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Performance of sheep in a spatial maze is impeded by negative stimuli

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ABSTRACT

Exposure to negative stimuli or stress can manifest in general changes in cognitive processing. This study aimed to investigate if a spatial maze task could be used to identify stress-induced differences in the cognitive performance of sheep. Two negative stimuli were used to test the hypothesis. For a negative pre-treatment ('dog' pre-treatment), sheep were moved individually to a holding yard at the beginning of the maze where they were exposed to a dog for 3 min, for 5 consecutive days. Alternative to the dog pre-treatment, sheep were moved in small groups to the same holding yard, for the same amount of time, where they received a feed reward ('food' pre-treatment). For a during-test stimulus, white noise was played as sheep moved through the maze ('noise' treatment). Sixty-four male castrated lambs were allocated to one of four groups: dog and noise, food and noise, dog and no noise, or food and no noise. Sheep traversed the maze on 3 consecutive days and the total time to complete the maze, the number and the duration of errors made were used to assess cognitive performance. Maze results were analysed using GLMM, LMM and linear contrasts. The noise increased both total time (140 s vs. 105 s, $P = 0.043$) and error time (67 s vs. 56 s, $P = 0.044$) on day 1. The dog pre-treatment increased error time compared to the food pre-treatment (81 s vs. 41 s, $P = 0.041$) and tended to increase the number of errors made on day 1 (1.5 errors vs. 1.2 errors, $P = 0.057$). Neither noise nor dog pre-treatment influenced cognitive performance on days 2 or 3. Results suggest that both stimuli affected cognitive performance in the maze by impeding initial problem solving. The maze used demonstrates the ability to identify differences in cognition.

1. Introduction

Cognition and affective state are closely intertwined. Cognition can influence the formation of affective states (Scherer, 2001; Desiré et al., 2002), and certain affective states can alter the processing of information (Paul et al., 2005). Exposure to negative stimuli or stress can also manifest in general changes in cognitive processing, affecting problem solving, learning and memory (for a detailed review see Mendl, 1999). Research in recent years has focused on identifying these changes in cognition in relation to affective states, and these tests have the ability to assess an animal's welfare.

Much animal welfare research is currently focused on developing tools to measure appraisal (how an animal perceives a certain stimulus; examples in sheep, Desiré et al., 2004, 2006; Greiveldinger et al., 2007; Deiss et al., 2009; Greiveldinger et al., 2009), and the influence affective states have on the interpretation of information (cognitive bias; examples include rodents, Harding et al., 2004; birds, Bateson and Matheson, 2007; sheep, Doyle et al., 2010; invertebrates, Bateson et al., 2011; dogs, Burman et al.,

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2011; primates, Bethell et al., 2012). These methods are particularly valuable at detecting more intricate details of an animal's affective state, as they have the potential to measure the valence of an animal's affective state.

Tests for emotional reactivity and cognitive bias can also be complex to interpret (for review see Mendl et al., 2009). In addition, these complex tests take significant time to train and test, and importantly, some current methods to test for cognitive bias cannot be applied to all individuals [e.g. in sheep (Doyle et al., 2011) and dogs (Muller et al., 2012)], leading to the exclusion of animals from tests. This potentially skews results, as animals may fail to complete training tasks because of factors like reduced coping ability or temperament-inhibiting learning (Pascual-Alonso et al., 2013). Based on this, having standardised tests that assess the effects of environmental stimuli on general cognition can be a valuable tool to measure affective state.

General cognitive changes in response to stress can occur for different reasons. For example, in times of stress animals move into a more automatic method of processing information rather than using cognitive control. As a result, their behaviour becomes more rigid and inflexible, preventing them from solving novel tasks effectively (Toates, 2002, 2006). An animal's attention may also be drawn from a task as the result of cognitive overload from either a large number of stimuli presented at once, or a particularly arousing stimulus. This attentional shifting can result in poorer task performance (Dukas and Kamil, 2000, 2001; Lavie, 2005; Shettleworth, 2010).

