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Can we use change in core body temperature to evaluate stress in sheep?

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ABSTRACT

Physical and psychological stressors are known to cause a transient increase in the core body temperature of mammals. We hypothesized that rise in core body temperature could be used to indicate the intensity of stress experienced by sheep. Based on established indices of stress, we ranked five treatments from very low to severe stress as follows: Holding with flock mates, Human presence, Visual isolation, Capture and inversion, and Simulated shearing. Change in core body temperature was measured remotely using thermal sensors placed in the ear canal of the sheep. Maximum change in core body temperature (*p=0.08*) and the area under the core temperature change curve (*p=0.09*) allowed differentiation of the least and most stressful treatments: Holding with flock mates and Simulated shearing. The intermediate treatments could not be differentiated using change in ear canal temperature. Therefore, given the levels of statistical significance, our hypothesis that stressors of different intensities could be discriminated by changes in ear canal temperature was only partially supported. Reasons for the inability to discriminate between all treatments include a lack of sensitivity of change in ear canal temperature as an index of stress, high individual variability in responses to stress, and thermal sensor malfunction.

Keywords: Animal stress; body temperature; sheep; handling.

INTRODUCTION

Physical and psychological stressors are known to cause a transient increase in the core body temperature of mammals (Nakamori *et al*., 1993). This thermoregulatory response has been documented in a range of species, experiencing a variety of physical and psychological stressors (Rats: Nakamori *et al.,* 1993; Cattle: Macaulay *et al.,* 1995; Humans: Marazziti *et al.,* 1992; Mice: Zethof *et al*., 1994; Rabbits: Snow & Horita, 1982; Pigs: Parrott *et al.,* 1988; Sheep: Drew, 1996), and may be useful as a stress indicator for welfare and productivity (Hahn *et al*., 1990; 1992; Parrott & Lloyd, 1995; Ingram *et al*., 2002). Rise in core temperature would be a convenient index to evaluate stress as it can be measured remotely, alleviating some of the problems associated with manual sampling (Cabanac & Briese, 1991).

Based on previous studies using a variety of established physiological and behavioural indices of stress, we selected five treatments expected to produce varying levels of stress in sheep. The stressors can be ranked from a very mild stress where a sheep is held with a group of flock mates, to a severe stress such as simulated shearing, with intermediate stressors including human presence, visual isolation, and capture and inversion (Kilgour & DeLangen, 1970; Rushen, 1986; Baldock & Sibly, 1990; Hargreaves & Hutson, 1990a; 1990b).

We hypothesize that the greater the intensity of the stress caused by the treatment, the greater the subsequent rise in core body temperature. Therefore, we expect that a stressful event like mock shearing will induce a higher core temperature than treatments considered less stressful to sheep, and that it is possible to differentiate between stressors of different intensities using the rise in core body temperature.

MATERIALS AND METHODS

Experimental design, animals and procedures

Core body temperature was measured remotely every 2:08 minutes in sheep subjected to one of five stressors for 15 minutes, and then held for at least one hour in a recovery room. Each sheep was randomly assigned to a test day and treatment, and was tested only once. Treatments were balanced across ten days of testing. Ambient temperature inside the testing facility was also measured on each testing day.

100 Romney cross rams (20 per treatment) were maintained as one group at pasture and were yarded and drafted into test groups (n=10) each morning between 6 and 7:30am (July-August). The indoor testing facility consisted of a pre-test room, a race leading to two treatment rooms and a recovery room (Figure 1). Animals were familiarized with the testing facility and procedures by moving them through the pre-test, treatment and recovery areas on each of 3 days before testing. Two handlers moved the sheep during habituation and attached the equipment; one of these handlers introduced and removed the sheep during testing.

On day 3, the Temptags (HortResearch, Ruakura, New Zealand) were attached and the sheep were returned to pasture to habituate to wearing them. The next day, the test group was drafted and left undisturbed in the pre-test room for one hour before testing to allow core body temperature to stabilize. Test animals were individually removed from the pre-test room by one handler, and immediately ushered into the treatment room, where they

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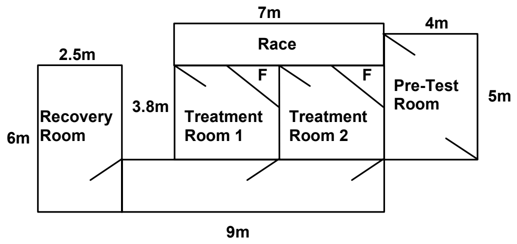
FIGURE 1: Experimental facility. F indicates the location of the four flock mates in the treatment rooms.



were left for 15 minutes. After treatment, they were taken from the treatment room directly to the recovery room. Groups of four additional sheep from the same flock (not tested) were placed in the pre-test and recovery rooms to serve as company for the first and last test animals. All test sheep remained in the recovery room for at least one hour after testing. The TempTags were removed on the day after testing.

