Meta-Analysis. PLos One 8.

Gaines, S., 2008. Kennelled dog welfare - effects of housing and husbandry, PhD Thesis University of Bristol.

Gaines, S.A., Rooney, N.J., Bradshaw, J.W.S., 2008. The effect of feeding enrichment upon reported working ability and behavior of kenneled working dogs. J. Forensic Sci. 53, 1400-1404.

Gazzano, A., Mariti, C., Notari, L., Sighieri, C., McBride, E.A., 2008. Effects of early gentling and early environment on emotional development of puppies. Appl. Anim. Behav. Sci. 110, 294-304.

Goddard, M.E., Beilharz, R.G., 1982. Genetic and environmental factors affecting the suitability of dogs as Guide Dogs for the Blind. TAG Theor. Appl. Genet. 62, 97-102.

Goddard, M.E., Beilharz, R.G., 1984a. A factor analysis of fearfulness in potential guide dogs. Appl. Anim. Behav. Sci. 12, 253-265.

Goddard, M.E., Beilharz, R.G., 1984b. The relationship of fearfulness to, and the effects of, sex, age and experience on exploration and activity in dogs. Appl. Anim. Behav. Sci. 12, 267-278.

Goddard, M.E., Beilharz, R.G., 1985. A multivariate analysis of the genetics of fearfulness in potential guide dogs. Behav. Genet. 15, 69-89.

Goddard, M.E., Beilharz, R.G., 1986. Early prediction of adult behaviour in potential guide dogs. Appl. Anim. Behav. Sci. 15, 247-260.

Gottesman, I.I., Gould, T.D., 2003. The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiat. 160, 636-645.

Grandin, T., Deesing, M., 2002. Distress in animals: is it fear, pain or physical stress?, American Board of Veterinary Practitioners, Manhattan Beach, California, USA.

Grant, K.A., McMahon, C., Austin, M.P., Reilly, N., Leader, L., Ali, S., 2009. Maternal Prenatal Anxiety, Postnatal Caregiving and Infants' Cortisol Responses to the Still-Face Procedure. Devel. Psychobio. 51, 625-637.

Gray, J., 1971. The Psychology of Fear and Stress. McGraw Hill, New York.

Grissom, N., Bhatnagar, S., 2009. Habituation to repeated stress: Get used to it. Neurobiol. Learn. Mem. 92, 215-224.

Gruen, M.E., Case, B.C., Foster, M.L., Lazarowski, L., Fish, R.E., Landsberg, G., Depuy, V., Dorman, D.C., Sherman, B.L., 2015. The use of an open-field model to assess sound-induced fear and anxiety-associated behaviors in Labrador retrievers. J.Vet. Behav. 10, 338-345.

Grundy, S.A., Feldman, E., Davidson, A. , 2002. Evaluation of infertility in the bitch. Clin Tech Small Anim. Pract. 17, 108-115.

Haverbeke, A., De Smet, A., Depiereux, E., Giffroy, J.-M., Diederich, C., 2009. Assessing undesired aggression in military working dogs. Appl. Anim. Behav. Sci. 117, 55-62.

Haverbeke, A., Laporte, B., Depiereux, E., Giffroy, J.M., Diederich, C., 2008. Training methods of military dog handlers and their effects on the team's performances. Appl. Anim. Behav. Sci. 113, 110-122.

Haverbeke, A., Messaoudi, F., Depiereux, E., Stevens, M., Giffroy, J.M., Diederich, C., 2010. Efficiency of working dogs undergoing a new Human Familiarization and Training Program. J. Vet. Behav. 112-119.

Heilig, M., 2004. The NPY system in stress, anxiety and depression. Neuropeptides 38, 213-224.

Hellemans, K.G.C., Sliwowska, J.H., Verma, P., Weinberg, J., 2010. Prenatal alcohol exposure: Fetal programming and later life vulnerability to stress, depression and anxiety disorders. Neurosci.and Biobehav. R. 34, 791-807.

Hennessy, M.B., Morris, A., Linden, F., 2006. Evaluation of the effects of a socialization program in a prison on behavior and pituitary-adrenal hormone levels of shelter dogs. Appl. Anim. Behav. Sci. 99, 157-171.

