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PERSPECTIVES ON ANIMAL BIOSCIENCES

Future application of an attention bias test to assess affective states in sheep

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ABSTRACT

The affective states of animals comprise a key aspect of welfare that can be difficult to assess. An attention-bias test was developed for sheep, which assessed allocation of attention between a predator threat and a food reward, as a potential measure of affective state. The method was pharmacologically validated as a measure of anxiety-like states, finding that 'anxious' sheep were more vigilant, less likely to feed and spent more time looking towards the previous location of a dog than did 'calm' sheep. Across six further validation studies, the method was modified and explored as a measure of other types of affective states. This perspective article aims to provide guidance on what the method can tell us about affective state and make recommendations for further research by using this approach. Evidence was strongest across the studies for the test as a measure of anxiety-like states, but it is clear that there are other factors affecting animal behaviour during testing that need to be further investigated. One study showed potential for a modified method to assess depression-like states in sheep, while the impact of chronic stress on affect and attention bias remains unclear. It is likely that the test cannot be used to measure positive affect in sheep without further modification, due to the fear-eliciting nature of the test. Versions of the method using food as a positive stimulus allow for a clearer interpretation of attention than do versions using a conspecific photograph, and are recommended for use in future studies where appetite is not expected to be a confounding factor. In this context, vigilance behaviour may indicate trait anxiety or fearfulness, while other measures of attention may be more sensitive to transient changes in affect. Modifications to the method are suggested to allow for a clearer characterisation of attention in livestock species and to improve the practical application of the test. Overall, the attention-bias test shows promise as a measure of negative affective states, but the method is still very new and further research is needed to better determine its potential use as a welfare-assessment tool.

Keywords: anxiety, behaviour, cognitive bias, depression, euphoria, fear, livestock, Merino, ruminant, sheep, threat, welfare.

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Introduction

Consumers, producers, industry bodies and regulators are demanding greater standards of welfare in livestock industries (Australian Government 2008; Kauppinen *et al*. 2010; Red Meat Advisory Council Ltd 2015). A key component of welfare that needs to be considered is the emotional or affective states of animals. The term 'affect' describes an animal's physiological and behavioural responses, that can vary in terms of intensity (arousal) and pleasantness or unpleasantness (valence) (Mendl *et al*. 2010). This can include short-term emotions that are triggered by specific events as well as longer-term moods. It is important that tools are available for researchers and producers to assess and benchmark the affective states of animals as part of a comprehensive welfare assessment.

One approach taken to measure affective states in animals is the assessment of affect-driven attention biases. An attention bias describes the tendency to process certain types of information before others, which can be altered by the affective state (Bar-Haim *et al*. 2007).

In humans, predictable changes in attention towards certain types of stimuli have been used to determine affective states and the presence of clinical affective disorders. For example, increased attention to threats is attributed to anxious states and generalised anxiety disorder (Bradley *et al.* 1995, 1997; Bar-Haim *et al.* 2007; Cisler and Koster 2010).

Several studies have explored the potential for attention bias to provide a measure of affect in livestock (Crump *et al.* 2018). The methodologies used have ranged in their complexity and the degree of animal training required. Lee *et al.* (2016) presented a rapid 'looking time' task to assess attention bias in sheep, which may have more practical applications for welfare assessment on farm. A considerable number of studies have since been conducted to refine and further validate this methodology in sheep and to adapt the method for use in other livestock species. There is now an opportunity to collate these studies and critically examine the potential of this approach to assess affect in livestock.

The aim of this perspective article is to provide guidance on the following question: 'which version of the attention-bias test methodology should I use and what will it tell me?' To address this question, we summarise the literature that used variations of the test methodology described by Lee *et al.* (2016) in sheep, to examine its potential use as a practical measure of affect. The findings of each study are tabulated for a clear comparison of treated- and control-animal responses. We then make recommendations for future application of the methodology in welfare research and highlight key gaps that need to be addressed moving forward.

Attention bias in livestock

Crump *et al.* (2018) provided a comprehensive overview of the methods used in animals to assess affect-driven attention biases. One approach is the use of eye-tracking and looking-time tasks that measure fixation of the gaze on competing images of emotional stimuli (Hermans *et al.* 1999; Eizenman *et al.* 2003; Kellough *et al.* 2008). Looking-time tasks have been applied to primates for the assessment of attention biases, using methodologies similar to those used in humans (e.g. Bethell *et al.* 2012; Howarth *et al.* 2021). Both Vögeli *et al.* (2015) and Raoult and Gygax (2018) developed looking-time tasks for sheep to assess attention, where attention towards valenced video stimuli was determined on the basis of head orientation, ear postures and frontal brain activity assessed using functional near-infrared spectroscopy. These approaches showed promise for assessing attention bias, but requirements to confine or habituate sheep limits the practical application of the test for the purpose of welfare assessment.

Another approach used to assess attention bias in non-human animals includes foraging or threat perception tasks. Brilot and Bateson (2012) assessed attention bias in starlings, by measuring the extent to which birds were distracted from feeding by the sound of a conspecific alarm call. Key behaviours included vigilance (head up) and latency to feed after the alarm call. This approach has also been applied to other bird species including parrots (Cussen and Mench 2014) and chickens (Campbell *et al.* 2019*a*, 2019*b*, 2022; Anderson *et al.* 2021).

The attention-bias task developed for sheep by Lee *et al.* (2016) sat somewhere between the looking time and foraging tasks described above. Sheep were tested in a novel arena, where they were exposed to a threat (a live dog sitting quietly behind a window) for a period of 10 s. The window was then covered, and the dog was removed, then the sheep stayed in the test arena for a further 3 min. Attention was assessed by measuring duration looking towards the previous location of the dog, vigilance behaviour defined by having the head at or above shoulder height, and latency to eat from a familiar feed bowl containing pellets that was located centrally within the arena. Lee *et al.* (2016) pharmacologically validated the method using anxiolytic and anxiogenic drugs, finding that 'Anxious' sheep spent more time looking towards the dog, were more vigilant and had a longer latency to eat than did 'Calm' animals. Thus, the authors showed that the test could be used to measure biases in attention towards a threat, which were related to anxiety-like states in sheep.

Eight studies have been conducted using the attention-bias test method described by Lee *et al.* (2016), or variations thereof, that applied pharmacological or environmental treatments to sheep prior to testing. Seven of these studies were conducted on Merino sheep at the same research station in Armidale, New South Wales (NSW), Australia, with the key results summarised in Table 1. The studies used sheep of varying ages ranging from 5 months to 7 years old and have used both male and female sheep. Overall, these studies have modelled chronic stress through environmental and pharmacological manipulation and have used pharmacological manipulations that attempted to model anxious, calm, depressed and euphoric-like states. Modifications made to the method over time included reducing the period of exposure to the threat and using a photograph of a conspecific in place of feed as a positive stimulus, to remove the potentially confounding effect of appetite on sheep responses. Hereafter, we broadly refer to methods using food as a positive stimulus as 'the food method' and methods using a conspecific photograph as the 'photograph method'. The repeatability of the food method has been assessed in sheep (Monk *et al.* 2023). The food method has also been adapted to present a human as the threatening stimulus instead of a dog (Atkinson *et al.* 2022). Variations of the methodology have also been applied to cattle (Lee *et al.* 2018; Kremer *et al.* 2021), pigs (Luo *et al.* 2019; Verbeek *et al.* 2021), goats (Neave and Zobel 2020) and chickens (Campbell *et al.* 2019*a*, 2019*b*, 2022; Anderson *et al.* 2021), although this review will primarily

Table 1. Validation studies using variations of the attention-bias test for sheep described by Lee *et al.* (2016).

