Effect of breed on thermal pain sensitivity in dogs

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Abstract

A problem in assessing pain sensitivity in animals is the variability among individuals within a species. Thermal nociceptive threshold (TNT) testing is used to measure pain sensitivity in animals. However, little research has been done on within species differences in pain sensitivity, with most studies focusing on the effectiveness of analgesics. This research was carried out to see if there was any variation in baseline TNTs in different dog breeds.

To determine TNTs, a heat stimulus was applied to the leg of a dog using a new device that could be remotely activated. This removed the need to restrain the dogs. The time and temperature at which the dog responded behaviourally was recorded. The TNT of dog was recorded six times in a one-hour session, once a week, for four consecutive weeks.

In the first experiment the repeatability of harrier hound (n= 11) TNTs over time and the effects of the initial thermode temperature were examined. The results indicated that TNTs were repeatable over the daily test, session however they were affected by week of testing, thermode and initial thermode temperature. It was concluded that using a consistent elevated initial thermode temperature was more consistent than the natural starting temperature.

The aim of the second experiment was to investigate differences in TNTs between three dog breeds: harrier hounds, greyhounds, and huntaways (n=10 per breed). A breed effect was found whereby huntaways took significantly longer to respond than harrier hounds and responded at higher temperatures than greyhounds and harrier hounds. There were no differences between greyhounds and harrier hounds. This study provides the first scientific evidence of breed differences in pain sensitivity in dogs.

It is concluded that there were differences in thermal pain thresholds between the three dog breeds tested. The study supported the use of TNT testing on dogs and offered new insight into ways to improve the reliability of threshold testing. Future work should use more breeds, evaluate pain sensitivity in other modalities, and assess the effect of analgesics on TNTs in dogs.

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1. Literature Review

Dogs settling down before testing commences

1.1 Introduction

Over a thousand dog breeds have been identified (Mehrkam and Wynne, 2014). They are the result of selective breeding by humans for different physical or behavioural traits. Many breeds were developed in the Middle Ages (12th to 15th century), due to the rise in popularity of hunting (Stafford, 2006). This resulted in a range of breeds that were bred for specific hunting tasks. In the nineteenth century, dog shows became popular and new breeds were developed on the basis of appearance rather than function.

Breeds differ in size and appearance but there are also variations in physiology (Fleischer et al., 2008). For any disease there is a variation in the risk between breeds. This significant variation in disease incidence is due to the underlying difference in genotypes that are responsible for the dog's anatomy and physiology (Lark and Chase, 2012).

Pain is an unpleasant physiological and emotional response to a noxious stimulus that can cause damage to the tissues and which results in behavioural responses (Molony and Kent, 1997). Pain may allow an animal to identify potentially damaging stimuli and thus learn to avoid such stimuli in the future (Weary et al., 2006). All dogs will experience pain in their lifetime. Millions of dogs are de-sexed annually and need appropriate pain relief during and after surgery (Sharkey, 2013). Moreover, approximately 20% of dogs will develop osteoarthritis, a common pain problem in dogs (Sharkey, 2013).

The alleviation of pain in dogs has evolved with an increased awareness of dog welfare and improvements in analgesia. However, there are difficulties in assessing pain in dogs. Unlike humans, dogs are unable to verbally describe their pain. Pain is alleviated in dogs when the veterinarian and owner deem the severity of the pain to be significant (Viñuela-Fernández et al., 2007).

One problem in assessing pain in animals is the variability among individuals within a species. Understanding the degree of individual variation in pain sensitivity is important for improving the selection and tailored application of analgesics. Factors believed to influence pain sensitivity include physical and genetic differences, ambient temperature, environmental stimuli and learning, sex and the sensitivity of

tissue (Le Bars et al., 2001). An overlooked factor is the influence of genetically determined pain sensitivity between individuals and breeds. Little research has been done on individual treatment of pain, and most pain studies have focused on the effectiveness of analgesics during and after specific surgical procedures rather than within species differences in pain sensitivity. Testing human pain sensitivity found that people are either class field into two groups, either they are pain sensitive or pain tolerant (Chen et al., 1989).The only animal studies that have investigated individual differences in pain sensitivity have used rodents. In rats and mice 41 to 72% of the variance in pain sensitivity between individual subjects was due to genetic heritability (Mogil et al., 1999). No studies have looked at the variation in baseline pain sensitivity in different dog breeds.

1.2 Pain in Mammals

1.2.1 Defining Pain

Pain is an essential experience and warns people and animals of potential and actual injury. The International Association for the Study of Pain (IASP) defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (ISAP, 1994). Humans have the benefit of being able to use language to give detailed information about pain. It is difficult to assess these experiences in other mammals due to the communication barrier. Molony and Kent (1997) came up with a definition of pain for animals “an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues. It changes the animal’s physiology and behaviour to reduce or avoid the damage, to reduce the likelihood of recurrence and to promote recovery.”

The term nociception is used in relation to stimuli that cause tissue injury. Nociception refers to the physiological aspect of pain and is separate from the awareness and emotional experience of pain (Cervero, 2012). Melzack and Wall (1999) note that “the activity induced in the nociceptor and nociceptive pathway by a noxious stimulus is not pain, which is always a psychological state”; pain is a conscious perception. Nociception involves the fundamental physiological system that alerts an animal to damage (Sneddon et al., 2014). It is the neural transduction and transmission of a noxious stimulus via the spinal cord to the brain via a pain

pathway and the resulting reflex behavioural response (Steeds, 2013). Pain involves both nociception and the conscious perception and recognition that one is in pain (Fox, 2013).

Pain can vary in severity. A pain threshold is the minimum intensity of a stimulus that is perceived as painful (ISAP, 1994). Pain tolereance is the maximum intensity of a pain-producing stimulus that a subject is willing to accept in a given situation (ISAP, 1994). Both are the subjective experience of an individual. One person or animal could have a higher pain threshold or pain tolerance than another individual.

1.2.2 Nociceptors

The physiological process of pain perception begins with the detection of a noxious stimulus by nociceptors. Nociceptors are sensory nerve cells that are distributed throughout the periphery of the body (White, 2013). They are free nerve endings of primary afferent Aδ and C fibres. Most nociceptors are classified according to modality: thermal, chemical, mechanical or electrical stimulus, or they may be polymodal (respond to several stimuli). Nociceptors have a threshold that, once exceeded by a noxious stimulus, will cause an action potential to be sent down the nerve fibres. These fibres carry the signal to the spinal cord and up to the brain where it can be processed further (Fox, 2013).

The main nociceptor fibres are made up of two afferent neuron types, Aδ and C, and non-noxious neuron Aβ (Reddi et al., 2013). Aδ fibres are lightly myelinated and have a diameter of 2 to 5 μm. Signals in Aδ fibres move at a fast speed (5 to 36 m/s) and are associated with acute pain. They are responsible in part for the reflex response of moving the body part away from a noxious stimulus. Aδ fibres can be subdivided into mechanosensitive and mechanothermal receptors (Reddi et al., 2013). Mechanosensitive receptors only respond to pressure and touch, while mechanothermal receptors respond to both thermal and mechanical stimuli. C fibres are unmyelinated and are the smallest type of primary afferent fibre (0.2 to 1.5 μm). The lack of myelination causes a slow conduction velocity of around 2 m/s. They are the most common fibre in mammals and are polymodal, responding to chemical, mechanical, thermal and electrical stimuli (Sneddon et al., 2014). Aβ fibres are highly myelinated and are the largest of the three fibres. They allow rapid transmission of a signal and are usually activated by a light touch and transmit non-noxious stimuli.