Hennessy, M.B., Williams, M.T., Miller, D.D., Douglas, C.W., Voith, V.L., 1998. Influence of male and female petters on plasma cortisol and behaviour: can human interaction reduce the stress of dogs in a public animal shelter? Appl. Anim. Behav. Sci. 61, 63-77.

Hiby, E.F., Rooney, N.J., Bradshaw, J.W.S., 2004. Dog training methods: their use, effectiveness and interaction with behaviour and welfare. Anim. Welf. 13, 63-69.

Hoffmann, G., Blackshaw, J.K., Smith, G.A., 1995. Puppy tests: intercorrelations and test-retest reliability, Proceedings of the 7th International Conference of Human-Animal Interactions, Geneva.

Holden, M.D., Gregory, J., Watkins, V., Radford, L., 2006. Operant-conditioning programme for White rhinoceros, Black rhinoceros and Indian or greater one-horned Asian rhinoceros Ceratotherium simum, Diceros bicornis and Rhinoceros unicornis at Whipsnade Wild Animal Park, Dunstable, UK. International Zoo Yearbook 40, 144-149.

Horvath, Z., Igyarto, B.Z., Magyar, A., Miklosi, A., 2007. Three different coping styles in police dogs exposed to a short-term challenge. Hormones and Behavior 52, 621-630

Houpt, K.A., Hintz, H.F., 1983. Some effects of maternal-deprivation on maintenance behavior, spatial relationships and responses to environmental novelty in foals. Appl. Anim. Ethol. 9, 221-230.

Howell, T.J., Bennett, P.C., 2011. Puppy power! Using social cognition research tasks to improve socialization practices for domestic dogs (Canis familiaris). J. Vet. Behav. 6, 195-204.

Hsu, Y.Y., Serpell, J.A., 2003. Development and validation of a questionnaire for measuring behavior and temperament traits in pet dogs. JAVMA 223, 1293.

Hsu, Y.Y., Sun, L.C., 2010. Factors associated with aggressive responses in pet dogs. Appl. Animal Behav. Sci.123, 108-123.

Imanaka, A., Morinobu, S., Toki, S., Yamamoto, S., Matsuki, A., Kozuru, T., Yamawaki, S., 2008. Neonatal tactile stimulation reverses the effect of neonatal isolation on open-field and anxiety-like behavior, and pain sensitivity in male and female adult Sprague-Dawley rats. Behav. Brain Res. 186, 91-97.

Jagoe, A., Serpell, J., 1996. Owner characteristics and interactions and the prevalence of canine behaviour problems. Appl. Anim. Behav. Sci. 47, 31-42.

Jarvis, S., Moinard, C., Robson, S.K., Baxter, E., Ormandy, E., Douglas, A.J., Seckl, J.R., Russell, J.A., Lawrence, A.B., 2006. Programming the offspring of the pig by prenatal social stress: Neuroendocrine activity and behaviour. Horm. Behav. 49, 68-80.

Jensen, P., Rushen, J., Forkman, B., 1995. Behavioral strategies or just individual variation in behaviour - a lack of evidence for active and passive piglets. Applied Animal Behaviour Science 43, 135-13

Jones, A.C., Gosling, S.D., 2005. Temperament and personality in dogs (Canis familiaris): A review and evaluation of past research. Applied Animal Behaviour Science 95, 1-53.

Jones, A.C., Josephs, R.A., 2006. Interspecies hormonal interactions between man and the domestic dog (Canis familiaris). Horm. Behav. 50, 393-400.

Jordan, W.P., Strasser, H.C., 2000. Contextual control of long-term habituation in rats. J. Exp. Psychol.-Anim. Behav. Process. 26, 323-339.

Karagiannis, C.I., Burman, O., H. P., , Mills, D.S., 2015. Dogs with separation-related problems show a "less pessimistic" cognitive bias during treatment with fluoxetine (Reconcile (TM)) and a behaviour modification plan. BMC Vet. Res. 11, 1-10.