Study	Age (sex)	Positive stimulus	Test duration (s)		Treatment	*n*	Findings relative to controls				Study conclusions
			Dog	No dog			Look at dog	Look at positive	Vigilant	Eat/sniff latency	
Verbeek *et al.* (2019)	1 year (female)	Pelleted ration in familiar bowl	30	180	Chronic stress (lying deprivation)	15	=	=	↓#	↓#	Lying deprivation caused reduced vigilance and increased attention to feed, suggesting a more positive state after chronic stress
Monk *et al.* (2019*a*)	1.5 years (female)	Pelleted ration in familiar bowl	30	180	Chronic stress (ACTH; 0.5 mg i.m. daily for 22 days)	14	=	na	=	=	Cortisol response alone may not explain previously observed changes in behaviour after lying deprivation
Lee *et al.* (2016)	2 years (female)	Pelleted ration in familiar bowl	10	180	Anxiogenic (mCPP; 2 mg/kg i.m.)[A]	20	↑*	na	=	=	The test method was sensitive to changes in anxious states, consistent with human literature
					Anxiolytic (diazepam; 0.1 mg/kg i.v.)[B]	20	↓*	na	=	↓*	
Monk *et al.* (2018*a*)	5 months (male)	Lucerne hay	3	180	Anxiogenic (mCPP; 2 mg/kg i.m.)[A]	20	=	na	↑*	↑*	The test could be shortened to 45 s and the habituation period to the feed bowl removed
					Anxiolytic (diazepam; 0.1 mg/kg i.v.)[B]	20	↓*	na	=	=	
	1 year (male)	Lucerne hay	3	180	Presentation of dog vs empty window	20	=	na	↑*	↑*	The dog was perceived as a threat; window movement alone also captured attention
Monk *et al.* (2018*b*)	2.5 months (female)	Photograph of a conspecific	3	180	Anxiogenic (mCPP; 2 mg/kg i.m.)[A]	16	↓*	↑^	↑*	↑^	The modified test was sensitive to and distinguished the negative states; a different interpretation of behaviour was required for the new method
					Depressant (pCPA; 20 mg/kg i.p. twice daily)	16	↑*	↓^	↑*	↑*	
Monk *et al.* (2019*b*)	7 years (female)	Photograph of a conspecific	3	180	Anxiogenic (mCPP; 1.5 mg/kg i.m.)[A]	20	↓^	=	=	↑*	The test was not sensitive to changes in positive states, however this may have been due to confounding factors
					Anxiolytic (diazepam; 0.1 mg/kg i.v.)[B]	20	↓^	=	=	=	
					Euphorigenic (morphine; 1 mg/kg i.m.)[C]	20	=	=	=	=	
Monk *et al.* (2020)	1 year (female)	Photograph of a conspecific	3	180	Anxiogenic (mCPP; 1.5 mg/kg i.m.)[A]	20	=	=	↑*	↑^	The test was not sensitive to changes in positive states
					Anxiolytic (diazepam; 0.1 mg/kg i.v.)[B]	20	=	=	=	=	
					Euphorigenic (morphine; 1 mg/kg i.m.)[C]	20	=	=	=	=	

The table summarises observed differences for key behavioural responses in each test, relative to control animals. Arrows (↑,↓) indicate the direction in which a treatment group containing *n* animals was different from the control animals, where the *P*-value was reported as <0.05 (*) or between 0.05 and 0.1 (^), or where models fitting treatment performed better than a model fitting the intercept only (#). The '=' denotes no difference between treatment and control groups, 'na' indicates the behaviour was not measured. Look at dog or positive: duration looking towards the dog window or positive stimulus. Vigilant: duration with the head at or above shoulder height. Eat/sniff latency: latency to eat food or sniff the photo. Footnotes (A, B, C) are used to group the same treatments used across studies. All studies used Merino sheep.

focus on sheep. Notably, other studies have used similar methodologies, such as a fear test developed for dairy cattle by Welp *et al.* (2004) and an emotionality test for sheep developed by Torres-Hernandez and Hohenboken (1979).

Comparison of study findings

It is clear from the studies presented in Table 1 that induction of some affective states has an impact on sheep behaviour during the attention-bias test. Differences among induced affective-state groups were often strong in the attention-bias studies, but the effects relative to control groups were sometimes inconsistent. There are also some important differences in the methodologies that need to be considered. Here, we examine the results of the studies to discuss the merits and limitations of this approach for measuring attention bias in sheep and its potential ability to measure different types of affective states.

Anxiety-like states

The most consistent findings across the studies shown in Table 1 support the method as an indicator of anxiety-like states in sheep. In three studies, treatment with the drug diazepam to decrease anxiety (anxiolytic) resulted in decreased attention to the dog window relative to control sheep. Use of the drug meta-chlorophenylpiperazine (mCPP) to increase anxiety (anxiogenic) resulted in an increased latency to eat or sniff the photograph in four studies and increased vigilance in three studies, relative to control animals. The effect of mCPP on attention bias was also replicated in cattle (Lee *et al.* 2018) and chickens (Campbell *et al.* 2019*b*). However, the differences between induced affective-state groups and control animals were sometimes inconsistent (Table 1). For example, Lee *et al.* (2016) reported a significant effect of mCPP on the duration looking towards the dog window, which was not replicated by Monk *et al.* (2018*a*). In contrast, Monk *et al.* (2018*a*) showed that sheep treated with mCPP spent significantly more time displaying vigilance behaviour than did control animals, but this effect was not observed by Lee *et al.* (2016). Overall, the test shows promise as a measure of anxious states in livestock, while highlighting behavioural variation that is not explained by the drug treatments alone. Examples of other sources of variation that might affect behaviour include the variable effect of drugs on individuals, variation in the animals' moods prior to treatment and testing, individuals' previous experiences or other aspects of animal temperament or personality.

Positive affect

Sheep treated with morphine to induce a euphoric-like state did not display an attention bias toward or away from the threat or a conspecific photograph presumed to be perceived as positive during testing (Monk *et al.* 2019*b*, 2020). This could suggest that morphine did not model positive affect or that the test could not discriminate positive affect induced by morphine. The effect of morphine on sheep behaviour in a food-based attention-bias test has not been examined. Sheep treated with the anxiolytic drug diazepam were often labelled as being in a calm-like state, which might be considered a positively valenced state. However, due to the nature of the attention-bias test involving social isolation and novelty, we propose it is likely that all animals tested were in a relatively high arousal, negatively valenced state, irrespective of their assigned pharmacological treatment. The drugs expected to induce positively valenced states may have been partially or completely over-ridden by the emotional response sheep have to isolation in the novel test environment and the threat of predation. As such, the 'Calm' sheep may have been less anxious than those in the 'Anxious' groups, but were not necessarily in a calm state (Fig. 1). The approach may still be useful as a measure of positive affect in species, breeds or individuals for which isolation is a less aversive stressor, or where the stress associated with testing and isolation is reduced through modification of the method or arena, as discussed further in the section 'Refinement of the test arena'. However, in its current form, we do not believe that this method can provide a measure of true positive affect in sheep.