1.2.3 Pain Pathway

The pain pathway, from stimulus to cognitive perception, is divided into four main stages: the pathway to the spinal cord (signal transduction), the signal moving up the spinal cord (signal transmission), processing of painful stimuli within the brain, and the descending analgesic pathway (Meintjes, 2012).

1.2.3.1 Signal Transduction

Signal transduction refers to the phase during which a noxious stimulus is converted into an electrophysiological signal (Bridgestock and Rae, 2013). The stimulation of a nociceptor by a noxious stimulus will cause an action potential in the nerve fibre. In the nerve fibre stimulus gated ion channels are opened, resulting in an influx of sodium or calcium ions along a diffusion gradient through pores (Meintjes, 2012). This causes a wave of depolarisation that moves along the nerve fibre to the spinal cord. The nerve fibres reach into the spinal cord through the dorsal roots and into the dorsal horn. The dorsal horn is made up of grey matter and is referred to as a grey column (Meintjes, 2012). The cells in the dorsal horn are divided into ten layers called rexed laminae. Aδ and C fibres transmit most information to the rexed laminae 1 and 2, however there are several projections into other deeper laminae. In the substantia gelatinosa, a part of the dorsal horn, primary afferent neurons synapse with secondary afferent neurons (Meintjes, 2012). The primary neurons release excitatory neurotransmitters to the secondary afferent neurons. Both large (Aβ) and small (C and Aδ) nerve fibres synapse in the dorsal horn with secondary neurons, which then reach the spinothalamic tract, and inhibitory interneurons. Here the interactions between the afferent neurons, interneurons and other pathways determine whether the pain impulse is amplified or inhibited (Reddi et al., 2013).

1.2.3.2 Signal Transmission

There are two main pathways that carry the nociceptive information to the brain, the spinothalamic tract and the spinoreticular tract. The spinothalamic tract transmits information about pain to the thalamus (Steeds, 2013). The spinothalamic tract starts at the spinal cord, where the secondary afferent neurons cross over to the other side of the spinal cord, via the anterior white commissure. The neurons then ascend the spinal cord via the contralateral spinothalmic tract to the brain stem and into the nuclei within the thalamus (Bridgestock and Rae, 2013).

There are two different parts of the spinothalamic tract; these are called the neospinothalamic tract and paleospinothalamic tract. The neospinothalamic tract goes to the ventral posterior lateral nucleus of the thalamus, and from there to the post-central gyrus (Steeds, 2013). It deals with fast pain and receives input from Aδ fibres. The paleospinothalamic tract, on the other hand, deals with slow acting pain and receives input from C fibres, with its fibres going to the medial thalamus (Bridgestock and Rae, 2013).

The spinoreticular tract follows a similar pathway to the spinothalamic tract, in that it also decussates and ascends the contralateral cord (Bridgestock and Rae, 2013). Instead of going to the thalamus, the spinoreticular tract leads to the reticular formation first before projecting onto the thalamus and hypothalamus. It conveys dull or burning pain and is usually involved with slow acting types of pain (Reddi et al., 2013).

1.2.3.3 Pathways in the brain

The processing of pain signals in the brain is complex (Reddi et al., 2013). The thalamus represents the main relay structure for sensory information destined for the cortex. The different projections from the thalamus to the cortex make up the core part of pain processing. The ascending spinothalamic tract carries the noxious signal directly to the thalamus. The thalamus comprises different nuclei, which are further subdivided. As noted previously the projections from the spinothalamic tract lead to different thalamic nuclei. The ventral posteromedial nucleus (VPM) projects to the prefrontal cortex and signals combine with information from the amygdala, hypothalamus and periaqueductal gray (PAG) to elicit the emotional aspect and autonomic responses of pain in humans (Almeida et al., 2004). The secondary neurons of the spinothalamic tract synapse with third order neurons in the ventroposterolateal (VPL) nucleus of the thalamus, which then travel up and terminate in the somatosensory cortex. This relays information about the location of damage in the body (Almeida et al., 2004).
The secondary neurons of the spinoreticular tract synapse with dendrites of neurons from the reticular formation. The third order neurons are distributed in different areas

throughout the cerebellum (Meintjes, 2012). This pathway is believed to be involved in the emotional aspects of pain (Reddi et al., 2013).

1.2.3.4 Descending pain pathway

Pain can be modulated, so there is not a linear relationship between the amount of tissue damage and the amount of pain experienced. The body has an endogenous analgesic system to counteract pain. The descending pathway originates in the somatosensory cortex and the hypothalamus, from which neurons project to the thalamus. Thalamic neurons descend to the midbrain and into the periaqueductal grey matter (PAG), which is the coordinating centre for the body's analgesic system (Stamford, 1995). From the PAG, neurons descend into the raphe nucleus, which is positioned between the pons and medulla of the brainstem. When the PAG neurons are activated, they send impulses down through the brain stem. From here the signal descends the spinal cord via dorsolateral tracts. The descending neurons then synapse onto neurons in the ascending pain pathways between the sensory (primary afferent) neuron and the secondary neuron. Once activated, the neurons in the dorsolateral tract release opioid neurotransmitters, e.g. dynorphin (Stamford, 1995). Both pre-synaptic sensory neurons and post-synaptic secondary neurons contain receptor sites for the opioid neurotransmitters. The opiate neurotransmitters block the impulses in the ascending pathway and stop a signal going to the secondary neuron and travelling up to the brain.

1.2.4 Types of Pain

1.2.4.1 Acute pain

Acute pain is associated with tissue damaging stimuli; for example, pain occurs after surgery, as operations cause tissue damage (Turk and Okifuji, 2001). Acute pain usually begins suddenly, can be mild and last for a few minutes or can be intense and last up to, but not exceeding, six months (Reddi et al., 2013). The source of the pain is usually nociceptive activation and it is associated with a behavioural response (Millan, 1999). Acute pain is useful and has a protective purpose. It warns of danger and limits the use of injured body parts (Cole, 2002).

1.2.4.2 Chronic pain

Chronic pain can persist for long periods of time, from weeks to months or even years. It can persist even after an injury has healed and, in some cases, people can suffer from it in the absence of any tramua or past injury (Hielm-Björkman, 2013). It is commonly linked in dogs with degenerative diseases such as osteoarthritis or abnormal formations of the bones like canine hip dysplasia (Wiseman-Orr et al., 2006). Unlike acute pain, chronic pain has little protective significance and ultimately interferes with normal activity (Cole, 2002).

1.2.4.3 Pain Sensitisation

Pain sensitisation is an increase in the neural responsiveness following a noxious stimulus (Baranauskas and Nistri, 1998). There are two types; peripheral sensitisation where the process occurs in skin receptors, or central sensitisation which occurs in the spinal cord. Peripheral sensitisation occurs during inflammation. Inflammation is a localised protective response elicited by injury which serves to wall off both the injurious agent and injured tissue. The excitation threshold of nociceptors drops to a point where stimuli that were previously insufficient to activate them can now do so (Schaible, 2007). In addition to this, silent nociceptors can become active. These are nociceptors that are typically unresponsive to noxious intensities but respond after inflammation in the surrounding tissue. The recruitment of these silent nociceptors amplifies the inflammatory nociceptive input (Schaible, 2007).

Central sensitisation is the increased excitability in the nociceptive areas of the central nervous system (Woolf, 2011). The threshold of nociceptive spinal cord neurons is lowered, as the nervous system gets upregulated in a persistent state of high reactivity (Schaible, 2007). Central sensitisation is the result of pain wind up. This occurs when there is an increase in the excitability of the spinal cord neurons via repeated stimulation of the non-noxious afferent C fibres (Herrero et al., 2000). There are also changes to the receptor sites at the dorsal horn and in the brain. Both environment and genetics factors are important in this phenomenon; however, it is still unknown why central sensitisation can occur in some people and not in others.