Temperature measurement

Temperature of the ear canal was measured remotely using TempTag ear tags developed at HortResearch (Ruakura, New Zealand). The TempTag consisted of a circuit board and data logger, housed in a circular plastic container (45mm diameter x 8mm wide), and weighed 17g. The housing was glued to the side of the animal's head beneath the ear using cyanoacrylate glue (Loctite 454 gel, Loctite Australia Pty Ltd, NSW, Australia). Two thermal sensors were attached to the logger by flexible wire. Temptags could record 2000 measurements from each attached sensor with an accuracy of 0.1°C and a resolution of 0.05°C. The core temperature sensor was incorporated into a foam outer ear canal plug (Pura-fit 6800, Moldex Metric Inc., Culver City, CA, USA) and inserted approximately 35mm into the ear canal. The anatomy of the sheep's ear made it difficult to place the sensor exactly at the tympanic membrane (Lowe *et al.,* 2001).

Treatments

The human stimulus was not involved in habituating sheep or attaching equipment, and was therefore unfamiliar at the onset of testing. The flock mates (not tested) were taken from the same flock as the test animals. The five treatments ranging from low to severe stress were:

Flock (F) (*very low stress*): Test sheep were left for 15 minutes with a group of 4 flock mates that were held behind a gate in the corner of the treatment room.

Human presence (H) *(mild stress):* Test sheep were introduced into a room with flock mates present. The human maintained a distance of approximately 1m from the test sheep by following it slowly in a non-threatening posture (eyes down) for the 15 minute duration of the treatment.

Visual isolation (I) *(moderate stress):* Test sheep were introduced into a room with no visual access to other sheep or humans for 15 minute duration of treatment.

Capture and inversion (IV) *(marked stress):* Test sheep were captured and inverted (by same person as in 'human presence') to sit on their haunches immediately after they entered the treatment room. Animals were held in this position for 5 minutes, then released, and the treatment continued as for 'human presence' for the final 10 minutes. No flock mates were present.

Simulated shearing (S) *(severe stress):* Test sheep were captured, inverted and shearing was simulated. Standard shearing clippers were used without blades attached. The clippers were turned on, placed in contact with the wool, and run over the body to mimic shearing. No wool was removed. After 5 minutes, the machinery was turned off, the animal was released, and the treatment continued as for 'human presence' for the final 10 minutes. No flock mates were present.

Statistical Analysis

Only those animals with complete and physiologically realistic recordings for the 48 hour attachment period were used in the analysis. Any animals with ear canal temperatures of greater than 41ºC were excluded; such high ear canal temperatures would indicate pathological heat stroke in sheep and no such clinical signs were displayed (Drew, 1996). In these cases it was assumed that the thermal sensors were malfunctioning. In addition, 6 animals that escaped en route to the recovery room were also excluded due to the potentially confounding effects of exercise-induced hyperthermia. Therefore 32 animals were included in the final analysis.

An experimental baseline temperature was estimated for each animal by averaging the temperatures from the 45 minute period before treatment was applied (undisturbed in pre-test room). This experimental baseline was used to calculate the change in core temperature after the start of treatment. In addition, maximum absolute temperature, time to reach maximum temperature and the area under the temperature change curve were analyzed using non-parametric one way ANOVA (Kruskal Wallis test) (data not normally distributed). Wilcoxon rank sum tests were used to determine whether maximum temperature differed from baseline for each treatment group. All statistical tests were performed using SAS Version 8.0 (SAS Institute, 1994).