Other negative states

Monk *et al.* (2018*b*) observed a significant effect of the depressant drug para-chlorophenylalanine (pCPA) on attention bias to threat, suggesting that the test may be sensitive to depressive-like states. Both pCPA and mCPP, inducing depressive-like and anxiety-like states respectively, resulted in increased vigilance compared with control animals. It was therefore proposed that, in the context of the test methodology, increased vigilance provides a measure of negative valence, which is consistent with the other attention-bias studies in sheep and cattle (Lee *et al.* 2018; Monk *et al.* 2018*a*, 2018*b*). Negative valence was also expected to result in increased attention towards the threat, which was the case for the depressed treatment group; however, the anxious treatment group unexpectedly spent less time looking towards the threat than did control animals. This finding contrasts with studies using the food method, where anxious sheep showed increased attention towards the threat. However, the tendency for anxious sheep to pay less attention towards the threat than for control animals using a conspecific photograph was also supported by Monk *et al.* (2019*b*). It was suggested that increased attention to the conspecific photo as a social stimulus aligned with the strong flocking instinct of sheep when faced with the threat of predation (Lynch *et al.* 1992). Together, this highlights the importance of considering a range of behavioural responses during testing to gain a more complete picture of affect and the need to carefully validate

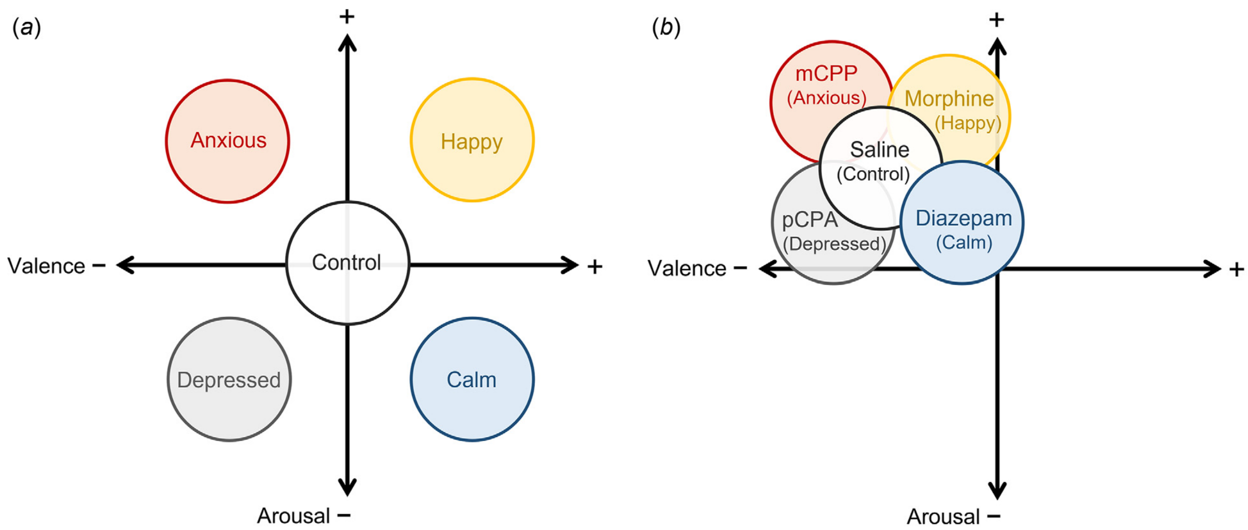

Fig. 1. Diagram depicting the (*a*) intended and (*b*) potential positions of pharmacologically treated sheep in the affective space, delineated by axes of valence and arousal. Positions depicted in (*b*) are not intended to be accurate, but rather to exemplify the potential mismatch between intended and actual affective outcomes due to environmental stressors or other external factors.

methods using different stimuli as attention biases are highly context specific. While the results of Monk *et al.* (2018*b*) are promising, further validation is needed using other models of depression in the attention-bias test.

Chronic stress induced through an environmental model using lying deprivation was found to reduce vigilance and result in a quicker approach to a feed reward, in contrast with the authors' expectations (Verbeek *et al.* 2019). A similar unexpected 'positive' response has also been observed in judgement-bias tasks following stress in sheep (Doyle *et al.* 2010; Sanger *et al.* 2011; Guldimann *et al.* 2015). Potential explanations for this included release from stressful conditions generating a positive mood or a general increase in motivation for rewarding stimuli under chronic stress conditions. Monk *et al.* (2019*a*) modelled chronic stress by using a pharmacological model, by administering synthetic adrenocorticotropic hormone to induce an exogenous stress response, which had no impact on attention bias compared with control animals (Table 1). The pharmacological model used by Monk *et al.* (2019*a*) suggested that the attention bias observed in sheep exposed to lying deprivation may not be explained by cortisol response alone. Together, the findings of Verbeek *et al.* (2019) and Monk *et al.* (2019*a*) suggested that the test may not be sensitive to changes in affective state resulting from induced chronic stress and that further work is needed to understand the effect of chronic stress on affective state in livestock.

Finally, Atkinson *et al.* (2022) adapted the method to assess attention to a human threat, by swapping the dog for a human, but otherwise following the protocol outlined by Monk *et al.* (2018*a*). Prior to testing, they applied two different treatments involving different types of human–animal interactions over a period of 7 weeks, with the aim to reduce human-directed fear in weaned lambs through either habituation (low intensity, predictable human behaviour) or stress inoculation (moderate intensity, active, unpredictable human behaviour). Neither intervention was shown to affect attention bias towards the human. It is difficult to determine what type of affective state may have been induced by the human exposure treatments. No pharmacological validation studies have been applied to a method using a human as the threatening stimulus.

Limitations of affective-state models

There is currently no way to directly measure affect in another living being, so there is no gold standard to which we can compare when validating new methods and models. Instead, measures and models can be incrementally validated against each other by drawing on human literature and by comparing a range of environmental and pharmacological models against a range of behavioural, physiological and neurological indicators. Environmental manipulations can be used to alter affective state in a way that more closely matches natural conditions. However, it can be unclear which affective states are being induced and the induced affective states are not always maintained during behavioural testing (Doyle *et al.* 2010; Sanger *et al.* 2011). Pharmacological models have an advantage as they can remain active during testing, be applied in a standardised manner and be easily paired with appropriate controls such as saline injections (Doyle *et al.* 2015). However, they are generally targeted towards a limited number of neurophysiological pathways and may not reflect naturally occurring affective states. They can also have unwanted side effects, such as the abnormal behaviours observed in sheep treated with mCPP, including head, tail and body shaking or ataxia (Doyle *et al.* 2015; Monk *et al.* 2018*a*). A relatively small number of studies have been conducted to find appropriate pharmacological models of affective states in livestock species and there is also often limited information available on the pharmacokinetic pathways of drug models. Further studies are required to understand the appropriate drugs, dose rates and dosing schedules for pharmacological models to have the desired outcome and to reduce unwanted side effects. While there are limitations for both environmental and pharmacological affective-state models, each can provide valuable information and a variety of models should be used to validate new welfare assessment methods.