Hyperalgesia is a result of sensitisation and is expressed in two forms: primary hyperalgesia, which is the result of peripheral sensitisation and occurs only at the

site of original injury, and secondary hyperalgesia, caused by central sensitisation where sensitivity is more widely distributed. Allodynia is a condition where pain is triggered by stimuli that normally do not provoke pain (Campbell and Meyer, 2006).

1.2.4.4 Visceral Pain

Pain from internal organs is termed visceral pain. The viscera are largely innervated by C fibres, and pain is triggered by smooth muscle contraction or distension, ischemia and inflammation (Arendt-Nielson and Yamitsky, 2009). Visceral pain is poorly localised and often appears to originate from an area of the surface of the body. This is because the density of the nociceptors in the internal organs is lower compared to the skin nociceptors (Steeds, 2013). Visceral pain shares the same pathway as somatic pain, and can be described as deep, sickening and dull (Arendt-Nielson and Yamitsky, 2009). Autonomic responses will occur, with vomiting, changes in heart rate and blood pressure.

1.3 Assessing pain in dogs

Assessing pain in non-human animals is difficult (Hansen, 2003). Placing a mammal in a situation that causes pain may result in vocal, behavioural and physiological responses (Fox, 2013). Several strategies have been implemented to quantify these pain responses in dogs. These include: behavioural observations, measurement of physiological responses, assessing responses before and after the administration of analgesia, and the use of quantitative sensory testing (measurement of nociceptive thresholds). An outline of the methods of evaluating pain in dogs is given below, together with an explanation of why pain sensitivity tests, such as nociceptive thresholds, were chosen as the primary method to assess pain in this thesis.

1.3.1 Behaviour-based pain assessment in dogs

Behaviour observations are the most commonly used pain assessment method in dogs (Fox, 2013). At its simplest level, this can be the observation of a single reflex response to a noxious stimulus. However, it is also possible to evaluate changes in general behaviours before and after a noxious stimulus, specific pain behaviours and abnormal behaviours (Sneddon et al., 2014). Stafford et al. (2002) outlined several key features of pain behaviour, a behaviour must be seen during and after a specific tissue damaging injury and not in a healthy animal or when local anaesthesia is used

Behavioural measurements are useful in that they are easy to do, cheap, and changes in behaviour are often immediate in their appearance, unlike physiological responses that take time to quantify.

Dogs in pain generally appear less alert and are normally quieter or hide away (Hansen, 2003). Dogs will often have mobility issues and appear quite stiff, unwilling to move or, if in severe pain, may adopt a static posture (Weary et al., 2006). Several behavioural cues should be used in judging what the pain behaviours are, including using the owner's knowledge of what normal behaviour is for their dog (Fox, 2013). Changes in posture, demeanour, activity and vocalisation of injured dogs are routinely used as indicators of pain (Fox, 2013).

1.3.1.1 Pain scales

Pain related behaviour in veterinary clinics is commonly evaluated through subjective measures, using the observer's personal judgement. Scoring systems based on such behaviours have been employed to quantify pain behaviour in dogs. This allows for standardised measures and creates a more reliable and validated way of assessing canine pain (Hansen, 2003). Validity refers to the ability to accurately measure what is supposed to be measured. Reliability is the extent in which the test yields the same or compatible results in different clinical areas or over repeated measures in the same situation (Hielm-Björkman, 2013). Benefits of a standardised test also include development of individuals skills, increased motivation to assess pain routinely and reduce individual assessor variation (Pascoe, 2012).

Uni-dimensional tests such as the visual analogue scale, the simple descriptive scale and the numeric rating scale are commonly used in clinical pain assessments for dogs (Hansen, 2003). However, there is evidence that these are actually poor indicators of pain (Holton et al., 2001; Weary et al., 2006). These ordinal scales, while simple to use, often have arbitrary numbers assigned to pain intensity and often do not define the difference between levels of pain. They are based on a subjective interpretation of pain behaviour (Mathews et al., 2014), which introduces variability due individual differences among observers, including gender, age and experience (Weary et al., 2006).

Composite pain scales (CPS) are advanced measures used to assess behavioural changes pre- and post-surgery (Minto et al., 2013; Morgaz et al., 2013; Rauser et al., 2013). These scales are made up of several parts, including physiological and behavioural responses, clinical and owner observations, and spontaneous or evoked responses (Holton et al., 2001). Researchers use these scales to explore pain relief with different analgesics and treatment regimes.

One validated CPS scale is the Glasgow Composite Measure Pain Scale, first introduced by Holton, et al. (2001). The original scale comprised a structured questionnaire and included seven behavioural categories: posture, activity, vocalisation, attention to wound or painful area, demeanour, mobility and response to touch. Each category had several worded answers to choose from, replacing the arbitrary numbers. This scale has been modified and validated over time to improve the efficiency and practicality of the test, including use in different countries and become quicker to use (Morton et al., 2005; Reid et al., 2007).

Other scales include the Colorado State University Pain Scale, Japanese Society of Study for Animal Pain Canine Acute Pain Scale and the University of Melbourne Scale (Firth and Haldane, 1999). Composite pain scales should be favoured in veterinary clinics, due to their quickness, ease of use, and validity. However, this does not seem to be the case, with one fifth of veterinarians surveyed in Queensland reported that they used a formal pain scoring system (Weber et al., 2012).

The Committee on Recognition and Alleviation of Pain in Laboratory Animals (2010), identified certain problems associated with scoring systems. These included; assessment criteria which were often highly subjective, and the reasons for using certain behaviours were not fully explained. Along with this there were problems with studies themselves in which they do not have any untreated controls, the studies did not include analgesic treatment, and lastly, when analgesics were administered only a single dosage is assessed rather than a range of doses (Criado, 2010).

1.3.1.2 Challenges in using behaviour for assessment of pain

Many of the assumptions about identifying pain behaviour are based on anthropomorphic projections of how humans experience and respond to pain (Hansen, 2003). Finding out whether a particular behaviour is indicative of pain relies

on research with and without the use of analgesics before and after testing (Stafford, 2006; Weary et al., 2006). This approach allows researchers to determine whether the behavioural change is due to tissue injury or due to environmental factors (Weary et al, 2006). However, there are ethical and practical problems with such studies, as the control groups receive less or no analgesia.

Assessing pain in dogs using behaviour is difficult as there are many factors that influence behavioural responses. Age, sex, breed, environment, and handling by a stranger or owner are all significant factors (Hansen, 2003). A dog placed in an unfamiliar, stressful place, such as a veterinary clinic, may not show typical pain behaviours due to fear (Fox, 2013). In such situations, normal pain-reducing behaviours might directly conflict with the animal's desire to remain inconspicuous (Broom, 2001). An injury or a disease can limit the dog physically and thus alter its expression of pain and making the initial identification of pain difficult (White, 2013). The incapacitation caused by damaged tissues may instead be measured rather than the actual pain. Pain behaviours can also be subtle and take a long time to notice, to such a degree that clinicians or owners may miss them completely (White, 2013). Certain behaviours (e.g. vocalisation) may be over-emphasised by pet owners. Animals will vocalize for a variety of reasons, including stress, attention and anxiety (Fox, 2013). Dogs that are severely injured often show little attention-seeking behaviour compared to those with a less severe injury (Hanson, 2003).