Both of these influential factors can affect problem solving, the process of learning, and memory formation and recall. For example, social isolation and environmental stressors negatively impact on the cognitive performance of rats in different tasks (Sandstrom and Hart, 2005; Harris et al., 2010; Alliger and Moller, 2011). The memory recall of pigs in a spatial cognition task was reduced by stressful factors including social isolation, exposure to an unfamiliar environment, and unpredictable events (Mendl et al., 1997). Laughlin et al. (1999) supported these results in an associated study, with the authors suggesting that this reduction in performance was the result of a deficit in attention rather than inhibition of memory retrieval. The performance of goats in a visual discrimination task was reduced after relocation to a new environment (Langbein et al., 2006). Cattle seemed to be unable to learn a reversal task when it was associated with a restraint stressor, and authors also noted that the calmer animals may have been more able to make accurate choices (Grandin et al., 1994). Calves displaying a greater level of fear following social isolation had poorer cognitive performance (Lensink et al., 2006). Perceived stressors are also enough to alter cognitive performance. Cattle were more distracted from feeding when they perceived a situation to be more threatening (Welp et al., 2004). Similarly, the grazing behaviour of sheep became increasingly interrupted with increased perceived risk of predation (Dumont and Boissy, 2000).

The aim of the current study was to investigate if a spatial task could be used to identify stress-induced differences in the cognitive performance of sheep. A spatial maze task designed by Lee and colleagues (2006) was used in the current study. It has previously been validated to assess spatial learning and memory, requires no prior training and relies on the innate flocking behaviours of sheep. Two stimuli were used to elicit a negative state in the sheep. The 'dog' pre-treatment was delivered in the 5 days prior to the maze task with the aim of inducing a negative affective state prior to the commencement of the maze task. The second was a novel auditory stimulus, white noise ('noise'). The novelty of a stimulus is a key component when forming an emotional response to it, and negative emotions like fear, anxiety and displeasure are associated with unfamiliar stimuli (Scherer, 2001; Desiré et al., 2002). In support of this, stress-related behavioural and physiological responses to novelty have been demonstrated in sheep (Desiré et al., 2006), and more specifically, white noise can generate an increased heart rate in naive sheep (Ames and Arehart, 1972). With this in mind, it was hypothesised that the noise stimulus would impede task performance more than the dog pre-treatment, as the sheep would be exposed to it while performing the task. Some sheep were exposed to both stimuli, and it was hypothesised that these animals would display the poorest cognitive performance.

2. Methods

The Charles Sturt University Animal Care and Ethics Committee, in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, approved all procedures in this experiment (protocol number 10/096).

2.1. Animals

Sixty-four castrated male lambs (6 months old Merino × Border Leicester) were used for the experiment. Sheep were housed outdoors in groups of five to six sheep per pen for the 2-week duration of the experiment. Sheep were fed a ration of mixed grain at a rate to maintain growth with lucerne hay and water provided ad libitum. All animals were habituated to the feed and housing conditions for 3 days before the experiment commenced.

2.2. Maze design

The maze used for the experiment (Fig. 1) was adapted from a previous study in sheep (Lee et al., 2006). The maze was 20 m × 9 m, with two error zones (EZ) and an additional holding yard, and was assembled in a large paddock out of visual and auditory range of other pen mates. The exterior walls were opaque and the internal walls were made from temporary fencing panels (ProWay Livestock Equipment, Bomen, NSW, Australia), allowing the sheep to see through to the end of the maze. Four conspecifics familiar to the test sheep were penned at the end of the maze. This encouraged the test sheep to move through the maze by engaging innate flocking behaviours.

2.3. Stimuli

Sheep were randomly allocated to either a dog pre-treatment group ($n = 31$), or food pre-treatment ($n = 33$).

