



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.

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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 (Lensing 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 ($n=33$).

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