Trait versus state affect

To determine how best to interpret and apply an attention-bias test, it is important to understand to what extent it is affected by emotions, moods or trait affect. Emotions are short-term states triggered by specific events, while moods occur over a longer time frame and are less context specific (Mendl *et al.* 2010; Kremer *et al.* 2020). Trait affect describes the propensity of an animal to experience a particular affective state, as an aspect of animal personality (Boissy and Erhard 2014), where personality traits are patterns of behavioural responses that are consistent across time and/or contexts (Réale *et al.* 2007). Measures of emotions may be best applied in research to determine the immediate impact of certain events or environments on welfare. Measures of moods may be applied in both research and on-farm welfare assessment to measure the cumulative effect of recent events on an animal's affective state. Measures of trait affect have the potential to be applied as a selection tool to identify animals with a less anxious or fearful personality. If the test is readily confounded by factors such as noise or weather, its practical application for welfare assessment may be limited.

Although the attention-bias studies have confirmed that the attention-bias test is to some extent influenced by affective state (Table 1), the extent to which it is affected by emotions, moods, personality and other factors remains unclear. Across three repetitions of an attention-bias test, Monk *et al.* (2023) showed consistency of vigilance behaviour in adult ewes by using the food method presented by Monk *et al.* (2018*a*). In cattle, Kremer *et al.* (2021) observed relationships between vigilance in an attention-bias test and a fearfulness personality trait that was characterised on the basis of behaviours across an open-field, novel object and runway test. Lee *et al.* (2018) demonstrated a tendency for more nervous cattle, as measured through flight speed and crush score, to show increased vigilance in an attention-bias test. Together these findings suggest that vigilance behaviour in the attention-bias test may be strongly driven by an underlying trait or aspect of personality, and thus may be considered to indicate the propensity of an animal to experience negative affect. However, further studies are still needed to examine the implications of increased vigilance during attention-bias testing for welfare outcomes more broadly.

Similar relationships were found between personality and attention to the threat during an attention-bias test in cattle (Lee *et al.* 2018) and consistency was observed in putatively 'fearful' and 'attentive' personality traits derived across two repetitions of an attention-bias test in goats (Neave and Zobel 2020). In cattle, Kremer *et al.* (2021) observed some relationships between attention to the threat and personality traits, as well as consistency in feeding behaviour between two repetitions of an attention-bias test. However, they did not observe consistency in threat-directed behaviours across the test repetitions and Monk *et al.* (2023) observed poor repeatability of looking and feeding behaviours over three repeated attention-bias tests in sheep. The inconsistency observed across repeated tests suggests that looking and feeding behaviours may be more strongly driven by transient affective states or other undetermined factors. Verbeek *et al.* (2019) demonstrated a positive response during attention-bias testing following a lying deprivation treatment, with reduced vigilance and latency to eat, which may suggest that the test is more sensitive to short-term emotions after release from a stressful condition, rather than a negative mood that the condition was expected to induce. However, further studies are needed to confirm this suggestion and to rule out other potential effects such as an increased motivation for rewarding stimuli (Verbeek *et al.* 2019).

Overall, these studies suggest that the attention-bias test is not only state-sensitive, but may also indicate trait affect, in a behaviour-dependent manner and in the absence of treatments that modulate affective state. Consideration of vigilance, looking and feeding behaviours independently may provide information on both trait and state affect within a single test. Importantly, however, it is likely that emotions, moods and personality interact and work together in some way to shape the responses of sheep during testing. Further studies should aim to manipulate emotion and mood independently, prior to attention-bias testing, so as to determine which of these aspects of affective state most strongly drive animal responses in the test.

Methodological considerations

Choosing the threat

Attention biases are highly context specific and so the choice of threatening stimulus should be carefully considered (Zvielli *et al.* 2014; Pergamin-Hight *et al.* 2015). In all the studies listed in Table 1, brief exposure to a predator threat (dog) was used as a threatening stimulus. Removal of the threat after a short time served two purposes. The first was to reduce the intensity of the threat that might otherwise prevent the animals from displaying attention towards other stimuli or the environment. The presence of a live dog in other behavioural tests is shown to be highly aversive for sheep, reducing and even eliminating the occurrence of exploratory behaviours (Torres-Hernandez and Hohenboken 1979; Beausoleil *et al.* 2005). The second was to remove the actual threat to the sheep, so that we could examine anxious states rather than fear states. The behavioural and physiological responses of fear and anxiety are largely the same but differ in the context of an actual versus an unknown threat respectively (Steimer 2002).

Brief exposure to a live dog has also been used as a threatening stimulus in an attention-bias test for both steers (Lee *et al.* 2018) and goats (Neave and Zobel 2020). While shown to be effective, the use of a live dog introduces more variation during testing, given the challenge of standardising the dog's behaviour. For dairy calves, Kremer *et al.* (2021) used a dog statue in conjunction with the scent of dog urine and audio of a dog growling as a threatening stimulus. Although they did not validate before testing that the dog model was perceived as threatening by heifers, behaviours displayed during the test were consistent with it being perceived as threatening. In pigs, Luo *et al.* (2019) used a combined visual and auditory threat of a squeaky door moving up and down to show a flashing light for 10 s, while Verbeek *et al.* (2021) used a 15 s audio recording of an aggressive dog barking. Other attention-bias test paradigms for sheep have shown variable success using video images (Raoult and Gygax 2018) and acoustic stimuli (Raoult and Gygax 2019) to represent predator threats and conspecifics. Other potential threats might include an air puff such as used by Salvin *et al.* (2020) in a startle test for sheep, or startling movements such as the opening of an umbrella (Coulon *et al.* 2011; Neave and Zobel 2020).

Alternatively, a human could be used as a threatening stimulus in sheep that are not accustomed to human handling. Atkinson *et al.* (2022) applied an attention-bias test to sheep using a human as the threat. The test was unable to differentiate sheep that had undergone different levels of human exposure to induce habituation or stress-inoculation, although it remains unclear whether this was due to a lack of sensitivity of the attention-bias test or the treatments not having the desired outcome on human-directed fear. Humans and even human-like models have been used as a fear-eliciting stimulus in behavioural tests such as the arena test, which induces conflict between approaching humans and conspecifics (e.g. Vandenheede and Bouissou 1994; Bouissou and Vandenheede 1995; Forkman *et al.* 2007) and are shown to be less aversive to sheep than dogs (Beausoleil *et al.* 2005). Importantly, the attention-bias test paradigm is known to be context specific, and the response that an individual sheep has to a dog threat may not be comparable to their response to a human. The induction of negative affect due to human interaction is something many producers may want to measure and reduce, either through improved management and environment or selective breeding programs. Conversely, increased attention and vigilance towards predator threats may be desirable in many extensive production environments where predators represent an actual danger to sheep (Dwyer 2009). Thus, in a context-specific test paradigm such as the attention-bias test, it is important to consider the production context and reasons for measuring attention and vigilance when choosing a threat.