1.3.2 Physiological measures of pain

Physiological responses to pain can be measured and are valuable when an animal does not show pronounced behavioural responses, for example due to fear or being physically restrained (Criado, 2010). The physiological responses to pain in dogs include increased activity of the sympathetic-adrenomedullary system, heart rate, respiratory rate and blood pressure. However, these parameters are not specifically related to pain, as similar changes can be observed in stressed animals (Criado, 2010). This is not surprising given that a wide range of factors can influence the activity of the autonomic nervous system, for example physical restraint (Weary et al., 2006).

Another physiological response to pain is increased cortisol levels. Cortisol is the main glucocorticoid hormone in mammals and it is released in response to stress

through activation of the Hypothalamo-Pituitary-Adrenal (HPA) axis. The HPA system influences metabolism and has anti-inflammatory effects (Stafford, 2006). Cortisol levels can be measured by taking a sample of plasma or saliva. In dogs, several studies have shown an association between cortisol level and pain, which was done by comparing behavioural and physiological measures (Hansen, 2003; Davila et al., 2013; Perez et al., 2013). Most studies combine measurement of cortisol concentrations with other parameters, such as behaviour, to evaluate pain.

There are limitations to using cortisol to assess pain. There are fluctuations in cortisol concentration due to circadian rhythms and events not associated with pain (Gardy-Godillot et al., 1989; Colborn et al., 1991). For example, stress can stimulate high levels of circulating cortisol. Hekman et al. (2012) found that there was variation in the salivary cortisol levels among populations of healthy dogs in a hospital setting. The implications are that high cortisol levels could actually be a consequence of stress rather than pain.

1.3.3 Nociceptive Threshold testing (Quantitative Sensory testing)

Nociceptive threshold testing (NTT) applies a quantifiable stimulus to a body part until a behavioural or physiological response is observed (Love et al., 2011). Nociceptive threshold tests measure the pain threshold, the point at which a stimulus triggers nociception. Nociception occurs and signals from the nociceptors may be delivered to the brain. The brain or the spine processes the signal and produces a behavioural or reflex response**.**

The threshold can be measured in two ways. The first is to measure the latency between application of the stimulus and the expression of the behavioural response. The second is measuring the intensity of the stimulus at which the behaviour response occurs. This behaviour is usually a withdrawal of the injured body part or movement of the head towards the area to which the stimulus is applied (Love et al., 2011).

The four main types of stimulus used are electrical, thermal, mechanical and chemical (Le Bars et al., 2001). For a stimulus to be sufficiently measured it has to stimulate nociceptors, be quantifiable, non-invasive and be reproducible (Lineberry, 1981). Chemical nociception is different from the others in that it is a very slow form

of stimulation and inescapable. In this case the threshold isn't measured, rather measurements are made using behavioural scores (Le Bars et al., 2001). In nature, animals are not often exposed to chemical stimuli, thus these measurements may not be accurate or relevant. It is because of these factors that few studies on dogs have ever looked at chemical nociception.

There are several advantages of using NTT rather than behavioural and/or physiological assessments of pain: the stimulus intensity and duration can be controlled (Arendt-Nielsen and Yarnitsky, 2009); the responses shown can be quantitatively assessed and compared over time; and the animals assessed do not have to have any tissue damage, injury, or surgery (Mellor et al., 2000). In contrast, pain scoring assessments using behavioural or physiological indicators of pain require tissue damage and thus are influenced by the location, type and degree of tissue damage (Stafford, 2006). As such, studies using measurements of NTT primarily focus on providing a better understanding of the inherent responsiveness of the pain processing system and the effectiveness of analgesics (Bergadano et al., 2006).

1.3.3.1 Electrical NTT

The nociceptive thresholds for an electrical stimulus are assessed by applying an electrical current to a body part, via electrodes. This stimulus produces a behavioural withdrawal reflex (Bergadano et al., 2007). The magnitude of this reflex is measured by an electromyogram (EMG), which monitors the muscle electrical activity of, for example, the upper leg when electrical stimulation is applied to the lower leg (Bergadano et al., 2007).

Electrical stimulation is an unnatural stimulus and is rarely encountered in the wild. Animals only receive electrical stimulation in laboratory situations. In addition, it is a non-specific stimulus; electrical stimulation activates different peripheral receptors, including the fine Aδ and C fibers, which are linked to thermal nociception (Le Bars et al., 2001). This may confound results, as it might not reflect the animals' real pain sensitivity.

1.3.3.2 Mechanical NTT

Noxious mechanical stimuli are generated using equipment that gradually increase pressure on a body part (Le Bars et al., 2001) or that distend hollow viscera (Love et al., 2011). The measurable aspect of these tests is the minimum amount of pressure that is needed for a threshold response behaviour to occur. Mechanical nociceptive threshold tests are commonly used in large animals such as cattle, horses and sheep (Chambers et al., 1994; Love et al., 2011). Several studies using mechanical threshold tests have been performed on dogs. Dixon et al. (2010) constructed a rolling diaphragm air pressure actuator to fit on dogs and cats. A syringe was used to manually inflate the pressure in the actuator, allowing a linear increase in applied pressure. Von Frey filaments, devices that apply the tip of a wire onto the skin at a consistent pressure, have also been used to assess mechanical pain thresholds in dogs (Moore et al., 2013; Briley et al., 2014). Moore et al. (2013) used these to compare sensitivity in neurologically normal dogs with and without cranial ligament rupture and in dogs with acute spinal cord injury. Dogs with acute spinal cord injury had higher sensory threshold values compared to normal dogs. Indicating that this test was able to accurately predict that dogs in pain had a higher pain sensitivity.

There are several limitations to mechanical pain threshold testing. Firstly, the device must remain in direct contact with the skin to form an even pressure distribution, a challenge in practical terms (Le Bars et al., 2001). For the pressure actuator device, the noise from the motor and pump can be distracting and, may result in dogs learning to associate the noise with pain (Coleman et al., 2014). The authors found that dogs were able to anticipate the stimulus and thus reacted at lower thresholds. In addition, it is difficult to perform the procedure on free moving animals and therefore restraint is needed. This could increase stress and influence nociceptive threshold levels (Imbe et al., 2005). Sensitisation or desensitisation can also play a part; repeated testing in an area can either reduce or increase the sensitivity of the body test site. Von Frey filaments also come in a large variety of probe styles and tip diameters (Le Bars et al., 2001). This means that there is a wide variability in values of mechanical thresholds among species and individuals of different size within a species.

1.3.3.3 Thermal NTT

Thermal nociceptive threshold (TNT) testing involves stimulating the thermal nociceptors with a cold or hot thermal source. For example, a thermode can be used; this consists of a probe containing a heating element and a temperature sensor which is held to a shaved area on the body (Dixon et al., 2002; Love et al., 2011; Hoffmann et al., 2012). Several other examples are; the CO_2 laser (Veissier et al., 2000; Farnworth et al., 2013), which uses a thermal beam focused onto an area of the skin; the use of heat plates (Bannon and Malmberg, 2007; Wegner et al., 2008), in which the animal stands on an area that heats up; or the use of the heat from a lamp source (Le Bars et al., 2001). A thermal stimulus is applied to the skin which heats up until the nociceptors are activated resulting in a behavioural response. The measurable variables are the temperature at which the response behaviour occurred and the latency from the start of heating till the expression of the response behaviour. The advantages of using heat as a stimulus are that it is considered a natural stimulus and that it uniquely activates heat-sensitive nociceptors (Arendt-Nielsen and Chen, 2003). Various factors, including ambient temperature, have been shown to influence nociceptive testing.