Kilgour, R., Foster, T.M., Temple, W., Matthews, L.R., Bremner, K.J., 1991. Operant technology applied to solving farm animal problems. An assessment. Appl. Anim. Behav. Sci. 30, 141-166.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., Blokhuis, H.J., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci.and Biobehav. R. 23, 925-935

Knuth ED and Etgen AM , 2007 Long-term behavioral consequences of brief, repeated neonatal isolation Brain Res., 1128: 139–147. Kutzler, M.A., 2012. Canine semen collection and management of male infertility, Fifth Congreso Latinoamericano de Emergencias y Cuidados Intensivos, Mexico.

Lambás-Señas L , Mnie-Filali O , Certin V , Faure, C., Lemoine, L. Zimmer, L., . Haddjeri H. 2009 Functional correlates for 5-HT(1A) receptors in maternally deprived rats displaying anxiety and depression-like behaviors Prog. Neuropsychopharmacol. Biol. Psychiatry, 33. 262–268

Landsberg, G.M., , Hunthausen, W.L., Ackerman, L.J., 2012. Behavior Problems of the Dog and Cat. Saunders Ltd.

Latham, N.R., Mason, G.J., 2008. Maternal deprivation and the development of stereotypic behaviour. Appl. Anim. Behav. Sci. 110, 84-108

Lefebvre, D., Diederich, C., Delcourt, M., Giffroy, J.M., 2007. The quality of the relation between handler and military dogs influences efficiency and welfare of dogs. Appl. Anim. Behav. Sci. 104, 49-60.

Leiner, L., Fendt, M., 2011. Behavioural fear and heart rate responses of horses after exposure to novel objects: Effects of habituation. Appl. Anim. Behav. Sci. 131, 104-109.

Levin, R., Nielsen, T.A., 2007. Disturbed dreaming, posttraumatic stress disorder, and affect distress: a review and neurocognitive model. Psychol. Bull.133, 482-528.

Levine, E.D., Ramos, D., Mills, D.S., 2007. A prospective study of two self-help CD based desensitization and counter-conditioning programmes with the use of Dog Appeasing Pheromone for the treatment of firework fears in dogs (Canis familiaris). Appl. Anim. Behav. Sci.105, 311-329.

Levine, S., 2000. Influence of psychological variables on the activity of the hypothalamic-pituitary-adrenal axis. Eur. J. Pharmacol. 405, 149-160.

Lin, H.-P., Lin, H.-Y., Lin, W.-L., Huang, A.C.-W., 2011. Effects of stress, depression, and their interaction on heart rate, skin conductance, finger temperature, and respiratory rate: sympathetic-parasympathetic hypothesis of stress and depression. J. Clin. Psychol. 67, 1080-1091.

Lippmann M , Bress A, . Nemeroff CB, . Plotsky PM,. Monteggia LM 2007. Long-term behavioural and molecular alterations associated with maternal separation in rats Eur. J. Neurosci., 25. 3091–3098

Lissek, S., Powers, A.S., McClure, E.B., Phelps, E.A., Woldehawariat, G., Grillon, C., Pine, D.S., 2005. Classical fear conditioning in the anxiety disorders: a meta-analysis. Behaviour Research and Therapy 43, 1391-1424.

Loftus, B.A., Rooney, N.J., Casey, R.A., 2012. Recognising fear and anxiety in dogs. http://www.bris.ac.uk/vetscience/services/behaviour-clinic/dogbehaviouralsigns/ Accessed 10 Sept 2016

Lord, M., Loftus, B., Blackwell, E.J. and Casey, R.A.in press. Environmental and general risk factors for human-directed aggression in a referral level clinical populatio. The Veterinary Record

Mackenzie, S.A., Oltenacu, E.A.B., Leighton, E., 1985. Heritability estimate for temperament scores in German Shepherd dogs and its genetic correlation with hip-displasia. Behav.Genet.15, 475-482.

Macri, S., Wurbel, H., 2007. Effects of variation in postnatal maternal environment on maternal behaviour and fear and stress responses in rats. Anim. Behav. 73, 171-184.

Macrì, S., Würbel, H., 2006. Developmental plasticity of HPA and fear responses in rats: A critical review of the maternal mediation hypothesis. Horm. Behav. 50, 667-680.

