



Genetic differences in temperament determine whether lavender oil alleviates or exacerbates anxiety in sheep

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ABSTRACT

Growing concerns about the risk of addiction to benzodiazepines have led to increasing interest in alternative therapies to treat anxiety and depression. Lavender oil (*Lavendula angustifolia*) is reportedly anxiolytic in a number of species but little is known about how it affects individuals that are more or less anxious when faced with a stressor. In this study, we used changes in locomotor activity and the plasma concentrations of cortisol to test whether lavender oil would reduce behavioral and endocrine correlates of anxiety in calm and nervous sheep exposed to an isolation stressor. During the non-breeding season, 'calm' or 'nervous' female sheep from the UWA temperament flock were exposed to a mask containing either 1 mL of 10% lavender oil (calm: n = 8; nervous: n = 8) or peanut oil (calm: n = 8; nervous: n = 8). After 30 min, each sheep was isolated for 5 min and then returned to the group. Blood was sampled prior to the mask, prior to isolation, 1 min and 30 min after isolation to profile changes in the plasma concentrations of cortisol. Agitation score, locomotor activity and vocalizations were recorded as correlates of anxiety associated with the isolation stressor. Irrespective of whether they were exposed to lavender oil, calm sheep had a lower agitation score ($P < 0.001$), crossed the central lines of the isolation box less frequently ($P < 0.001$), expressed fewer vocalizations ($P < 0.001$) and had lower plasma concentrations of cortisol immediately after isolation ($P < 0.001$) than nervous sheep. Exposure of calm sheep to lavender oil decreased the agitation score ($P < 0.001$), frequency of vocalizations ($P < 0.05$), decreased the number of crosses of the central lines of the isolation box ($P < 0.05$), and the plasma concentrations of cortisol prior to isolation ($P < 0.05$) (after mask application) compared to calm control sheep. Exposure of nervous sheep to lavender oil increased the frequency of vocalizations ($P < 0.05$), the number of sheep attempting to escape ($P < 0.05$) and the plasma concentrations of cortisol 30 min after isolation ($P < 0.05$) compared to nervous control sheep. We conclude that genetic differences in temperament determine whether lavender oil alleviates or exacerbates the behavioral and/or endocrine correlates of anxiety in sheep.

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1. Introduction

Anxiety can be interpreted as the emotional capacity of an individual to anticipate an aversive situation that is unpredictable and outside of their control [1]. Abnormally high levels of anxiety are associated with dysfunction of the hypothalamic–pituitary–adrenal (HPA) axis, and, in humans, affective disorders such as pathological anxiety and depression [1]. Anxiety disorders affect 40 million people annually in the USA, and are often co-morbidly expressed with depression [2].

Pathological anxiety and depression are frequently treated with benzodiazepines which act on the gamma-aminobutyric acid complex (GABA) to suppress neural activity in regions of the brain that mediate

anxiety [3]. In the short term, benzodiazepines are effective at reducing anxiety but long term use can be associated with a number of adverse side effects and a relatively high risk of abuse and addiction (review; [4]). Furthermore, polymorphisms in the enzymes and receptors responsible for mediating the effects of anti-depressant or anti-psychotic drugs reduce the reliability and predictability of the drug response [5].

In recent years, there has been increasing interest in the use of alternative therapies to treat a number of medical conditions, including anxiety disorders and depression [6]. Lavender oil (*Lavendula angustifolia*) is commonly used in aromatherapy and allegedly has anxiolytic effects in a number of species including rats [7], mice [8], Mongolian gerbils [9], dogs [10] and humans [11]. Both the mechanism of action [12] and anxiolytic effect of lavender oil are reputedly similar to the benzodiazepines, making lavender oil a potentially viable alternative to benzodiazepines for the treatment of anxiety [9,13,14].

In humans, lavender oil appears to be calmativ for young infants [15] and reduces the anxiety associated with visiting the dentist [16]

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or taking examinations [17]. However, interpretation of human studies can be difficult because lavender oil is often combined with other relaxation strategies such as massage [18], foot-baths [19] or baths [15,20]. A recent study also showed that humans expect lavender oil to be calming and that this expectation, rather than the aroma *per se*, was responsible for mediating the relaxing effects of lavender oil [21]. These issues, combined with the difficulty in sourcing an adequate placebo, make it difficult to differentiate between the real and perceived effects of lavender oil on anxiety in humans [21].

Animal models provide a viable alternative to test the anxiolytic effects of lavender oil because we can directly measure behavioral and endocrine correlates of anxiety and expectation is unlikely to be an issue. We can also use natural variation in anxiety within a population to select animals for extremes in this trait, thus allowing the anxiolytic effects of lavender oil to be tested in individuals that are more or less anxious when faced with a stressor. HAB/LAB rats have been selected for decades for extremes in anxiety related behavior when faced with a mild stressor [1,22,23]. Specifically, HAB rats have a lower propensity to enter the open arms of the elevated plus maze than LAB rats and secrete more corticosterone when forced to do so [24]. The repeatability and heritability of anxiety related behavior of the two lines within and between generations thus provides predictability over the level of anxiety associated with a stressor.