1.3.3.3.1 Ambient temperature

The effects of ambient temperature on thermal nociceptive testing are conflicting. Variations in ambient temperature could have an effect on skin temperature in terms of cutaneous vasodilation and vasoconstriction and impact the dissipation of heat during thermal nociceptive tests (Love et al., 2011). In rats, there have been reports that variations in the skin temperature affect the latency to respond in the tail flick test (Hole and Tjølsen, 1993; Vítková et al., 2015). Another study that used the same tail flick test on rodents found that ambient temperature had no effect on temperature of the skin of which the pain response behaviour occurred (Lichtman et al., 1993). Poller et al. (2013) found that in horses, the temperature at which the end point behaviour occurred varied according to cold (<10 ^{o}C) and warm (>20 ^{o}C) ambient temperature. The study found that the temperature of the skin after response was significantly lower at warm ambient temperatures compared to cold. It seems that nociceptors require more energy to reach the activation threshold and generate an action potential in the cold, thus there is a larger latency for the

withdrawal behaviour to occur. In calves, it was observed that there was no effect of ambient temperature on the temperature of the skin at response between the 5 ^{o}C and 27^{o}C (Whay, 1998).

1.3.3.3.2 Remote thermal NTT

Remote thermal NTT may be performed with a thermal beam focused onto an area of the skin without touching it. Thermal threshold research with remote heating has been used on laboratory animals (Le Bars et al., 2001), horses (Queiroz-Neto et al., 1998; Carregaro et al., 2007), cats (Farnworth et al., 2013), sheep (Guesgen et al., 2011) and cows (Veissier et al., 2000). In dogs heat plates have been used (Wegner et al., 2008), during which dogs were restrained in a fabric sling and stood on a glass plate. Individual focused projection bulbs were placed below the glass plate. The metatarsal pad of the left and right hind paw of the dog was then placed over each light, and the light bulb heated until a behavioural response occured. Radiant heating presents difficulties in terms of nociception activation as the skin is able to both reflect and absorb radiation (Love et al., 2011). The conduction properties of the skin influence the amount of applied radiation needed to increase the temperature of the skin to get to the threshold level i.e. to activate the nociceptors (Love et al., 2011). The initial temperature of the skin and the area chosen for testing must be taken into consideration, as they can both influence the thermal nociceptive activation. The close proximity of researchers to the animal required to apply some radiant heat can also lead to an increase in stress and confound results and responses (Criado, 2010).

The use of a carbon dioxide (CO_2) laser can reduce some of the problems associated with nociceptive thermal heating, as only a small amount of energy is lost via reflection from the skin, i.e. most thermal energy is absorbed. Thermal nociceptive tests with the CO_2 laser the technique has been validated with the use of cats (Farnworth et al., 2013) and cows (Veissier et al., 2000). In the Farnworth et al. (2013) study, the latency for a behavioural response (skin twitch or turn of the head) was measured. Skin temperature was not measured as it was expected to continually increase after testing in response to CO_2 laser stimulation (Carregaro et al., 2007). A problem arising from the use of CO_2 lasers is that the animal must be contained to limit movement and thus placed in a confined space. This may also

increase stress and influence the behavioural responses shown. The CO_2 laser may be dangerous for the users, is specialised equipment and is hard to use in a clinical setting.

1.3.3.3.3 Direct thermal NTT using thermodes

This method involves placing a thermode and temperature sensor onto a shaved area of skin. The thermode is heated at a constant rate and the temperature and latency at when a response behaviour is shown is measured. Thermode devices in nociceptive threshold testing have been validated and proven to be a useful tool in assessing pain sensitivity. For example, Dixon et al. (2002) used a direct contact thermode to evaluate analgesics in cats. Several studies using similar thermodes on cats have been undertaken (Steagall et al., 2007; Taylor et al., 2007; Millette et al., 2008), and the device has been modified for the use on horses (Robertson et al., 2005; Love et al., 2008; Poller et al., 2013). Hoffmann et al. (2012) used thermodes on dogs and looked at reproducibility with the device and the antinociceptive properties of analgesics. The research found that thermodes were a suitable method for assessing pain sensitivity in dogs as saline placebo thermal thresholds were stable over a six month period.

Thermodes can be controlled very precisely, and the rate of heating is adjustable (Love et al., 2011). Thermode testing is relatively simple and moderately cheap. An issue with using these devices for NTT is that restraint is needed to put the device on and then to manually check the temperature of the skin on the testing site. This may cause stress and influence the nociceptive responses of the dog (Criado, 2010). The thermode must remain in contact with the skin at all times, a problem as the shape of the thermode is flat and fixed. To overcome this problem an elastic band can be used to ensure the thermode has consistent pressure on the skin. Dixon et al. (2002) used an inflatable pressure bladder to adjust the amount of pressure the thermode exerted onto the skin. It was believed that the mechanical contact with the skin by the thermodes activate mechanosensitive afferents (Svensson et al., 1997). However, McMullan et al. (2004) argued that once contact has been made between the thermode and skin, inputs from the mechanical afferents will have a low effect on the thermal thresholds due to fatigue and adaptation to the stimulus.

1.4 NTT differences with-in mammalian species

Reseach involving quantitative sensory testing has shown that within species there are individual differences in pain senstivity (Le Bars et al., 2001; Love et al., 2011). In rodents, there is evidence that variation in pain sensitivity is partially mediated by genes (Shir and Seltzer, 2001). Eleven inbred strains of mice were tested using twelve separate measures of nociception (Mogil et al., 1999). All nociceptive assays displayed moderate-to-high levels of heritability (h^2 = 0.30–0.76). The behavioural traits have a significant heritable competent in mice and each strain had its own pattern of responsiveness (Mogil et al., 1999). However, Nielsen et al. (2008) indicated that there is also a considerable environmental influence. Subtle features in laboratory environments can have an impact on the pain responses in mice (Crabbe et al., 1999). One of the largest influences appears to be the experimenter performing the testing. Crabbe et al, (1999) used three different sites for testing the same strain of mice and had identical equipment, husbandry and testing protocols. The behaviours differed greatly from site to site. It seems likely that this effect could be due to differences in animal handling and thus different levels of stress in the subject (Chesler et al., 2002).

Nociceptive tests on humans have shown that ethnicity can influence pain perception (Woodrow et al., 1972; Zatzick and Dimsdale, 1990). Sheffield et al. (2000) reported that African-Americans had lower thermal pain tolerances and rated it as more unpleasant than Caucasians. The reason for this may be due to the activity in higher nervous centres that modulate and inhibit pain (Edwards et al., 2001). African-Americans could be more disposed to hypertension and have a larger cardiovascular reactivity than Caucasians, which could influence pain perception. However, several social and psychological factors such as, gender and age differences, race of experimenter, socioeconomic status, and stress could also be the cause of the difference between ethnic groups in pain perception (Edwards et al., 2001).

1.5 Physiological differences between dog breeds

Dogs do differ physiologically, due to their genetic makeup. Many breeds have genetically based diseases, with over 370 inherited disorder identified in purebred dogs (Fleischer et al., 2008). Immune differences were found between police Labrador retrievers and German shepherds (Villaescusa et al., 2012), with

differences in the blood leukocytes subsets. Age, sex, environment, housing, diet and exercise were similar in both breeds and thus it could be concluded that the initial findings show a difference in the immune system. Breed, age and sex, had significant effects on many everyday behaviour traits (Asp et al., 2015). Working breeds were about 10% more trainable, showed 30% more interest in playing with humans and were 10 – 60% less fearful. Other research has found breed differences in behaviour, however there also appears to be significant within breed differences in behaviour (Mehrkam and Wynne, 2014). Thus breed differences in behaviour are influenced by both the environment and genetics. Research in the physiological differences between dogs has involved investigation of coat pigmentation, the texture and length. The coat types have been the result of dogs under closed breeding lines with strong selection for desired traits (Kaelin and Barsh, 2012). Across breeds, similar traits have identical genetic determinants; however there are some exceptions with this (Kaelin and Barsh, 2012).