Macri, S., Wurbel, H., 2007. Effects of variation in postnatal maternal environment on maternal behaviour and fear and stress responses in rats. Anim. Behav. 73, 171-184.

Macri, S., Zoratto, F., Laviola, G., 2011. Early-stress regulates resilience, vulnerability and experimental validity in laboratory rodents through mother-offspring hormonal transfer. Neurosci.and Biobehav. R. 35, 1534-1543.Macri, S., Laviola, G., Leussis, M.P., Andersen, S.L., 2010. Abnormal behavioral and neurotrophic development in the younger sibling receiving less maternal care in a communal nursing paradigm in rats. Psychoneuroendocrino 35, 392-402.

Macri, S., Granstrem, O., Shumilina, M., dos Santos, F., Berry, A., Saso, L., Laviola, G., 2009. Resilience and vulnerability are dose-dependently related to neonatal stressors in mice. Horm. Behav. 56, 391-398.

Malinowski, K., Hallquist, N.A., Helyar, L., Sherman, A.R., Scanes, C.G., 1990. Effect of separation protocols between mares and foals on plasma cortisol and cell-mediated immune response. . J. Equine Vet. Sci. 10, 363.

Manteuffel, G., Langbein, J., Puppe, B., 2009. From operant teaming to cognitive enrichment in farm animal housing: bases and applicability. Anim. Welf. 18, 87-95.

Mariti, C., Gazzano, A., Moore, J.L., Baragli, P., Chelli, L., Sighieri, C., 2012. Perception of dogs' stress by their owners. J. Vet. Behav. 7, 213-219.

Marshall-Pescini, S., Barnard, S., Branson, N.J., Valsecchi, P., 2013. The effect of preferential paw usage on dogs' *(Canis familiaris)* performance in a manipulative problem-solving task. Behav. Process. 100, 40-43.

Masini, C.V., Day, H.E.W., Campeau, S., 2008. Long-term habituation to repeated loud noise is impaired by relatively short interstressor intervals in rats. Behav Neurosci 122, 210-223.

Massar, S.A.A., Mol, N.M., Kenemans, J.L., Baas, J.M.P., 2011. Attentional bias in high- and low-anxious individuals: evidence for threat-induced effects on engagement and disengagement. Cognition Emotion 25, 805-817.

McCall, C.A., Potter, G.D., Kreider, J.L., 1985. Locomotor, vocal and other behavioural responses to varying methods of weaning foals. Appl. Anim. Behav. Sci. 14, 27-35.

McCall, C.A., Potter, G.D., Kreider, J.L., Jenkins, W.L., 1987. Physiological responses in foals weaned by abrupt or gradual methods. J. Equine Vet. Sci. 7, 368-374.

Memon, M.A., 2007. Common causes of male dog infertility. Theriogenology, 68, 322-328.

Mendl, M., Brooks, J., Basse, C., Burman, O., Paul, E., Blackwell, E., Casey, R., 2010. Dogs showing separation-related behaviour exhibit a `pessimistic' cognitive bias. Curr.Biol. 20, R839-R840.

Meunier, L.D., 2006. Selection, acclimation, training, and preparation of dogs for the research setting. ILAR J. 47, 326-347.

Mills, D., 2005. Management of noise fears and phobias in pets. In Practice 27, 248-255.

Mills, D., Karagiannis, C., Zulch, H., 2014. Stress-Its Effects on Health and Behavior: A Guide for Practitioners. Vet. Clin. N. Am.-Small 44, 525.

Morgan, C.A., Southwick, S., Hazlett, G., Rasmusson, A., Hoyt, G., Zimolo, Z., Charney, D., 2004. Relationships among plasma dehydroepiandrosterone sulfate and cortisol levels, symptoms of dissociation, and objective performance in humans exposed to acute stress. Arch Gen Psychiatr 61, 819-825.

Mornement, K.M., Coleman, G.J., Toukhsati, S.R., Bennett, P.C., 2015. Evaluation of the predictive validity of the Behavioural Assessment for Re-homing K9's (BARK) protocol and owner satisfaction with adopted dogs. Appl. Anim. Behav. Sci. 167, 35-42.