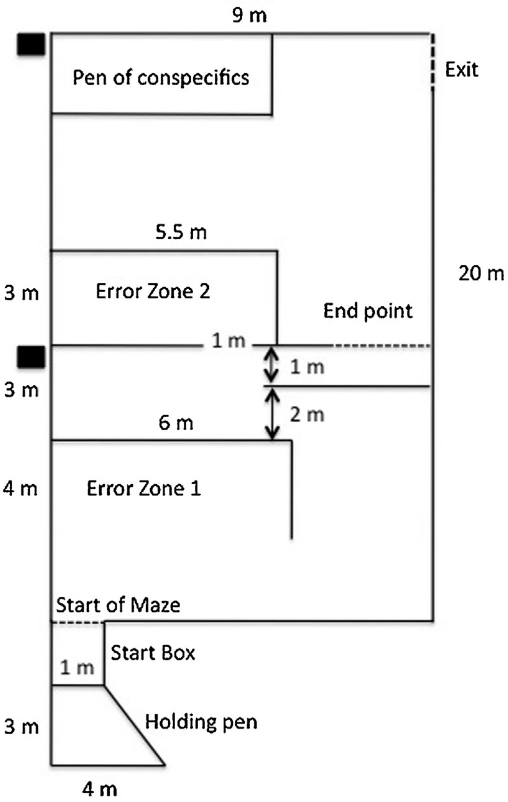

Fig. 1. Diagram of the spatial maze. ■ Indicates locations of speakers.

Following this, sheep were allocated to receive the auditory stimulus during the test or not in a 2 × 2 factorial design. The numbers for each group were as follows: dog and noise = 15 sheep, dog and no noise = 16 sheep, food and noise = 17, food and no noise = 16.

The dog pre-treatment involved moving the sheep individually from their home pen to the holding yard at the beginning of the maze where they were exposed to a dog (standing silently) and a noise recording of barking dogs for 3 min. This was repeated on 5 consecutive days. An audio recording of barking dogs was used to ensure each sheep received the same frequency and duration of the stimulus. The barking dog audio was recorded using the Voice Memos application (Apple iPhone iOS 4, Cupertino, United States of America), and was played at a maximum volume of 85 Hz through portable speakers (JBL, Northridge, United States of America). The 'food' pre-treatment group were moved in small groups (two or three sheep) from their home pen to the same holding yard where they received a feed reward (20 g grain/sheep and lucerne hay) for 3 min on 5 consecutive days.

For the second stimulus, a sample of white noise ('noise') was played intermittently (3 s every 10 s) for the duration of the spatial maze test on all 3 days. The locations of the speakers are indicated in Fig. 1 and the maximum volume emitted from each was 115 Hz. The white noise was generated using the program Audacity® 1.3.12 Freeware. No white noise was played for the 'no noise' sheep as they traversed the maze.

2.4. Maze testing

Following the 5 days of pre-treatment, sheep were individually tested in the maze on 3 consecutive days. The behaviour of all sheep in the maze was recorded in real time video (Bosch Securities, Huntingwood, New South Wales, Australia). Total time taken to complete the maze, the amount of time spent in EZ 1 and 2, and the number of entries into each EZ were recorded. Any sheep that failed to complete the maze within 5 min (300 s) was moved through the maze by a research technician slowly walked behind the animal.

2.5. Serum cortisol analysis

Sheep were gently restrained and blood samples were collected via jugular venepuncture immediately prior to entering the maze and 10 min after completing the maze. Those sheep that failed to complete the maze had their second blood sample collected 10 min after being moved through the maze. Blood was collected into 8 mL serum separating tubes using 18 gauges, 25 mm needles (both BD Diagnostics, North Ryde, Australia). Samples were then centrifuged at 3000 rpm and 4 °C for 10 min and serum was stored at −20 °C for cortisol analysis.