Choosing a positive stimulus

To be able to categorise how the individuals in the test arena divide their attention, an alternative positive attractant can be used alongside the threatening stimulus. Most attention-bias tests in sheep have used food as the positive stimulus (Table 1, Atkinson *et al.* 2022), where measures such as duration eating and latency to eat are used to indicate attention. Other studies have used conspecific photographs (Table 1), videos of conspecifics (Vögeli *et al.* 2015; Raoult and Gygax 2018) or audio of sheep bleating (Raoult and Gygax 2019), where attention is measured through behaviours such as looking, vigilance and ear postures. As acute stress responses typically involve allocation of resources away from non-essential functions such as feeding behaviour (Sherwood *et al.* 2005), using food as a positive stimulus provides a clear contrast against the predator threat. However, this contrast may become less clear depending on the testing context and the level of hunger experienced by an individual. Fraser and Duncan (1998) described how negative affect evolves from a 'need situation' where action is required for survival or reproductive success. In contrast, positive affect evolves from an 'opportunity situation' where performance of certain pleasurable behaviours such as play occur only when the cost of performing such behaviours is low. Feeding has the potential to fall under either category depending on the context.

Across the attention-bias studies using food, Lee *et al.* (2016) provided sheep with *ad libitum* access to pasture overnight prior to testing, while others withheld or limited access to food (Monk *et al.* 2018*a*, 2023; Atkinson *et al.* 2022). Given that feeding behaviour may arise from either a 'need' or 'opportunity' situation, relating to negative or positive affective states respectively (Fraser and Duncan 1998), it follows that the clearest interpretation of feeding behaviour as a contrast against the threat of predation would occur when the test sheep are not hungry. However, a complete absence of hunger may reduce the likelihood that sheep are willing to feed during the test, thus increasing the number of animals that fail to eat and limiting the ability of this measure to distinguish individuals. This may have been the case for Lee *et al.* (2016) where 85% of the control sheep failed to eat during the test, although other factors could have also contributed to a lack of feeding. Finding the right balance between hunger and satiation prior to testing may be difficult and presents a potential avenue for further validation of the test. Identifying feeds that are most rewarding and palatable for any given species may also be useful to increase positive interest in food during testing.

It is also important to control variation in appetite within a cohort during testing. Across all the attention-bias studies, sheep were housed in yards without feed while attention-bias testing was undertaken, which may have resulted in increased hunger over the course of the day. Atkinson *et al.* (2022) attempted to account for this by providing a half ration overnight to sheep that would be tested in the afternoon, while sheep tested in the morning were fasted. It is recommended that a similar approach is adopted for all further research using the food method to standardise hunger as much as possible across the cohort being tested.

Monk *et al.* (2018*b*) changed the positive stimulus from a food reward to a photograph of a conspecific to remove the potential influence of appetite on behaviour during testing. This removed appetite as a confounding factor, but sheep no longer had a strong incentive to become non-vigilant to feed. Additionally, a shift in attention towards a social stimulus represents an important strategy for sheep to cope with the threat of predation through flocking behaviour (Dwyer 2004; Wemelsfelder and Farish 2004). Thus, duration looking towards the positive stimulus alone may not be enough to discriminate positive affiliative motivations from flocking behaviour and therefore may not indicate the valence of the affective state of an animal being tested without also considering other behavioural responses. Overall, it is suggested that using food as a positive stimulus allows for a clearer interpretation of behaviour than does the photograph method and is the preferred approach in a context where appetite is not expected to confound results.

It is also important to consider the sensory capabilities of a species when selecting any stimulus, whether it is positive or negative. When selecting models, videos and photographs, researchers should consider the visual acuity of the target species and their abilities to perceive colour, luminance, depth and motion (Winters *et al.* 2015). Likewise, auditory and olfactory capabilities must be considered when using sounds and scents. Photographs or models of conspecifics and threats may not always be perceived by sheep in the expected way. For example, Franklin and Hutson (1982) found that the use of a taxidermy sheep as an attractant was unsuccessful as test sheep showed fear responses to the taxidermy model rather than affiliative responses. Together, these findings once again have highlighted the need to carefully validate the stimuli used for attention-bias tests.

Stimulus duration and intensity

The attention-bias methods listed in Table 1 present two stimuli, which are presumed to have contrasting emotional valence qualities, with the dog being perceived negatively and either food or a conspecific photograph being perceived positively. Other studies of attention bias in livestock have described a necessity to balance dually presented stimuli with regards to their presentation times and intensities, such that the stimuli differ only in emotional valence (Raoult and Gygax 2019). The stimulus presentation times used in the attention-bias test developed by Lee *et al.* (2016) are not balanced between the positive and negative stimuli, nor have they been in any variation of the method used thereafter. Further, it is difficult to determine and balance the intensity of a threatening stimulus when compared with a feed reward. In the context of this test paradigm, an attention bias is interpreted as increased attention towards a given stimulus relative to other tested animals. This is opposed to increased attention towards a given stimulus relative to other the stimuli presented. We argue that by comparing behavioural responses among and not within individuals, balancing of the positive and negative stimulus durations and intensity is not essential, so long as the test remains consistent for all tested animals in a population. Nevertheless, the presentation of stimuli for different durations has the potential to introduce new confounding factors that may affect animal responses. For example, spatial memory or learning may confound animal responses if a test subject no longer associates the previous location of the dog with the threat of a dog and does not localise their attention accordingly. This potentially confounding factor is important to consider when using pharmacological models that may have an impact on spatial memory or learning, as may be the case for diazepam (Brioni and Arolfo 1992; Sasaki-Hamada *et al.* 2013). It may be worthwhile exploring options to balance stimulus presentation times in the attention-bias test in a way that does not increase the intensity of the threat, such as by using a photograph of a dog instead of or in conjunction with a live dog.

Quantifying attention

To measure a bias in attention, we first need to be able to accurately quantify attention, which can be difficult in livestock species. Measures such as vigilance defined by having the head at or above shoulder height and latency to feed provide a very crude measure of attention compared with the eye-tracking studies used in humans and primates. A key problem with removing the visual threat of a dog after a short period of time is that sheep can no longer localise their gaze towards the threatening stimulus itself, only to the last known location of the threat. While some attention-bias studies have shown that this approach can be effective (Lee *et al.* 2016; Monk *et al.* 2018*b*), the use of a threatening stimulus that remains visually present for the entire test duration would be likely to allow for a clearer characterisation of visual attention. Importantly, however, in the presence of an actual threat, the test may be considered as a measure of fearful rather than anxious states. Consideration is needed as to whether this distinction is functionally important, given that studies in humans tend to focus on anxiety states with regards to attention bias, rather than fear. The test duration must also be carefully considered as the animals' responses may become extinct as the threat is not further reinforced throughout the test period (Erhard *et al.* 2006).

Irrespective of the stimuli used, measuring direction of looking with binocular vision in a species with a wide visual field may not effectively characterise direction of attention. Expanding the definition of attention to incorporate other sensory modalities, and adjusting the stimuli presented accordingly, may help determine to which stimuli sheep are allocating their attention with a greater accuracy. Auditory stimuli have been used in an attention-bias test for sheep developed by Raoult and Gygax (2019). In their study, the direction of attention towards contrasting audio stimuli was determined by the orientation of the head while a sheep was restrained; however, to be considered attentive, sheep also needed to have their heads up in an alert position and their ears in a non-passive posture (i.e. the ears were both forward, both backward or asymmetrical). Incorporation of ear posture to the ethogram used in the attention-bias test could help better define direction of attention and may also give an indication of the affective state itself (Reefmann *et al.* 2009; Boissy *et al.* 2011; Lee *et al.* 2018). The collection of ear postures may be more meaningful if auditory cues were used instead of or in addition to the visual stimuli presented during testing. However, observations of ear postures is a labour-intensive and time-consuming process which would limit the practical application of the method, unless using an automated tracking system such as the one developed for sheep by Vögeli *et al.* (2014). Overall, modifications to the ethogram and stimuli, alongside the use of automated ear- and/or gaze-tracking technologies, may help to more clearly characterise attention in sheep and make the test more practical to apply.