Murphree, O.D., Dykman, R.A., 1965. Litter patterns in the offspring of nervous and stable dogs. 1. Behavioral-tests. J. Nerv. Ment. Dis. 141, 321-332.

Murphy, J.A., 1995. Assessment of the temperament of potential guide dogs. Anthrozoos 8, 224-228.

Nyhuis, T.J., Sasse, S.K., Masini, C.V., Day, H.E.W., Campeau, S., 2010. Lack of Contextual Modulation of Habituated Neuroendocrine Responses to Repeated Audiogenic Stress. Behav Neurosci 124, 810-820.

Odendaal, J.S.J., Meintjes, R.A., 2003. Neurophysiological correlates of affiliative behaviour between humans and dogs. Vet.J. 165, 296-301.

Ogata, N., Kikusui, T., Takeuchi, Y., Mori, Y., 2006. Objective measurement of fear-associated learning in dogs. J. Vet. Behav. 1, 55-61.

Parker, K.J., Buckmaster, C.L., Sundlass, K., Schatzberg, A.F., Lyons, D.M., 2006. Maternal mediation, stress inoculation, and the development of neuroendocrine stress resistance in primates. Proc. Natl. Acad. Sci. U. S. A. 103, 3000-3005.

Pfaffenberger, C.J., Scott, J.P., 1959. The relationship between delayed socialization and trainability in guide dogs. J. Genet. Psychol. 95, 145-155.

Pfaffenberger, C.J., Scott, J.P., 1976. Early rearing and testing, in: Pfaffenberger, C.J., Scott, J.P., Fuller, J.L., Ginsburg, B.E., Bielfelt, S.W. (Eds.), Guide Dogs for the Blind : Their Selection, Development, and Training, Elsevier, Amsterdam, pp. 13-37.

Pich, E.M., Agnati, L.F., Zini, I., Marrama, P., Carani, C., 1993. Neuropeptide-Y produces anxiolytic effects in spontaneously hypertensive rats. Peptides 14, 909-912.

Pluijmakers, J.J.T.M., Appleby, D.L., Bradshaw, J.W.S., 2010. Exposure to video images between 3 and 5 weeks of age decreases neophobia in domestic dogs. Appl. Anim. Behav. Sci. 126, 51-58.

Podberscek, A.L., Gosling, S.D., 2000. Personality research on pets and their owners: Conceptual issues and review, in: Podberscek, A.L., Paul, E.S., Serpell, J.A. (Eds.), Companion Animals and Us: Exploring the Relationships between People and Pets, Cambridge University Press, Cambridge, pp. 143-167.

Podberscek, A.L., Serpell, J.A., 1997. Aggressive behaviour in English cocker spaniels and the personality of their owners. Vet. Rec. 141, 73-76.

Poore, K.R., Boullin, J.P., Cleal, J.K., Newman, J.P., Noakes, D.E., Hanson, M.A., Green, L.R., 2010. Sex- and age-specific effects of nutrition in early gestation and early postnatal life on hypothalamo-pituitary-adrenal axis and sympathoadrenal function in adult sheep. J. Physiol.-London 588, 2219-2237.

Reefmann, N., Kaszas, F.B., Wechsler, B., Gygax, L., 2009a. Physiological expression of emotional reactions in sheep. Physiol. Behav. 98, 235-241.

Reefmann, N., Wechsler, B., Gygax, L., 2009b. Behavioural and physiological assessment of positive and negative emotion in sheep. Anim. Behav. 78, 651-659.

Rooney, N., Bradshaw, J.W.S., Almey, H.A., 2002. Specialist Serach Dogs: An inter-agency review and critique of selection, Report to DERA.

Rooney, N.J. (2012). Welfare of racing greyhounds – prioritisation of issues. Poster presented at Universities Federation for Animal Welfare Portsmouth UK.

Rooney, N., Bradshaw, J.W.S., Gaines, S., 2003. Investigation into the effects of rearing conditions on the ability of specialist search dogs, Report to Defense Science and Technology Laboratory, pp. 1-229.

Rooney, N.J., Almney H.A., Bradshaw, J.W.S. 2004. Attributes of specialist search dogs - a questionnaire survey of UK dog handlers and trainers. Journal of Forensic Sciences. 49(2) 300-306.