Serum cortisol concentrations were analysed by enzyme immunoassay using an IMMULITE® 1000 (Siemens, IL, USA), which has been previously validated for ovine cortisol at other laboratories (Poore et al., 2010). Analysis was conducted according to the manufacturers recommendation. Briefly, 10 μL of each serum sample was analysed in duplicate in a competitive chemiluminescent enzyme immunoassay to detect total cortisol. Three quality control (QC) samples are used to gauge the long-term accuracy of the assay. For the three QCs (115 nmol/L, 334 nmol/L and 1127 nmol/L) the coefficients of variations were 8.57%, 5.89% and 6.70%, respectively.

2.6. Statistical analyses

All analyses were performed using GenStat 14th edition (VSN International Ltd., Hemel Hempstead, United Kingdom). In all analyses, the full model including all interactions was fitted first, and then any non-significant terms (at 5% level) were sequentially removed until, in the final reduced model, all explanatory variables were statistically significant.

Sheep from all groups failed to complete the maze in the allocated 300 s. To investigate any influence of noise or pre-treatment on failure to complete, a binomial Generalised Linear Mixed Model (GLMM) was fitted using day, pre-treatment (dog or food) and noise (noise or no noise) as fixed effects, and sheep/day as a random effect.

Total time, time in EZ 1 and time in EZ 2 were analysed using linear mixed models (LMM) with day, pre-treatment and noise as fixed effects, and sheep/day as random effect. In a second series of analyses of total time, time in EZ 1 and time in EZ 2, two linear contrasts were used in place of day in the fixed effects. These compared means on day 1 with days 2 and 3 combined (contrast 1), and day 2 to day 3 (contrast 2). Contrasts were run to provide a more focused analysis of differences over days (Rosenthal and Rosnow, 1985). For both analyses, events where sheep failed to complete the maze were removed from the total time results, but incomplete trial data were used for EZ 1 and 2 analyses. To ensure that the assumptions of normality and homogeneity of variance were met, total time was log-transformed and data for EZ 1 and 2 were both quarter root transformed.

The number of entries into EZ 2 was analysed using a Poisson GLMM with day, pre-treatment and noise as fixed effects, and sheep/day as random effects. No sheep entered EZ 1 more than once, so these data were not analysed. Because of these differences in the number of entries, and the difference in time spent in the error zones, the results for EZ 1 and 2 were analysed separately.

Log-transformed serum cortisol concentrations were analysed using linear mixed models with day, pre-treatment, noise and time of sample (pre- or post-maze) as fixed effects and sheep/day as random effects.

3. Results

3.1. Number of completions

There were no significant influences of noise, pre-treatment or day on the probability of sheep completing the maze. Results for successful completions are as follows: day 1 = 77%, day 2 = 81%, day 3 = 91% ($F_{2,187.0} = 2.14$, $P = 0.12$); noise = 84%, no noise = 83% ($F_{1,187.0} = 0.03$, $P = 0.85$); dog = 85%, food = 83% ($F_{1,187.0} = 0.14$, $P = 0.71$). Only one individual failed to complete the maze on all 3 days.

3.2. Total time

There was a significant effect of day on total time taken to complete the maze ($F_{2,144.1} = 39.98$, $P < 0.001$), and completion time improved as the test progressed: back transformed means for days 1–3 are 122 s, 47 s and 37 s, respectively (transformed averages: 4.8, 3.8 and 3.6, SED ± 0.140).

Neither pre-treatment nor noise had a significant effect of completion time. There was a significant interaction between contrast 1 and noise (Wald statistic = 4.11, $P = 0.043$). No other interactions were significant. The mean total maze times of the noise and no noise groups on all 3 days of testing are shown in Fig. 2; contrasts indicated that noise affected performance on day 1, but not days 2 or 3.

3.3. Error zone 1 time

Day had a significant effect on time spent in EZ 1 ($F_{2,180.2} = 14.14$, $P < 0.001$), and again decreased over the 3 days of the experiment, the back transformed means being: 4.1 s on day 1, 0.9 s on day 2 and 0.0 s on day 3 (transformed averages: 1.4, 1.0 and 0.4, SED ± 0.20). Noise had no effect on time in EZ 1, nor did pre-treatment. Neither linear contrast indicated pre-treatment or noise effects on time in EZ 1.