Modifications to the attention-bias test arena may also allow for a clearer assessment of the direction in which attention is being directed. The original method positioned food in a way that allowed sheep to continue looking in the direction of the threat while feeding. Further, during the observation of video footage, the authors anecdotally noticed that the sheep may be remaining alert and attentive to their surroundings while their heads are lowered to a non-vigilant position and even while they are feeding. To prevent this from occurring, the food could be positioned against the wall opposite the threatening stimulus, where the photograph was positioned during later studies, or following a design similar to that used by Kremer *et al.* (2020) where the food was positioned in the corner of the test arena. Alternatively, a small visual barrier could be created between the food and the threatening stimulus so that sheep cannot remain visually attentive towards the threat while feeding or becoming non-vigilant. This approach was taken by Welp *et al.* (2004) when measuring vigilance towards a human in dairy cattle. Importantly, however, as a more fearful species, removing the ability of sheep to remain somewhat vigilant while feeding could reduce the number of sheep that are willing to feed during testing and may therefore limit the ability of the test to detect affective states.

Refinement of the test arena

A number of modifications could be made to the test arena and method to improve its practical application, standardisation and interpretation. The first is to have sheep enter through a narrow chute rather than a large gate used in the current method, to standardise the angle at which sheep enter the arena and, consequently, the angle and time that they see the stimuli. The second is to explore options for the automation of behavioural analysis using on-animal sensors or video-analysis software, as manual video observations are a time-consuming and labour-intensive process that may limit the test's application to larger populations of animals. The third is to modify the test method or arena to allow attention bias to be assessed using existing handling or housing facilities on-farm. Currently, the time and equipment required to conduct attention-bias testing are likely to limit its application to research settings. Together, automation of behavioural annotation and adapting the method to existing handling facilities might allow the method to be applied as a welfare assessment tool on-farm.

Modifications to the method that reduce the fear-eliciting nature of the test environment and isolation may allow for a clearer assessment of attention and potentially allow for assessment of positive affect without the confounding effects of fear and stress. This could be undertaken by adapting the test to existing housing facilities if applicable to the production system, or by using habituation periods to reduce the stress caused by being in a novel environment; however, the latter will reduce the practical application of the test. Live conspecifics could be introduced to reduce the effect of social isolation on a test animal. However, the test arena would need to be carefully designed so that the conspecifics are not also exposed to the threatening stimulus, to reduce the potential effect of social contagion (Salvin *et al.* 2020). Alternatively, it may be useful to have sheep spend a short period of time in the test arena to get a baseline of behaviour prior to exposure to the valenced stimuli. Rather than remove the fear-eliciting elements of the test, this may allow researchers to account for individual variation in fearfulness as a covariate in the analysis. This could be standardised as a set time period (Verbeek *et al.* 2021) or could be based on feeding behaviour, whereby the sheep is exposed to a threat only after eating, following a design similar to that used in starlings (Brilot and Bateson 2012) or goats (Neave and Zobel 2020); however, the latter design would exclude any sheep that are not willing to feed in the novel environment. Due to the highly context-specific nature of attention biases, further modifications to the method or stimuli used during attention-bias testing should be carefully validated, taking into consideration the basic ethology of the species being tested.

Concluding remarks: which version should I use and what will it tell me?

The attention-bias test method is still new and further research is needed to properly answer this question. However, on the basis of the discussion above, we can make some recommendations moving forward. It is suggested that measures of interest in food more clearly represent a shift in attention away from the threat of predation than do methods that use a conspecific stimulus. Thus, in the absence of treatments that have a large influence on appetite, we recommend using the methodology presented by Monk *et al*. (2018*a*) or a variation thereof, that uses food as the positive stimulus instead of a photograph. Hunger should be standardised across the cohort being tested as best as possible. We believe that in this context, vigilance behaviour can provide an indication of trait anxiety, fearfulness or negative affect more broadly, while other measures of attention such as looking duration and feeding may be more sensitive to transient changes in anxiety-like states. The method does not appear to be appropriate for measuring positive affect in a prey species such as sheep, without further modifications to the method or arena. Changes in mean behavioural responses were evident across repeated attention-bias tests in sheep, as the sheep habituated to the novel test environment (Monk *et al*. 2023). Therefore, it is suggested that all animals being tested should have the same prior experience with the attention-bias test, to ensure a valid comparison of individual responses. Overall, the attention-bias test provides another valuable tool for researchers to better understand the impact that management practices and the environment have on livestock welfare.

However, it is important to note here that the method has been applied only to Merino sheep raised under similar conditions, and that all but one study have been conducted on the same research station, using the same or similar dogs as a threatening stimulus. Further studies are still needed to explore the relative influence of emotions, moods and personality on animal responses in differing populations and contexts, to enable a clearer interpretation of behaviour during the attention-bias test. The specific effects of age and sex have not been examined and there is currently not enough data available to draw meaningful conclusions on the potential impact that these factors have on attention bias. Modifications to the ethogram or test arena discussed throughout this review could be made to more clearly characterise the direction of attention towards the chosen stimuli during testing, and to automate the collection of behavioural data for a more practical application of the test. There is also a need to further validate the pharmacological models used across these studies to ascertain their effect on affect in sheep and other non-human animals.

References

Anderson MG, Campbell AM, Crump A, Arnott G, Newberry RC, Jacobs L (2021) Effect of environmental complexity and stocking density on fear and anxiety in broiler chickens. *Animals* **11**, 2383. doi:10.3390/ani11082383

Atkinson L, Doyle RE, Woodward A, Jongman EC (2022) Exposure to humans after weaning does not reduce the behavioural reactivity of extensively reared Merino lambs. *Behavioural Processes* **201**, 104709. doi:10.1016/j.beproc.2022.104709

Australian Government (2008) 'The Australian animal welfare strategy.' Revised edn. (Australian Government)

Bar-Haim Y, Lamy D, Pergamin L, Bakermans-Kranenburg MJ, van IJzendoorn MH (2007) Threat-related attentional bias in anxious and nonanxious individuals: a meta-analytic study. *Psychological Bulletin* **133**, 1–24. doi:10.1037/0033-2909.133.1.1

Beausoleil NJ, Stafford KJ, Mellor DJ (2005) Sheep show more aversion to a dog than to a human in an arena test. *Applied Animal Behaviour Science* **91**, 219–232. doi:10.1016/j.applanim.2004.10.008

Bethell EJ, Holmes A, MacLarnon A, Semple S (2012) Evidence that emotion mediates social attention in rhesus macaques. *PLoS ONE* **7**, e44387. doi:10.1371/journal.pone.0044387

Boissy A, Erhard HW (2014) How studying interactions between animal emotions, cognition, and personality can contribute to improve farm animal welfare. In 'Genetics and the behavior of domestic animals'. (Eds T Grandin, MJ Deesing) pp. 81–113. (Elsevier) doi:10.1016/B978-0-12-394586-0.00003-2

