**Development of a thermal threshold tester for use in evaluating chronic pain in horses**

Horses suffer from a variety of painful conditions that are challenging to treat. Laminitis and osteoarthritis in horses are diseases that have no cure and can cause significant chronic pain and suffering to horses. The possible treatment options for painful conditions are limited, partly due to the complications associated with traditional pain medications but also due to the difficulty in diagnosing painful conditions in horses. To control and treat pain effectively requires a good understanding of the physiology of pain, the behavioural signs of pain and also the actions and durations of the analgesics used. However, pain is a difficult sensation to quantify, even in humans where a person can state that they feel pain and grade it.  In animals, observers have to look for behavioural changes to tell if an animal is in pain or has become painful. One way to quantify pain and the response to analgesia is to provide a reproducible painful stimulus and attempt to determine the “nociceptive threshold.” This can be done with either a mechanical or thermal stimulus where either the pressure or temperature slowly increases on the skin surface until nociceptive receptors (pain receptors) are triggered and a painful sensation is induced. A behavioural change is then expected in the animal which is noted by an observer and the stimulus stopped. This makes it possible to determine a reproducible nociceptive threshold. This can be increased by an analgesic or may be decreased due to acute or chronic pain. Being able to detect pain more accurately should allow better treatment of the condition and development of better analgesic drugs for horses.

The **first objective** of this project was to construct and validate a thermal threshold tester for use in horses. The requirements were that the heating element and temperature sensor (combined together in a “thermode” connected to a controller) could be attached to different sites on the horse; the thermode would heat at a linear rate and would either be shut down automatically by the control unit at a predetermined temperature or by an observer when the horse responded to the thermode heating. The control unit would be wirelessly connected (via Bluetooth) to a computer that would record the temperature of the thermode several times per second and also record the time taken for the horse to respond to the heating thermode as recorded by an observer. The lack of a physical connection between the thermode and the observer would allow the horse to be unrestrained and to behave normally. The equipment was to be built from off the shelf parts.

A device was built consisting of a 40mm square aluminium plate with a Peltier chip (which heats on one side and cools on the other when electrical current is passed through it) as the heating element, and a thermocouple to detect the temperature of the thermode. This was controlled by an programmable Arduino microprocessor connected wirelessly to a laptop computer by a Bluetooth modem. Since horses respond to thermal stimuli at higher temperatures than other species, the device was programmed to reverse the current to the Peltier chip at the end of a run to ensure that the skin cooled rapidly and was not damaged.

Testing showed that the thermode did not give as reproducible results as expected in horses, although it worked very well in sheep. The size of the thermode was increased to 50mm square and the thermocouple was replaced by a Dallas temperature sensing chip. The larger size made active cooling at the end of the run necessary so a large heat sink and fan was fitted to the thermode. The larger Peltier chip also made larger batteries necessary and a harness capable of carrying two 12V lead acid batteries was constructed. This design gave reproducible results on the thorax or upper foreleg of horses and two devices of this type were built and validated.

**Objective two** was to complete validation of the device and carry out initial testing of analgesic drugs in normal healthy horses. A MSc student was recruited for this. In the course of this trial we assessed the effect of three main manipulations on the thermal nociceptive threshold of five gelded horses (observer consistency and accuracy, artificially elevated and maintained baselines, and acepromazine and butorphanol’s effects).

Observer consistency was assessed by comparing trials where the commencement of heating was known by the observer to runs where they were unaware (by programming a random delay to the start of heating). Analysis of these results indicated that an attentive observer would produce consistent results. The effects of artificially elevated and maintained baselines were observed by comparing the consistency of results obtained with no manipulation of the skin baseline temperature of the horse to results where the thermal heating device heated the skin to 35°C and maintained it there between runs. Analysis of these results indicated that there is insufficient evidence to suggest that elevated baselines improve the consistency of results, and this was abandoned.

Acepromazine and butorphanol were administered intravenously either as an Ace/Saline control or an Ace/But treatment. The data indicated that butorphanol had a negligible effect on thermal threshold, but acepromazine had a small but significant effect – contrary to the literature which indicated that it would be an unobtrusive control, hence the failure to use a saline only control.

On the basis of these results, this device was shown to be able to assess thermal nociceptive thresholds reliably, although the specific behaviours each horse to painful heat was slightly different. Other than the unexpected effects of acepromazine, none of the manipulations appeared to affect the consistency or temperature at which a thermal nociceptive threshold was detected.

**Objective three** was to measure thermal nociceptive thresholds in horses in chronic pain to assess if thresholds were lowered compared to healthy horses. Eleven Thoroughbred mares undergoing unilateral radiocarpal osteochondral fragment (OCF) creation (n = 5) and unilateral sham surgery (n = 6) as part of another trial were used. All were given the same analgesic protocol. Skin temperature (Temp) and thermal nociceptive thresholds (TNTs) were measured in both forelimbs before (week 0) and weekly for 8 weeks (week 1-8) after arthroscopic surgery. The contralateral limb was used as control. Percentage thermal excursion (%TE) values were calculated: %TE = 100 \* (TNT - skin Temp)/(cut-off Temp - skin Temp). Differences between operated and non-operated limbs at each time point and for each group (i.e., OCF and sham surgery) were analysed using a two-way ANOVA followed by Bonferroni test. Differences between time points for operated and non-operated limbs in each group were analysed using a two-way ANOVA followed by Tukey test.

One mare from the OCF group was euthanized due to hip fracture during recovery from anaesthesia. Skin Temp was significantly higher in both limbs at week 7 compared to values at weeks 1, 2, 3 and 8 in sham-operated horses and at weeks 1, 2 and 8 in OCF horses (P < 0.05). Skin Temp was significantly lower at week 2 than at weeks 5 and 6 in the non-operated limb of sham-operated horses (P < 0.05). One week after surgery, TNTs and %TE in operated limbs of OCF horses were significantly reduced as compared to values for their contralateral control limb (P < 0.01) and to values in the operated limb at weeks 0, 4-6 and 8 (P < 0.05); TNTs at week 1 were also significantly lower than at week 7. Other than significantly lower TNTs values in the operated limb at week 1 as compared to week 2 (P < 0.05), no significant differences for TNTs and %TE in sham-operated horses were found.

We concluded that thermal sensitivity in horses increased one week after surgical creation of an OCF on a radiocarpal bone. This hyperalgesic effect was transient with TNTs returning to pre-surgery levels in week 2 and seemed to be peripherally, rather than centrally, mediated since TNTs were not affected in the non-operated limb. The small size of the effect was probably because the pain was mild, as shown by only minor lameness.

The equipment withstood use on unbroken thoroughbred yearlings remarkably well. The cooling fans on the thermodes were most susceptible to mechanical damage, but were cheap ($3) and easy to replace. The Bluetooth modem chips on both devices also failed, but again were relatively cheap ($25) to replace. Bluetooth technology has advanced recently and replacement chips are expected to be more reliable.

We now have two large devices and one small one, and plan to carry out analgesic drug trials on horses with clinical pain with them in the future.