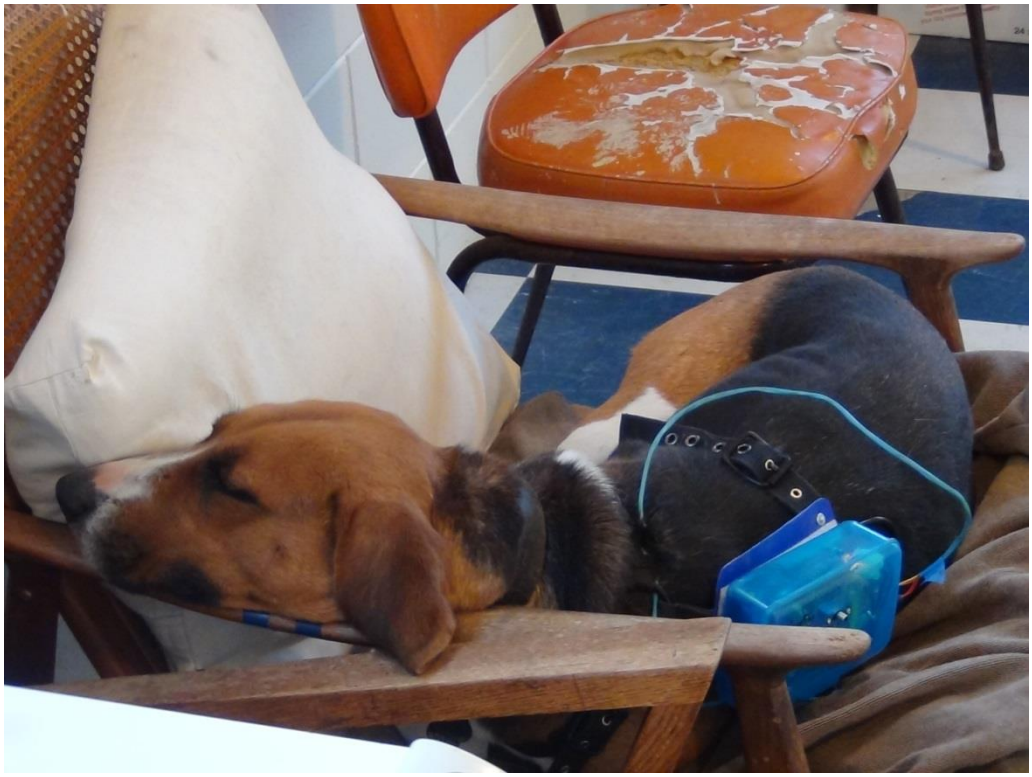
1.6 Conclusions

Pain is a complex experience, involving both physiological and emotional elements. While pain is generally an adaptive trait, it can cause considerable welfare issues, and the management of pain is important. In dogs, there has been an increased focus on the provision of appropriate pain-relief. This is reflected by the dozens of research trials in the last decades investigating comparative analgesic effects post-surgery (Stafford, 2006). While most dogs are provided with analgesics post-operatively, this does not mean that pain relief is effective. Very little work has been done to look at differences in individual pain sensitivity in dogs, with most pain studies focusing on the effectiveness of analgesics. No studies have looked at variability in baseline pain sensitivity among dog breeds.

The following research investigated whether there was any difference in TNTs between three dog breeds. TNTs was objectively assessed using a novel thermal device. This device removed the need to constrain the dog's movement and allowed all data to be instantly transmitted to a computer.

This thesis comprises two studies. The first study aimed to validate the nociceptive threshold device and determine the most appropriate procedure for further testing. The second examined the differences in NTT between three dog breeds.

2. Repeatability of thermal nociceptive thresholds measured with a new remotely activated device and the effect of initial thermode temperature on thermal thresholds of harrier hounds



Harrier hound sleeping after testing

2.1 Introduction

Nociceptive threshold testing (NTT) is used to objectively assess pain sensitivity in animals. The nociceptive threshold is the minimum intensity of a stimulus that is perceived as painful (ISAP, 1994). NTT evaluates pain sensitivity by measuring either the latency to respond or the strength of the stimulus when a threshold behavioural response occurs or both (Love et al., 2011). The advantages of using NTT to measure pain sensitivity are threefold: the stimulus intensity and duration can be controlled (Arendt-Nielsen and Yarnitsky, 2009); the responses shown can be quantitatively assessed and compared over time; and the animals assessed do not have to have any prior injury (Le Bars et al., 2001).

In dogs, nociceptive tests have been performed to provide a better understanding of the effectiveness of analgesics (Bergadano et al., 2006). Nociceptive thresholds in dogs have been assessed using thermal (Wegner et al., 2008; Hoffmann et al., 2012), electrical (Bergadano et al., 2006; Bergadano et al., 2007), and mechanical (Dixon et al., 2010) stimuli.

Thermal testing involves increasing the temperature of the peripheral tissue until cutaneous thermo-sensitive nociceptors are activated and ultimately a behavioural response occurs. Thermal nociceptive thresholds (TNT) in dogs have been assessed using thermodes (Hoffmann et al., 2012) and with heat plates (Wegner et al., 2008). Thermodes consists of a probe containing a heating element and a temperature sensor and are held to a shaved area on the body. With heat plates, the dogs are restrained in a fabric sling and stood on a glass plate. Focused projection bulbs are placed below the glass plate and metatarsal pad of the left and right hind paw of the dog are placed over each light. The light bulb heats the dogs paw until a behavioural response occurs (Wegner et al., 2008). Thermodes have the benefit of being able to be controlled very precisely, and the rate of heating is adjustable (Love et al., 2011). Thermode testing is relatively simple and moderately cheap.

The use of thermodes for testing nociceptive thresholds has been validated for cats (Dixon et al., 2002; Robertson et al., 2003; Steagall et al., 2007; Slingsby et al., 2009). However, the thermal devices used so far for threshold testing in dogs often place the animal in a stressful situation. Often the dogs need to be restrained (Wegner et al., 2008) or have limited movement (Hoffmann et al., 2012). In addition

the device must be attached to the animal and a person must touch or be very close to the animal to deliver the stimulus and monitor the temperature of the skin (Veissier et al., 2000). All of these factors may elicit a stress response and influence the nociceptive response the dog displays (Criado, 2010). Thus there is a need to evaluate the repeatability of TNTs in dogs measured using a remotely activated thermode based system.

One problem with nociceptive testing is that environmental factors can influence the threshold response. For example ambient temperature has been shown to influence thermal nociceptive thresholds in some studies (Love et al., 2011). A correlation was found between initial tail-skin temperature on the latency to respond in rats with ambient temperatures set at 18, 20, 24 or 26 ^{o}C (Vítková et al., 2015). However, another study on rodents with temperatures between 5^{o}C and 38 ^{o}C found no evidence of this effect (Lichtman et al., 1993). In cattle it was found that the ambient temperature affected the response latencies only when the temperature was below 7^{o}C (Veissier et al., 2000). Poller et al. (2013) observed in horses that response temperature was significantly lower (i.e. there was greater sensitivity) at warm ambient temperatures than cold. They suggested that it might be necessary to heat the probe before commencing testing to reduce this effect. However, the effect of initial skin temperature on TNTs in dogs has not been assessed.