Boissy A, Aubert A, Désiré L, Greiveldinger L, Delval E, Veissier I (2011) Cognitive sciences to relate ear postures to emotions in sheep. *Animal Welfare* **20**, 47–56. doi:10.1017/S0962728600002426

Bouissou MF, Vandenheede M (1995) Fear reactions of domestic sheep confronted with either a human or a human-like model. *Behavioural Processes* **34**, 81–92. doi:10.1016/0376-6357(94)00056-M

Bradley BP, Mogg K, Millar N, White J (1995) Selective processing of negative information: effects of clinical anxiety, concurrent depression, and awareness. *Journal of Abnormal Psychology* **104**, 532–536. doi:10.1037/0021-843X.104.3.532

Bradley BP, Mogg K, Lee SC (1997) Attentional biases for negative information in induced and naturally occurring dysphoria. *Behaviour Research and Therapy* **35**, 911–927. doi:10.1016/S0005-7967(97)00053-3

Brilot BO, Bateson M (2012) Water bathing alters threat perception in starlings. *Biology Letters* **8**, 379–381. doi:10.1098/rsbl.2011.1200

Brioni JD, Arolfo MP (1992) Diazepam impairs retention of spatial information without affecting retrieval or cue learning. *Pharmacology Biochemistry and Behavior* **41**, 1–5. doi:10.1016/0091-3057(92)90050-P

Campbell DLM, Dickson EJ, Lee C (2019*a*) Application of open field, tonic immobility, and attention bias tests to hens with different ranging patterns. *PeerJ* **7**, e8122. doi:10.7717/peerj.8122

Campbell DLM, Taylor PS, Hernandez CE, Stewart M, Belson S, Lee C (2019*b*) An attention bias test to assess anxiety states in laying hens. *PeerJ* **7**, e7303. doi:10.7717/peerj.7303

Campbell AM, Johnson AM, Persia ME, Jacobs L (2022) Effects of housing system on anxiety, chronic stress, fear, and immune function in Bovan brown laying hens. *Animals* **12**(14), 1803. doi:10.3390/ani12141803

Cisler JM, Koster EHW (2010) Mechanisms of attentional biases towards threat in anxiety disorders: an integrative review. *Clinical Psychology Review* **30**, 203–216. doi:10.1016/j.cpr.2009.11.003

Coulon M, Hild S, Schroeer A, Janczak AM, Zanella AJ (2011) Gentle vs. aversive handling of pregnant ewes: II. Physiology and behavior of the lambs. *Physiology & Behavior* **103**, 575–584. doi:10.1016/j.physbeh.2011.04.010

Crump A, Arnott G, Bethell E (2018) Affect-driven attention biases as animal welfare indicators: review and methods. *Animals* **8**, 136. doi:10.3390/ani8080136

Cussen VA, Mench JA (2014) Personality predicts cognitive bias in captive psittacines, *Amazona amazonica*. *Animal Behaviour* **89**, 123–130. doi:10.1016/j.anbehav.2013.12.022

Doyle RE, Fisher AD, Hinch GN, Boissy A, Lee C (2010) Release from restraint generates a positive judgement bias in sheep. *Applied Animal Behaviour Science* **122**, 28–34. doi:10.1016/j.applanim.2009.11.003

Doyle RE, Lee C, McGill DM, Mendl M (2015) Evaluating pharmacological models of high and low anxiety in sheep. *PeerJ* **3**, e1510. doi:10.7717/peerj.1510

Dwyer CM (2004) How has the risk of predation shaped the behavioural responses of sheep to fear and distress? *Animal Welfare* **13**, 269–281. doi:10.1017/S0962728600028384

Dwyer CM (2009) Welfare of sheep: providing for welfare in an extensive environment. *Small Ruminant Research* **86**, 14–21. doi:10.1016/j.smallrumres.2009.09.010

Eizenman M, Yu LH, Grupp L, Eizenman E, Ellenbogen M, Gemar M, Levitan RD (2003) A naturalistic visual scanning approach to assess selective attention in major depressive disorder. *Psychiatry Research* **118**, 117–128. doi:10.1016/S0165-1781(03)00068-4

Erhard HW, Elston DA, Davidson GC (2006) Habituation and extinction in an approach–avoidance test: an example with sheep. *Applied Animal Behaviour Science* **99**, 132–144. doi:10.1016/j.applanim.2005.10.008

Forkman B, Boissy A, Meunier-Salaün M-C, Canali E, Jones RB (2007) A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology & Behavior* **92**, 340–374. doi:10.1016/j.physbeh.2007.03.016

Franklin JR, Hutson GD (1982) Experiments on attracting sheep to move along a laneway. III. Visual stimuli. *Applied Animal Ethology* **8**, 457–478. doi:10.1016/0304-3762(82)90059-1

Fraser D, Duncan IJH (1998) 'Pleasures','pains' and animal welfare: toward a natural history of affect. *Animal Welfare* **7**(4), 383–396. doi:10.1017/S0962728600020935

Guldimann K, Vögeli S, Wolf M, Wechsler B, Gygax L (2015) Frontal brain deactivation during a non-verbal cognitive judgement bias test in sheep. *Brain and Cognition* **93**, 35–41. doi:10.1016/j.bandc.2014.11.004

Hermans D, Vansteenwegen D, Eelen P (1999) Eye movement registration as a continuous index of attention deployment: data from a group of spider anxious students. *Cognition & Emotion* **13**, 419–434. doi:10.1080/026999399379249

Howarth ERI, Kemp C, Thatcher HR, Szott ID, Farningham D, Witham CL, Holmes A, Semple S, Bethell EJ (2021) Developing and validating attention bias tools for assessing trait and state affect in animals: a worked example with *Macaca mulatta*. *Applied Animal Behaviour Science* **234**, 105198. doi:10.1016/j.applanim.2020.105198

Kauppinen T, Vainio A, Valros A, Rita H, Vesala KM (2010) Improving animal welfare: qualitative and quantitative methodology in the study of farmers' attitudes. *Animal Welfare* **19**, 523–536. doi:10.1017/S0962728600001998

Kellough JL, Beevers CG, Ellis AJ, Wells TT (2008) Time course of selective attention in clinically depressed young adults: an eye tracking study. *Behaviour Research and Therapy* **46**, 1238–1243. doi:10.1016/j.brat.2008.07.004

Kremer L, Klein Holkenborg SEJ, Reimert I, Bolhuis JE, Webb LE (2020) The nuts and bolts of animal emotion. *Neuroscience & Biobehavioral Reviews* **113**, 273–286. doi:10.1016/j.neubiorev.2020.01.028

Kremer L, Bus JD, Webb LE, Bokkers EAM, Engel B, van der Werf JTN, Schnabel SK, van Reenen CG (2021) Housing and personality effects on judgement and attention biases in dairy cows. *Scientific Reports* **11**, 22984. doi:10.1038/s41598-021-01843-w

Lee C, Verbeek E, Doyle R, Bateson M (2016) Attention bias to threat indicates anxiety differences in sheep. *Biology Letters* **12**, 20150977. doi:10.1098/rsbl.2015.0977