Rooney, N.J., Morant, S., Guest, C. 2013. Investigation into the Value of Trained Glycaemia Alert Dogs to Clients with Type I Diabetes. PLoS ONE 8(8):e69921. doi:10.1371/journal.pone.0069921.

Rooney, N., Gaines, S., Hiby, E., 2009. A practitioner's guide to working dog welfare. J. Vet. Behav. 4, 127-134.

Rooney, N.J., Cowan, S., 2011. Training methods and owner-dog interactions: Links with dog behaviour and learning ability. Appl. Anim. Behav. Sci. 132, 169-177.

Rooney, N.J., Paul, E.S., Haig-Ferguson, A.J., Bradshaw, J.W.S., 2007a. Human factors affecting the performance of dog-handler teams, Report to Dstl.

Rooney, N.J., Gaines, S.A., Bradshaw, J.W.S., 2007b. Behavioural and glucocorticoid responses of dogs (Canis familiaris) to kennelling: Investigating mitigation of stress by prior habituation. Physiol. Behav. 92, 847-854.

Sankey, C., Richard-Yris, M.A., Henry, S., Fureix, C., Nassur, F., Hausberger, M., 2010. Reinforcement as a mediator of the perception of humans by horses (Equus caballus). Animal Cogn. 13, 753-764.

Savastano, G., Hanson, A., McCann, C., 2003. The Development of an Operant Conditioning Training Program for New World Primates at the Bronx Zoo. J. Appl. Anim. Welf. Sci. 6, 247-261.

Schilder, M.B.H., van der Borg, J.A.M., 2004. Training dogs with help of the shock collar: short and long term behavioural effects. Appl. Anim. Behav. Sci. 85, 319-334.

Scott, J.P., Bielfelt, S.W., 1976. Analysis of the puppy testing program, in: Pfaffenberger, C.J., Scott, J.P., Fuller, J.L., Ginsburg, B.E., Bielfelt, S.W. (Eds.), Guide Dogs for the Blind : Their Selection, Development, and Training, Elsevier, Amsterdam, pp. 39-76.

Scott, J.P., Fuller, J.L., 1965. Genetics and the Social Behavior of the Dog. University of Chicago Press: Chicago.

Seksel, K., Mazurski, E.J., Taylor, A., 1999. Puppy socialisation programs: short and long term behavioural effects. Appl. Anim. Behav. Sci. 62, 335-349.

Serpell, J.A., Hsu, Y.Y., 2001. Development and validation of a novel method for evaluating behavior and temperament in guide dogs. Appl. Anim. Behav. Sci. 72, 347-364.

Sherman, B.L., Mills, D.S., 2008. Canine anxieties and phobias: An update on separation anxiety and noise aversions. Vet. Clin. N. Am.-Small 38, 1081-1106.

Sherman, B.L., Gruen, M.E., Case, B.C., Foster, M.L., Fish, R.E., Lazarowski, L., DePuy, V., Dorman, D.C., 2015. A test for the evaluation of emotional reactivity in Labrador retrievers used for explosives detection. J. Vet. Behav. 10, 94-102.

Shors, T.J., 2004. Learning during stressful times. Learn Mem 11, 137-144.

Silver, R.C., Holman, E.A., McIntosh, D.N., Poulin, M., Gil-Rivas, V., 2002. Nationwide longitudinal study of psychological responses to September 11. Jama-Journal of the American Medical Association 288, 1235-1244.

Siniscalchi, M., Bertino, D., Quaranta, A., 2014. Laterality and performance of agility-trained dogs. Laterality 19, 219-234.

Sinn, D.L., Gosling, S.D., Hilliard, S., 2010. Personality and performance in military working dogs: Reliability and predictive validity of behavioral tests. Appl. Anim. Behav. Sci. 127, 51-65.

Slabbert, J.M., Odendaal, J.S.J., 1999. Early prediction of adult police dog efficiency—a longitudinal study. Appl. Anim. Behav. Sci.64, 269-288.

Slabbert, J.M., Rasa, O.A.E., 1993. The effect of early separation from the mother on pups in bonding to humans and pup health. Journal of the South African Veterinary Association-Tydskrif Van Die Suid-Afrikaanse Veterinere Vereniging 64, 4-8.