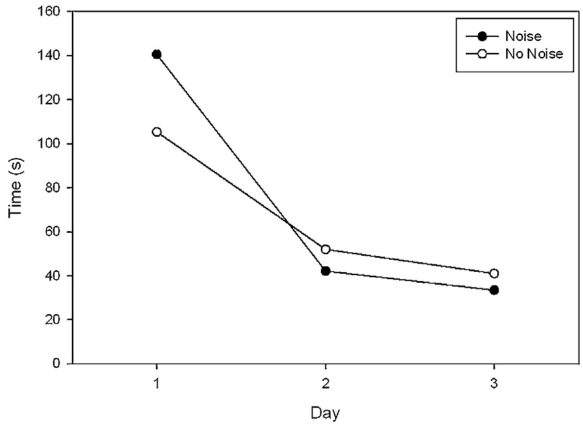

Fig. 2. Back-transformed averages for total time to complete the maze are presented (transformed averages: noise 4.94, 3.74, 3.51; no noise 4.66, 3.95, 3.17; SED ± 0.20). A significant contrast interaction was found between noise and no noise on day 1 compared to days 2 and 3.

Table 1
Number of entries into error zone 2 on all 3 days for dog pre-treatment ($n = 33$) and food pre-treatment ($n = 31$) groups (transformed averages in parentheses, SED ± 0.19).

Day	Dog	Food
1	1.5 (0.41)	1.2 (0.19)
2	0.8 (−0.26)	1.1 (0.13)
3	0.7 (−0.36)	0.8 (−0.27)

3.4. Error zone 2 time

Day was again the only significant term in the analysis ($F_{2,179.0} = 28.03$, $P < 0.001$). The average time spent in EZ 2 on day 1 was 61 s, 14 s on day 2, and 4 s on day 3 (transformed averages: 2.8, 1.9 and 1.5, SED ± 0.18). There was a trend towards the noise influencing time spent in EZ 2 with sheep exposed to noise spending an average of 14 s over all 3 days of testing, compared to 23 s for sheep not exposed to the noise ($F_{2,179.8} = 3.62$, $P = 0.059$; transformed averages: 1.9 and 2.2, SED ± 0.15). Pre-treatment did not affect performance (both = 18 s, $P = 0.937$). There was a significant pre-treatment × contrast 1 interaction (Wald statistic = 4.17, $P = 0.041$) and a significant noise × contrast 1 interaction (Wald statistic = 4.05, $P = 0.044$). The mean EZ 2 times of dog and food groups on all 3 days of testing are shown in Fig. 3, contrasts indicated that dog pre-treatment affected performance on day 1, but not days 2 or 3. The mean EZ 2 times of noise and no noise groups on all 3 days of testing are shown in Fig. 4, contrasts indicated that noise affected performance on day 1, but not days 2 or 3.

3.5. Number of entries into error zone 2

The interaction between pre-treatment and day was marginally significant (Table 1; $F_{2,183} = 2.91$, $P = 0.057$), with dog pre-treatment sheep making more errors on day 1 than control sheep, but less on days 2 and 3. There was no effect of noise on the number of entries into EZ 2

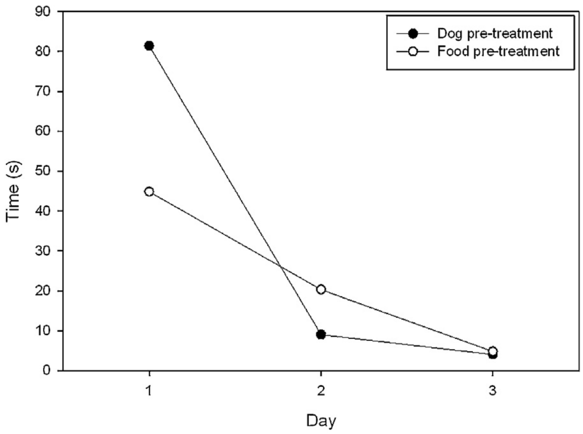

Fig. 3. Back-transformed averages for time spent in error zone 2 according to pre-treatment are presented (transformed averages: dog 3.00, 1.73, 1.41; food 2.59, 2.12, 1.48; SED ± 0.26). A significant contrast interaction was found between dog and food pre-treatments on day 1 compared to days 2 and 3.