This study investigated whether the initial temperature of the thermode had any effect on TNTs in dogs and examined the repeatability of thresholds measured using a new remotely operated thermode device on dogs.

2.2 Materials and Methods

2.2.1 Animal Ethics Approval

All procedures were approved by the Massey University Animal Ethics Committee (Protocol 14/28).

2.2.2 Animals and facilities

Eleven adult harrier hounds (Table 2.1) from the Massey University Nutritional Research Unit (MUNRU), Palmerston North, New Zealand were used in the study. All of the males were castrated and all females were entire. All procedures were

conducted on site at the MUNRU, where the dogs lived permanently. Outside of testing and the habituation period, dogs were maintained at the unit according to normal husbandry practices. The dogs slept indoors overnight in pairs. They were fed their standard diet in the morning and let out in fenced paddocks during the day. The experiment was conducted from the 2nd June to the 28th June 2014.

Testing was carried out in a naturally lit room approximately 5m x 3.2m x 2.5m. It was approximately 12m away from the dogs' sleeping pens. During the habituation and testing phase two dog mats, a bowl of water and a table for a laptop were brought into the room. The room was not heated and the ambient temperature ranged from 14.5 to 20 degrees Celsius.

The dogs' Golly and Royal were on heat in week 3 and week 4 respectively. Another male (Odin) was going to be used, but removed due to restlessness throughout testing.

Table 2.1: Dogs used in the first study.

Harrier hound names	Sex	Age (years)
Quick	M	2.5
Nemo	M	3.8
Gloman	M	5.8
Royal	F	2.5
Neat	F	3.6
Jade	F	4.6
Golly	F	5.8
Fay	F	6.4
Dawn	F	8.4
Chrissy	F	9.4
Chorus	F	9.4

2.2.3 Habituation

Dogs were habituated and tested in familiar pairs. Habituation to the testing room, procedure and the researcher occurred over two weeks. In the first week of habituation each pair of dogs were bought into the testing room and had a single one hour session during the course of a week. The purpose of this was to habituate the dogs to the testing room and equipment that was used. During this session a 25 x 25

mm area was shaved on the medial surface of their left foreleg, distal to the elbow joint and proximal to the carpal joint. Three thermal nociceptive trial tests with a 10 minute gap in-between tests were performed. This was done to make sure the device worked and the dogs were comfortable with the heating process. After this they were returned to the colony. The harrier hounds are commonly used as training for veterinary students and the greyhounds were all regular blood donors. Thus, both were familiar with human handling and being shaved. The huntaways had less socialisation with humans compared to the other two breeds, however every day they had handling by a familiar handler. They only thing they were not familiar with was the shaver, which is why habituation involved getting the dogs used to the shaver.

A trial was intended to start after this first week of habituation, however there were several complications with the device where no data was recorded. Thus, this second week was used as an additional week of habituation. The dogs were tested in the same pairs as the first week of habituation. Once a day over the week a pair of dogs was brought into the testing room and six nociceptive tests were performed on each dog. Nociceptive testing alternated between each dog, with a five minute gap between each test. Each pair was only tested once during this week and the testing lasted between one hour and an hour and a half. The leg was shaved as per the first week of habituation. This extra week however also allowed the researcher to learn the individual behaviour responses of the dogs. The device was adjusted and fixed so that the experiment could start in the following week.

2.2.4 Experimental design

During the test period, six tests were performed on each dog on one day per week for four consecutive weeks (total of 24 tests). Each dog was tested on the same day each week and in the same pair as the habituation period. Tests were performed under two different conditions: the normal baseline temperature condition, where the thermode was activated without changing the initial thermode temperature; and the elevated baseline temperature condition, where the temperature of the thermode was raised to 35^{o}C before each test began. Each temperature condition was tested three times; the three normal baseline condition tests were completed first and then the three elevated baseline conditions tests were done.

2.2.5 Thermal nociceptive threshold testing device

The device used in this experiment was a small animal thermal nociceptive threshold tester (SA-TNT), designed and constructed by Dr Michael A. Gieseg. It had several key design features:

1. The thermode was set to heat a metal disc measuring 12 mm in diameter from 20°C to 60°C at a rate of 0.5°C per second.
2. It had a built in safety mechanism that automatically shuts down heating once a maximum temperature had been reached.
3. It could be controlled remotely so the test animal could be free in an enclosure and not require restraint.
4. It had a self-contained power supply that could run for around 10 hours.

The equipment used was based on a device designed for use on cats by Dixon et al. (2002).

2.2.5.1 Thermode

Two thermodes were used in the trial, a third one was kept in reserve. The thermodes were called Dallas 1, 2 and 3. The thermode consisted of a copper disc (12 mm in diameter and 0.2 mm thick), a Dallas DS18B20 digital temperature sensor and a 10 ohm 0.25 W resistor. The resistor and temperature sensor were bonded to the disc using an Electrolube Thermal Bonding system. This was smoothly encased to a depth of ~ 5 mm with hard setting polymer. The thermode was positioned on the animal's leg using a nylon-webbing strap (35 mm) held in position with Velcro. A small compressed rectangle foam (35 x 25 mm) glued to the nylon strap helped to compress the thermode against the leg and keep it firmly positioned (Figure 2.1). After one day of testing the Dallas 2 thermode wiring was chewed through and Dallas 1 and 3 were used throughout the rest of testing.

2.2.5.2 Controller

Two controllers were used. They consisted of an Arduino Uno board running an Atmega328P microprocessor and MOSFET transistor used to amplify or switch the electronic signal. These devices controlled the heating of the thermode using pulse width modulation and a BlueSMiRF Silver Bluetooth Modem (SparkFun Electronics).

Connections and wiring to the Arduino Uno were custom built on a Protoshield Basic (Freetronics). The Arduino was programmed to increase or decrease the power to the resistor in the thermode to get linear heating. The Bluetooth modem allowed the controller to connect to a laptop computer and provide an update on the thermode temperature at 0.5 sec intervals and to receive commands from the observer. Everything was housed in a polycarbonate box measuring 130 x 95 x 45 mm with a single on-off switch on the side. The Dallas thermodes could be disconnected from the controller (Figure 2.1). Connections from the controller to the thermode were made with a 3.5 mm stereo plug (to the temperature sensor) and a 2.1 mm DC power plug (to the resistor).

2.2.5.3 Power supply

The thermode and controller were powered by two Lithium Polymer 3.7 V 3000 mAh batteries (Ultrafire 18650) running in series.

2.2.5.4 Harness

A large dog harness consisting of nylon webbing and buckles was used. It was modified so that the controller could be attached to the harness on the dog's back using Velcro (Figure 2.2). The harness had two loops, one that encircled the neck and the other surrounding the torso with connecting straps between them. Each loop could be adjusted to ensure the controller was balanced correctly. The wire of the thermode was wrapped around the harness while the thermode was attached to the forelimb.

2.2.5.5 Software

The software that controlled heating of the thermode was written in the Arduino programing environment IDE, version 1.0.5 (Arduino LLC) and uploaded using a USB serial port. Communication with the controller was established by a Bluetooth connection on a laptop computer running the software CoolTerm, version 1.4.2 (Roger Meier's Freeware). The software allowed data to be received from the controller and to send commands back to the controller. Coolterm allowed the data to be saved as a txt file, so that it could be reviewed later.

2.2.5.6 Commands

Once ready, the observer pressed ‘a’ on the computer triggering the thermode to begin heating. The controller software heated the thermode at a rate of 0.5°C per second. Testing was stopped by the observer pressing ‘b’ once the dog’s behavioural cue was observed.