Slabbert, J.M., Rasa, O.A.E., 1997. Observational learning of an acquired maternal behaviour pattern by working dog pups: an alternative training method? Appl. Anim. Behav. Sci. 53, 309-316.

Strong, V., Brown, S.W., Walker, R., 1999. Seizure-alert dogs - fact or fiction? Seizure 8, 62-65.

Svartberg, K., Forkman, B., 2002. Personality traits in the domestic dog (Canis familiaris). Applied Animal Behaviour Science 79, 133-155.

Svobodová, I., Vápeník, P., Pinc, L., Bartos, L., 2008. Testing german shepherd puppies to assess their chances of certification. Appl. Anim. Behav. Sci.113, 139-149.

Tami, G., Gallagher, A., 2009. Description of the behaviour of domestic dog (Canis familiaris) by experienced and inexperienced people. Appl. Anim. Behav. Sci. 120, 159-169.

Tata, D.A., Markostamou, I., Ioannidis, A., Gkioka,M., . Simeonidou, C., Anogianakis, G., . Spandou, E. 2015. Effects of maternal separation on behavior and brain damage in adult rats exposed to neonatal hypoxia-ischemia Behav. Brain Res., 280, 51–61

Taylor, K.D., Mills, D.S., 2006. The development and assessment of temperament tests for adult companion dogs. J. Vet. Behav.h 1, 94-108.

Terlouw, E.M.C., Schouton, W.G.P., Ladewig, P., 1997. Physiology, in: Appleby, M.C., Hughes, B.O. (Eds.), Anim. Welf., CAB International, Wallingford.

Thomas, S., 2011. Development and Epigenetics - The New Nature-Nurture Discussion, Defining, Developing and Documenting Success in Working Dogs, Pearl River, New York.

T

Tuber, D.S., Sanders, S., Hennessy, M.B., Miller, J.A., 1996. Behavioral and glucocorticoid responses of adult domestic dogs (Canis familiaris) to companionship and social separation. J. Comp. Psychol.110, 103-108.

Vincent, I.C., Leahy, R.A., 1997. Real-time non-invasive measurement of heart rate inworking dogs: a technique with potential applications in the objective assessment of welfare problems. Vet. J. 153, 179-183.

Walker, J., Waran, N., Phillips, C., 2014. The effect of conspecific removal on the behaviour and physiology of pair-housed shelter dogs. Appl. Anim. Behav. Sci. 158, 46-56.

Wells, D.L., Hepper, P.G., 2006. Prenatal olfactory learning in the domestic dog. Anim.B ehav.72, 681-686.

Willis, M.B., 1995. Genetic aspects of dog behaviour with particular reference to working ability, in: Serpell, J. (Ed.), The Domestic Dog: Its Evolution, Behaviour, and Interactions with People, Cambridge University Press, Cambridge, pp. 51-64.

Wilson, D.S., Clark, A.B., Coleman, K., Dearstyne, T., 1994. Shyness and boldness in humans and other animals. Trends Ecol. Evol. 9, 442-446.

Wilsson E., Sinn, D.L. , 2012   Are there differences between bahaviral measurement methods? A comparison of predictive validity of two rating methods in a working dog program

Appl. Anim. Behav. Sci. 141(3-4), 158-172.

Wilsson, E., Sundgren, P.-E., 1997. The use of a behaviour test for the selection of dogs for service and breeding, I: Method of testing and evaluating test results in the adult dog, demands on different kinds of service dogs, sex and breed differences. Appl. Anim. Behav. Sci 53, 279-295.

Yehuda, R., Flory, J.D., Southwick, S., Charney, D.S., 2006a. Developing an agenda for translational studies of resilience and vulnerability following trauma exposure. Annals of the New York Academy of Sciences 1071 (Psychobiology of Posttraumatic Stress Disorder: A Decade of Progress), 379-396.

Yehuda, R., Brand, S., Yang, R.K., 2006b. Plasma neuropeptide y concentrations in combat exposed veterans: Relationship to trauma exposure, recovery from PTSD, and coping. Biol. Psychiat 59, 660-663.