Sheep at the University of Western Australia have been divergently selected for 17 generations on the basis of their behavioral responses to social isolation and human presence. Isolation and human presence elicit a strong fear response in sheep [26], that can be quantified by increased locomotor activity and vocalization frequency [26,27]. The scores from the behavioral tests used to select the sheep are repeatable over time ($r=0.40-0.76$) [28] and moderately heritable ($h=0.45$) [28]. A recent cross-fostering study found the phenotype of calm and nervous sheep to be evident at 1 week of age and minimally affected by non-genetic factors [29]. Fear and anxiety are closely related emotions that describe the reaction of an individual to an actual or potential threat [30]. The stressors used to select the UWA temperament flock pose no actual, physical danger to the sheep, so one could argue that nervous sheep are predisposed to view an ambiguous situation as more threatening than calm sheep. This type of negative bias is characteristic of high anxiety in rats, which combined with the divergence in the behavioral [29,31,32] and endocrine [32] responses of calm and nervous sheep to social isolation supports the notion that nervous sheep are more 'anxious' than calm sheep when faced with an isolation stressor. The calm and nervous sheep of the UWA temperament flock thus draw many parallels to the HAB/LAB rats (strong phenotypic differences of genetic origin that are evident early in life) [25] and as such may provide an opportunity to study anxiety in a non-rodent species.

Genetic factors play an integral role in determining the level of anxiety associated with a stressor [22] and the phenotypic expression of the drug response [33] so may, in turn, affect the anxiolytic properties of lavender oil. In this study, we tested the effect of lavender oil on the behavioral and endocrine correlates of anxiety in calm and nervous sheep exposed to an isolation stressor. The nervous sheep have been genetically selected for high expression of locomotor activity [29,31] when faced with an isolation stressor that is associated with significantly higher plasma concentrations of cortisol than those observed in calm sheep [32]. Therefore, we hypothesized that exposure to lavender oil would reduce locomotor activity and plasma concentrations of cortisol in sheep selected for nervous temperament during exposure to an isolation stressor. Calm sheep express low levels of locomotor activity when faced with an isolation stressor [29,31] that are associated with significantly lower plasma concentrations of cortisol than those observed in nervous sheep [32]. Therefore, we hypothesized that exposure to lavender oil would be unlikely to further reduce the behavioral and endocrine correlates of anxiety in calm sheep during exposure to an isolation stressor.

2. Materials and methods

2.1. Animals

These experiments were carried out in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition, 2004) and were approved by the Animal Ethics Committee of The University of Western Australia (RA3/100/947).

2.1.1. The UWA temperament flock

The UWA temperament flock are Merino sheep kept at the University of Western Australia (UWA) research farm in Wundowie (latitude 31°46'S, longitude 116°29'E; 330 m in altitude) that have been divergently selected for 'calm' or 'nervous' temperament for 17 generations [29,31,34]. In this context, we define 'temperament' as the behavioral reactivity of sheep to the psychosocial stress of social isolation in the presence or absence of humans [31,34]. For the purpose of genetic selection, behavioral reactivity to stress is measured at 14–16 weeks of age using a series of tests that assess the animal's relative ability to cope with stress. The first test is a 3 min arena test that quantifies the expression of locomotor activity and vocalizations when faced with the conflict of approaching a human to gain contact with a small group of con-specifics [31,34]. In the second test, the animal is placed in visual isolation in a solid plywood box (1.5 m³) for 1 min. Physical activity inside the box (agitation score) is recorded using a digital agitation meter that is calibrated prior to the test for low, medium and high levels of activity [31,34]. The scores from the two tests are then used in a selection index to formulate a score for the temperament of the sheep as described in detail by Beausoleil et al. [35]. Males with the most extreme scores within the calm and nervous lines are used for breeding the next generation of sheep when they reach sexual maturity. Calm and nervous sheep are maintained as one flock at pasture and subject to conventional Australian farm practice.

We have screened over 10,000 sheep across Australia using the isolation box test (test 2) and found that the progeny bred from unselected or 'wild type' sheep that were 'more or less reactive' to the isolation stressor, were also 'more or less' reactive to the isolation stressor, as indicated by differences in their agitation score. These data indicate that the genes underlying calm and nervous temperament in this model are likely to be common in the general population, thus supporting the heritability, relevance and validity of our animal model [28].

2.1.2. Experimental animals

During August 2010 (non-breeding season; southern hemisphere), females from the calm ($n=16$) or nervous ($n=16$) lines of the UWA temperament flock were allocated to one of four treatments; calm and nervous sheep exposed to 10% lavender oil (Calm Lav; $n=8$; Nerv Lav; $n=8$) or exposed to peanut oil as a control (Calm Con; $n=8$; Nerv Con; $n=8$). The groups were balanced for age (14–16 months) and live weight within temperament (Table 1). The sheep were initially selected based on their parental temperament (i.e. calm or nervous) and their agitation score in the standard isolation box at weaning (3–4 months). Before they were finally allocated to their treatment groups, the sheep were screened during a 1 min test in a smaller (0.5 m × 1.2 m × 1.2 m), portable version of the isolation box (screening agitation score) to ensure that the screening agitation scores were balanced within temperament across the lavender oil and control groups (Table 1).

2.2. Experimental procedures

The isolation box was set up in an enclosed shed under natural light with access to yards for holding the sheep before and after the isolation stressor. The isolation box was stood on a base of four tires of equal size and pressure to provide suspension and stability for movement within the box. On the day of the test, a mechanical device

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