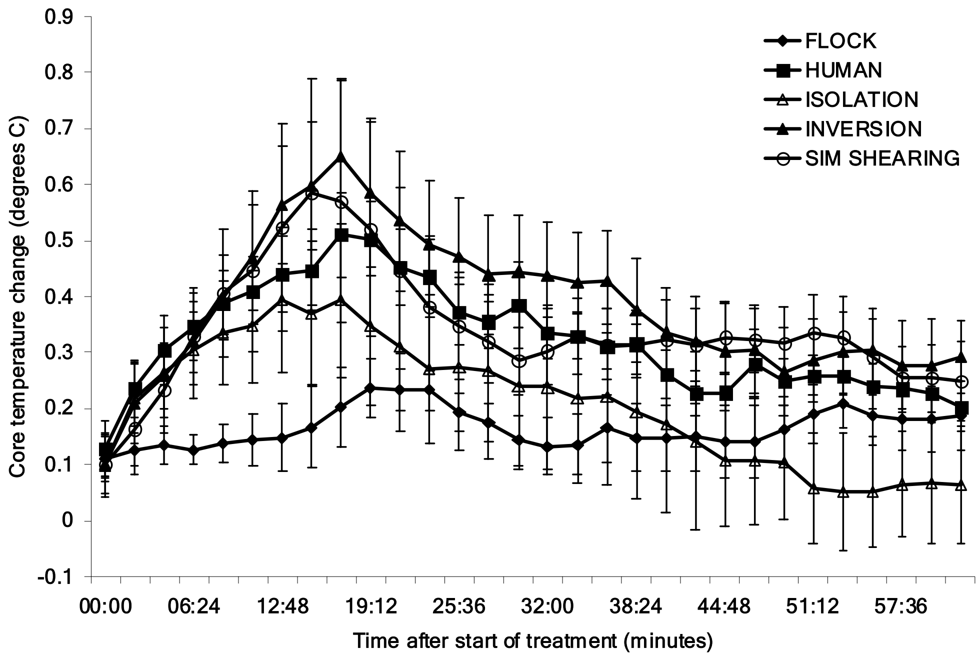
RESULTS

The maximum rise in core temperature from the start of treatment until the end of recovery was higher for Simulated shearing than for Flock ($\mathbf{X}^2$=8.44 $p=0.08$ Table 1). In addition, the total area under the temperature change curve (to one hour after start of treatment) was also higher for Simulated shearing than Flock ($\mathbf{X}^2$=8.01 $p=0.09$).

TABLE 1: Maximum change in core temperature and time to reach maximum core temperature in sheep exposed to one of five stress treatments (Mean ± SEM).

Treatment	n	Maximum core temperature change (ºC)	Time to maximum core temperature (minutes)
Flock	5	0.38 ±0.07	34:08 ± 6:40
Human Presence	8	0.61 ± 0.06	21:36 ± 3:06
Visual Isolation	6	0.49 ± 0.11	13:52 ± 3:41
Capture and Inversion	8	0.73 ±0.11	21:04 ± 2:44
Simulated Shearing	5	0.85 ± 0.16	34:59 ± 8:00
		X^2=8.44 *p=0.08*	X^2=8.68 *p=0.07*

FIGURE 2: Average change in core temperature (º Celsius) every 2:08 minutes up to one hour after the start of treatment in sheep exposed to one of five stress treatments (Mean ± SEM).



The time to reach maximum temperature was shorter for visually isolated sheep than for those held with flock mates or mock sheared (**X^2**=8.68 *p=0.07* Table 1). None of the other temperature parameters differed between treatments. The maximum temperature reached after treatment differed from baseline for all groups: Flock (**X^2**=3.15 *p=0.08*), Human presence (**X^2**=3.58 *p=0.06*), Isolation (**X^2**=3.69 *p=0.05*), Inversion (**X^2**=6.35 *p=0.01*) and Simulated shearing (**X^2**=5.77 *p=0.02*). Figure 2 shows the average time course of core temperature change for each of the five treatments. Because each point on the graph is a summation of temperatures measured at that time (within each treatment), the maximum temperature change and time to reach maximum temperature do not reflect the statistical averages calculated for these parameters.

DISCUSSION

The hypothesis that core body temperature could be used to indicate the intensity of the stress experienced was partially accepted. The highly stressful treatment that mimicked shearing caused a much greater rise in core temperature than the mild treatment of being left with flock mates. The total thermal response to stress indicates the same, that is, the area under the curve was greater when shearing was simulated than when sheep were left with flock mates. However the intermediate treatments of human presence, visual isolation and capture/inversion could not be differentiated from each other or from the two extremes using rise in core body temperature.