Lee C, Cafe LM, Robinson SL, Doyle RE, Lea JM, Small AH, Colditz IG (2018) Anxiety influences attention bias but not flight speed and crush score in beef cattle. *Applied Animal Behaviour Science* **205**, 210–215. doi:10.1016/j.applanim.2017.11.003

Luo L, Reimert I, de Haas EN, Kemp B, Bolhuis JE (2019) Effects of early and later life environmental enrichment and personality on attention bias in pigs (*Sus scrofa domesticus*). *Animal Cognition* **22**, 959–972. doi:10.1007/s10071-019-01287-w

Lynch JJ, Hinch GN, Adams DB (1992) 'The behaviour of sheep: biological principles and implications for production.' (CSIRO: Melbourne, Vic., Australia)

Mendl M, Burman OHP, Paul ES (2010) An integrative and functional framework for the study of animal emotion and mood. *Proceedings of the Royal Society B: Biological Sciences* **277**, 2895–2904. doi:10.1098/rspb.2010.0303

Monk JE, Doyle RE, Colditz IG, Belson S, Cronin GM, Lee C (2018*a*) Towards a more practical attention bias test to assess affective state in sheep. *PLoS ONE* **13**, e0190404. doi:10.1371/journal.pone.0190404

Monk JE, Belson S, Colditz IG, Lee C (2018*b*) Attention bias test differentiates anxiety and depression in sheep. *Frontiers in Behavioral Neuroscience* **12**, 246. doi:10.3389/fnbeh.2018.00246

Monk JE, Belson S, Lee C (2019*a*) Pharmacologically-induced stress has minimal impact on judgement and attention biases in sheep. *Scientific Reports* **9**, 11446. doi:10.1038/s41598-019-47691-7

Monk JE, Lee C, Belson S, Colditz IG, Campbell DLM (2019*b*) The influence of pharmacologically-induced affective states on attention bias in sheep. *PeerJ* **7**, e7033. doi:10.7717/peerj.7033

Monk JE, Lee C, Dickson E, Campbell DLM (2020) Attention bias test measures negative but not positive affect in sheep: a replication study. *Animals* **10**, 1314. doi:10.3390/ani10081314

Monk JE, Colditz IG, Clark S, Lee C (2023) Repeatability of an attention bias test for sheep suggests variable influence of state and trait affect on behaviour. *PeerJ* **11**, e14730. doi:10.7717/peerj.14730

Neave HW, Zobel G (2020) Personality of dairy goats affects competitive feeding behaviour at different feeder heights. *Small Ruminant Research* **192**, 106222. doi:10.1016/j.smallrumres.2020.106222

Pergamin-Hight L, Naim R, Bakermans-Kranenburg MJ, van IJzendoorn MH, Bar-Haim Y (2015) Content specificity of attention bias to threat in anxiety disorders: A meta-analysis. *Clinical Psychology Review* **35**, 10–18. doi:10.1016/j.cpr.2014.10.005

Raoult C, Gygax L (2018) Valence and intensity of video stimuli of dogs and conspecifics in sheep: approach–avoidance, operant response, and attention. *Animals* **8**, 121. doi:10.3390/ani8070121

Raoult CMC, Gygax L (2019) Mood induction alters attention toward negative–positive stimulus pairs in sheep. *Scientific Reports* **9**, 7759. doi:10.1038/s41598-019-44330-z

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. *Biological Reviews* **82**, 291–318. doi:10.1111/j.1469-185X.2007.00010.x

Red Meat Advisory Council Ltd (2015) Meat industry strategic plan MISP 2020. Red Meat Advisory Council, Canberra, ACT, Australia.

Reefmann N, Bütikofer Kaszàs F, Wechsler B, Gygax L (2009) Ear and tail postures as indicators of emotional valence in sheep. *Applied Animal Behaviour Science* **118**, 199–207. doi:10.1016/j.applanim.2009.02.013

Salvin H, Cafe L, Lees A, Morris S, Lee C (2020) A novel protocol to measure startle magnitude in sheep. *Applied Animal Behaviour Science* **228**, 104996. doi:10.1016/j.applanim.2020.104996

Sanger ME, Doyle RE, Hinch GN, Lee C (2011) Sheep exhibit a positive judgement bias and stress-induced hyperthermia following shearing. *Applied Animal Behaviour Science* **131**, 94–103. doi:10.1016/j.applanim.2011.02.001

Sasaki-Hamada S, Sacai H, Sugiyama A, Iijima T, Saitoh A, Inagaki M, Yamada M, Oka J-I (2013) Riluzole does not affect hippocampal synaptic plasticity and spatial memory, which are impaired by diazepam in rats. *Journal of Pharmacological Sciences* **122**, 232–236. doi:10.1254/jphs.13052SC

Sherwood L, Klandorf H, Yancey PH (2005) 'Animal physiology: from genes to organisms.' (Thomson/Brooks/Cole: Australia)

Steimer T (2002) The biology of fear- and anxiety-related behaviors. *Dialogues in Clinical Neuroscience* **4**, 231–249. doi:10.31887/DCNS.2002.4.3/tsteimer

Torres-Hernandez G, Hohenboken W (1979) An attempt to assess traits of emotionality in crossbred ewes. *Applied Animal Ethology* **5**, 71–83. doi:10.1016/0304-3762(79)90008-7

Vandenheede M, Bouissou MF (1994) Fear reactions of ewes to photographic images. *Behavioural Processes* **32**, 17–28. doi:10.1016/0376-6357(94)90024-8

Verbeek E, Colditz I, Blache D, Lee C (2019) Chronic stress influences attentional and judgement bias and the activity of the HPA axis in sheep. *PLoS ONE* **14**, e0211363. doi:10.1371/journal.pone.0211363

Verbeek E, Dicksved J, Keeling L (2021) Supplementation of *Lactobacillus* early in life alters attention bias to threat in piglets. *Scientific Reports* **11**, 10130. doi:10.1038/s41598-021-89560-2

Vögeli S, Wechsler B, Gygax L (2014) Welfare by the ear: comparing relative durations and frequencies of ear postures by using an automated tracking system in sheep. *Animal Welfare* **23**, 267–274. doi:10.7120/09627286.23.3.267

Vögeli S, Wolf M, Wechsler B, Gygax L (2015) Housing conditions influence cortical and behavioural reactions of sheep in response to videos showing social interactions of different valence. *Behavioural Brain Research* **284**, 69–76. doi:10.1016/j.bbr.2015.02.007

Welp T, Rushen J, Kramer DL, Festa-Bianchet M, de Passillé AMB (2004) Vigilance as a measure of fear in dairy cattle. *Applied Animal Behaviour Science* **87**, 1–13. doi:10.1016/j.applanim.2003.12.013

Wemelsfelder F, Farish M (2004) Qualitative categories for the interpretation of sheep welfare: a review. *Animal Welfare* **13**, 261–268. doi:10.1017/S0962728600028372

Winters S, Dubuc C, Higham JP (2015) Perspectives: the looking time experimental paradigm in studies of animal visual perception and cognition. *Ethology* **121**, 625–640. doi:10.1111/eth.12378

Zvielli A, Bernstein A, Koster EHW (2014) Dynamics of attentional bias to threat in anxious adults: bias towards and/or away? *PLoS ONE* **9**, e104025. doi:10.1371/journal.pone.0104025

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