(back-transformed counts: White Noise = 0.9 entries, No Noise = 1.1 entries, $P = 0.309$).

3.6. Cortisol

Both the time of sample (before or after the maze) and pre-treatment had a significant effect on cortisol concentration ($F_{1,357} = 51.63$, $P < 0.001$ and $F_{1,357} = 6.5$, $P = 0.011$, respectively), but no interaction between the two. Cortisol concentrations were lower before the maze test than afterwards (back-transformed concentrations: 43.0 nmol/L vs. 60.8 nmol/L, transformed data: 3.76 vs. 4.01, SED ± 0.05); food pre-treatment sheep also had a significantly lower cortisol concentration overall compared to dog pre-treatment sheep (back-transformed concentrations: 48.0 nmol/L vs. 54.4 nmol/L; transformed data: 3.87 vs. 4.00, SED ± 0.05). Neither noise nor day had an effect on cortisol.

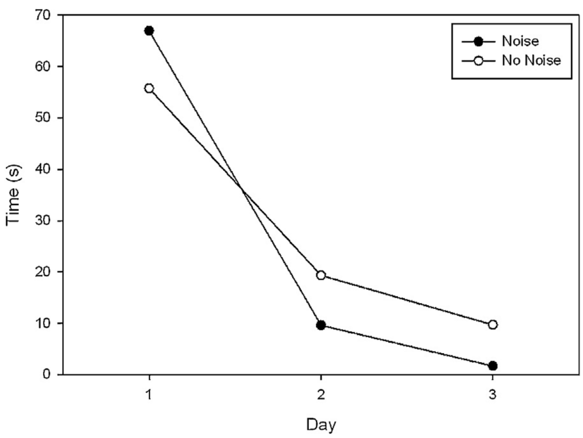

Fig. 4. Back-transformed averages for time spent in error zone 2 for noise or no noise are presented (transformed averages: noise 2.86, 1.76, 1.14; no noise 2.73, 2.07, 1.76; SED ± 0.26). A significant contrast interaction was found between noise and no noise groups on day 1 compared to days 2 and 3.

4. Discussion

The results from this study demonstrate that the cognitive processing of sheep was impaired by both noise and dog treatments on day 1 of testing, providing evidence that this spatial maze may be a useful method of identifying the effect of stress on cognition. It was not possible to identify which stimulus was more stressful however, and so the hypothesis that the stimulus delivered during the maze (white noise) would be more adverse than the dog pre-treatment cannot be confirmed. There was also no evidence of a cumulative effect of the stimuli (dog and noise) on cognition.

Relying on the strong flocking motivation of sheep, the test has a high completion rate with no prior training required (Lee et al., 2006). The number of completions and repeated significance of the factor 'day' in the results strongly support the spatial maze task as a useful cognitive test in sheep. While not all sheep managed to complete the task, a majority did so on the first day without any prior training. Data from sheep that failed to complete the test were still included in the analyses on frequency and duration of errors made. Analyses of these data from all sheep prevent potentially influencing results by the elimination of individuals. Error data are also reported to be the most useful measures of task performance (Spear et al., 1990; Lensink et al., 2006; Shettleworth, 2010). An overall difference in EZ performance was also noted in the current study.