We do see step-wise increases in the core temperature response with putative increases in stressor intensity, however the changes are not discrete. In addition, there seems to be some disparity between the presumed and actual order of intensity. It appears that on average, these sheep found the presence of a human more stressful than visual isolation, and that inversion alone caused a larger change in temperature than inversion plus simulated shearing. It would have been extremely useful to include an independent measure of stressor intensity to confirm the rankings of the treatments used. In future, hormonal (e.g. catecholamines, cortisol) and/or behavioural responses should be used to independently verify the rankings.

This would allow for better interpretation of core temperature change results.

There are several possibilities why our measurements of core body temperature did not discriminate between the treatments. First, changes in temperature in the external ear canal may not accurately reflect changes in core body temperature in sheep. Ingram *et al.* (2002) report that the change in ear canal temperature of sheep during transport more closely reflected changes in ambient temperature than changes in rectal temperature. However, the changes in ambient temperature during transport were relatively large. Ambient temperature did not differ between treatment groups in the present study. Lowe *et al*. (2001) found that change in ear canal temperature in undisturbed sheep was a good measure of change in vaginal temperature, and conclude that ear canal temperature probably closely follows core body temperature.

If ear canal temperature does accurately reflect core body temperature, the failure to resolve the treatments might be because core body temperature is not a sensitive enough index to differentiate between these types of psychological stressors. The fact that ear canal temperature rose significantly after all treatments indicates that they were causing some degree of stress to the animals, and agrees with previous reports of stress-induced rise in core temperature in sheep (Drew, 1996; Parrott *et al.,* 1999; Ingram *et al.,* 2002). However, physiological measurements are not always sensitive enough to discriminate between stressors of different quality or intensity (Jephcott *et al.,* 1986; Natelson *et al*., 1987; 1988).

Isolation may be more stressful than human presence, but not in a way that can be discerned using change in core body temperature. The difference in the quality of the stressors is supported by the fact that maximum temperatures were reached more quickly after visual isolation than after mock shearing or holding with flock mates. In addition, simulated shearing and capture/inversion may not differ enough to elicit different temperature change responses. Wool removal, reported to be the most stressful component of shearing (Hargreaves & Hutson, 1990b), did not occur during the mock shearing treatment. It must also be noted that the degree of physical activity may have differed in response to each stressor. Unfortunately the contribution of exercise to temperature increase could not be differentiated from psychological stress-induced rise in core temperature in this experiment. In future, the use of a single stressor applied at varying levels of intensity would remove problems of stressor-specific responses.

Another reason for the lack of resolution between treatments may be the high variability in temperature change between individuals. Natelson *et al.* (1987) note that variability between individuals is a major limitation to the use of hormone responses as tools for quantifying stress. We tried to compensate for individual differences by using each animal as its own control. However, stress responses reflect an individual's own subjective experience of an event. This experience, and hence the response, is likely to vary between individuals.

The settling period of 45 minutes used to calculate experimental baseline may have been inadequate to achieve a meaningful, stable temperature. During the settling period, the temperature would be expected to be declining after a yarding effect (Ingram *et al.,* 2002). Therefore, this may not have been the most appropriate baseline to use. A baseline temperature measured when the sheep were at pasture (at the same time of day as treatment) might be more appropriate for calculating subsequent change in core temperature.

The fact that two thirds of the data were discarded is disconcerting. The cut off point of 41ºC was chosen based on previous reports of stress-related temperature change in sheep (Drew, 1996; Ingram *et al*., 2002). It is possible that the exclusion criteria selected were inappropriate, and artificially forced down the treatment means. However, analyses were also run including all data, and at varying levels of data exclusion, with no significant changes to the results, implying that these results are realistic.

Finally, the TempTags and thermal sensors were recycled after use. It is possible that the sensors were damaged during removal and subsequently gave inaccurate ear canal temperatures. Accordingly, we recommend that the thermal sensors be replaced after every use.

CONCLUSIONS

Ear canal temperature allowed a reasonable degree of discrimination between the least and most stressful of the five treatments. However, it did not allow resolution of those stressors that were intermediate to simulated shearing and holding with flock mates. Core body temperature, as estimated by ear canal temperature, may not be a sensitive enough index to differentiate between psychological stressors such as those used in this experiment. Alternatively, high individual variability or thermal sensor malfunction may have contributed to the lack of resolution between treatments. Future work should include an independent measure of stressor intensity, or should use a single stressor at varying intensities to evaluate the usefulness of change in ear canal temperature as an indicator of stress in sheep.

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