The results for day 1 were significantly different to days 2 and 3, with these results supporting the findings of Lee and colleagues (2006). Both current and previously published results (Lee et al., 2006) reflect different aspects of cognitive performance being measured on day 1 to days 2 and 3, with initial problem solving demonstrated on day 1, and then learning and memory recall on days 2 and 3. This spatial maze task has been used to successfully identify differences in memory acquisition and memory retention (Dwyer et al., 2012). In the study by Dwyer et al. (2012) prenatal undernutrition and breed altered memory retention. Results were mixed with slower performance times for Suffolk lambs from dams experiencing undernutrition during pregnancy, but faster completion times for Scottish Blackface lambs whose dams were exposed to the same nutritional pressures. Mendl (1999) states that it is difficult to determine how a stressor has influenced cognitive performance, whether it is influencing initial task performance, learning or memory. Being able to identify how stressors influence these different aspects of cognition may be a useful purpose of this maze test for future studies. Both the dog pre-treatment and noise showed signs of impeding cognitive performance on day 1. Exposure to the aversive noise was associated with a slower completion time, and more time spent in EZ 2, compared to unexposed sheep. Dog pre-treatment resulted in sheep spending more time in EZ 2, a trend towards more entries into EZ 2, and higher cortisol concentrations. As these significant differences were only seen on day 1, it suggests that both stimuli impeded initial problem solving, but that learning or memory recall were not altered. Since both stimuli had an effect on cognition, this maze test may be useful to test stimuli to which sheep have been previously exposed, as well as stimuli experienced whilst performing the test.

White noise was chosen as a stimulus because it was unfamiliar to the sheep. While it is well known that novelty is a key component in the formation of negative emotions (Scherer, 2001; Desiré et al., 2002), and that it is associated with fear in sheep (Boissy, 1995; Bickell et al., 2011), it cannot be assumed that a negative state was generated in sheep in the current study as a result of the white noise. A previous study has shown that the heart rate of naïve sheep increased when they were exposed to white noise more so than other auditory stimuli, suggesting an activation of the sympathetic nervous system (Ames and Arehart, 1972). In the current study, white noise did not elicit any significant increases in cortisol concentrations, and so further conclusions on the degree of aversion of white noise cannot be drawn. Despite this, differences in maze performance suggest that the white noise was distracting to the sheep at least. Further studies may be able to conclude whether this distraction is associated with vigilance and fear.

The dog pre-treatment used a natural predator to induce fear in sheep (Dwyer, 2004; Beausoleil et al., 2005). This did result in overall higher cortisol concentrations for the dog pre-treatment sheep, suggesting that the dog pre-treatment activated the hypothalamo-pituitary-adrenal stress response. As no baseline cortisol concentrations were collected from the sheep however, it cannot be conclusively stated that this difference is the result of the dog pre-treatment.

Based on current results, it cannot be determined which of these dog or noise stimuli were perceived as more aversive. The different results for both stimuli are of interest however. Day 1 results indicate that white noise increased overall completion time, whereas dog pre-treatment tended to increase entries into EZ 2. The differing results may suggest two different effects on cognitive processing. A slower overall completion time may be more reflective of attentional shifting from a task that occurs with the presence of arousing secondary stimuli (Dukas and Kamil, 2000; Dumont and Boissy, 2000). Repeated entries into the error zone suggest repeated attempts at problem solving, but unwillingness to try a new solution (Erhard et al., 2004; Hernandez et al., 2009; Morton and Avanzo, 2011). This could reflect the type of behavioural rigidity and low levels of active cognitive problem solving described at times of intense stress (Grandin et al., 1994; Toates, 1998, 2002). The data presented in the current study are not able to distinguish these differences, but further studies may be warranted to investigate if different aspects of the maze do in fact indicate different levels of arousal.

In conclusion, the spatial maze test can identify changes in cognition based on environmental stimuli and does not exclude any animals based on cognitive ability. For these reasons, and with further validation, the maze test may have the potential to measure welfare. Investigation of this specific test with both experimental and common on-farm stressors may further validate it as a measure of welfare.

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