If no behavioural signs of nociception were observed by the time the thermode reached 55°C, a sub-programme set in the software would be initiated and heating was terminated automatically. The aim of this cut off temperature was to avoid causing burns to the dogs skin.The cut off temperature was determined by previous studies (Gieseg, unpublished data; Hoffmann et al, 2012).

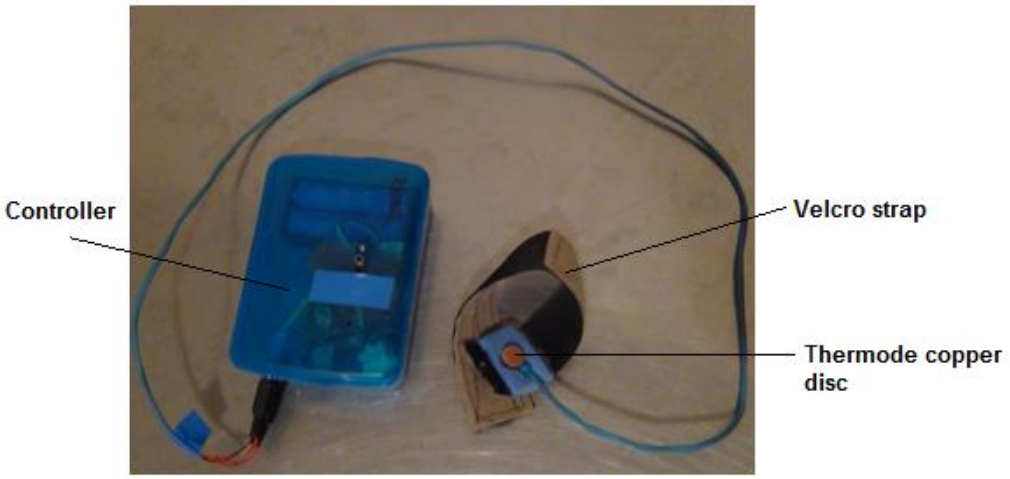


Figure 2.1: The thermal nociceptive threshold device, showing the custom made controller and thermode.

Figure 2.2: The thermal nociceptive threshold device, showing the dog harness with the controller attached.

2.2.6 Experimental procedure

Testing started at approximately 15:00h each day, with a pair of dogs being bought into the test room. Once in the test room, the dogs were given a few minutes to adjust to the situation. An area of skin on the dog's left foreleg measuring approximately 25 x 25 mm was clipped and washed with swabs and water to remove debris. Occasionally the leg would have to be switched to the right leg if there were any injuries or burn marks on the left. The harnesses were put on the dogs and then the controller box were attached (Figure 2.2). The thermode was placed onto the clipped skin area and then the wire was wrapped around the harness and connected to the controller. Each controller was turned on and connected via Bluetooth to the laptop. Two coolterm programmes would be running simultaneously, with each one linked to a separate device. The same thermode was used for each dog every week apart from several exceptions: Chorus during week one because this was the only data obtained from Dallas two before it was chewed through; Golly and Gloman also during week one because it was a different thermode to that used for the rest of their testing. There were also two missing data points: Golly in week four, third elevated test baseline and Chrissy in week two, third elevated baseline test.

TNTs were determined by measuring the latency of the dog to respond and the temperature at which the dog responded. A dog was determined to have reached its TNT when it showed a pain response behaviour, moving the leg with the attached

thermode, and/or turned its head towards the thermode. Testing was stopped once one of these behaviours was observed. If no obvious behavioural indication of nociception was shown before the thermode reached temperature of 55°C the test was terminated and the thermode turned off automatically. The temperature measured was the thermode temperature rather than skin temperature. The ambient temperature was measured after each individual test.

With each individual dog there was a 10 minute break between each test to allow the thermode to cool. To ensure this, testing alternated between the two dogs, with a 5 minute gap between each dog. For example, if dog A was tested first, after five minutes dog B was tested, and then after another five minutes the testing would return to dog A. When no obvious behavioural signs were reached due to lack of pressure of the thermode onto the skin and the test reached the cut off it was listed as a fail. The thermode was readjusted and the test was repeated after a 10 minute resting period which allowed the thermode to cool down. The devices were also left on the dogs in between testing sessions. Testing would take around one hour to complete for both dogs. Once the tests were completed the pair of dogs would be returned to their pen.

2.2.7 Statistical analysis

Raw data were initially investigated graphically to see if any trends existed. This was used as a basis for subsequent analysis. Preliminary analysis investigated the effects of the condition of the tests and the two different thermodes. Twenty individual observations for latency to respond were removed and nineteen for response temperature (Chrissy week two, third elevated baseline test was available for temperature). The remaining raw data was analysed without transformation as it met assumptions for parametric analyses and thus was approximately normally distributed. Twenty tests failed due to lack of pressure of the thermode and had to be repeated ten minutes later.

Latency, response temperature and initial thermode temperature variables were analysed using a linear mixed model analysis in SAS version 9.4 (SAS Institute Inc. Cary, NC, USA). The best model was selected using Akaike's information criterion. The model had the fixed effects of thermode (Dallas 1 & 3), condition (normal baseline, elevated baseline), week (1-4), test (1-3), and dog as a random effect and

test as a grouping variable. It included the interactions of test and condition, week and test, condition and device, and week and device. Effects were considered significant at $p<0.05$. When a significant effect was found, post hoc tests were undertaken with a manual correction for multiple comparisons, where by the p value was multiplied by the number of comparisons made.

2.3 Results

2.3.1 Latency to Respond

The significant effects on latency to respond are shown in Table 2.2. The mean of latency to respond for the nociceptive thermal threshold of eleven dogs for all time points was 29.0 ± 0.3 seconds.

There was a week by condition interactive effect; in week two and three, dogs responded more quickly when the initial thermode temperature was elevated than when it was normal. Comparison between the weeks within each condition showed that in the normal condition the dogs responded more quickly in week one than week two and three (Figure 2.3). There was no difference among weeks in the elevated condition.

There was a significant interaction between condition and thermode on latency to respond. At the normal baseline condition, dogs were quicker to reach their response with the Dallas three thermode than Dallas one ($t=-5.68$, $P= 0.0003$). However, there was no significant difference between the thermodes when starting temperature of the thermode was elevated ($t= -2.18$, $P=0.06$). Overall dogs were quicker to reach their response with both thermodes when the start temperature was elevated (Dallas 3: $t=3.65$, $P= 0.02$; Dallas 1: $t=7.58$, $P= <0.0001$).

There was no effect of test nor any interactions between test and other variables on latency to respond.

Table 2.2 Results of statistical analysis for latency to respond (seconds), response temperature (^{o}C), and initial thermode temperature (^{o}C) using raw data. Only significant interactions between variables at $p<0.05$ are shown.

Variables	Week		Condition		Thermode		Test		Condition x thermode		Week x condition		Test x condition	
	F (3,24)	P	F (1,9)	P	F (1,9)	P	F (2,18)	P	F (1,9)	P	F (3,27)	P	F (2,20)	P
Latency to respond	7.73	0.0007	61.07	<0.0001	30.71	0.0004	0.12	0.89	5.98	0.04	4.22	0.01	2.85	0.08
Response temperature	6.18	0.03	0.72	0.42	9.01	0.01	1.48	0.25	0	0.96	3.48	0.03	1.33	0.29
Initial thermode Temperature	4.03	0.02	388.34	<0.0001	44.63	<0.0001	7.85	0.0004	39.74	0.0001	4.17	0.02	7.7	0.003

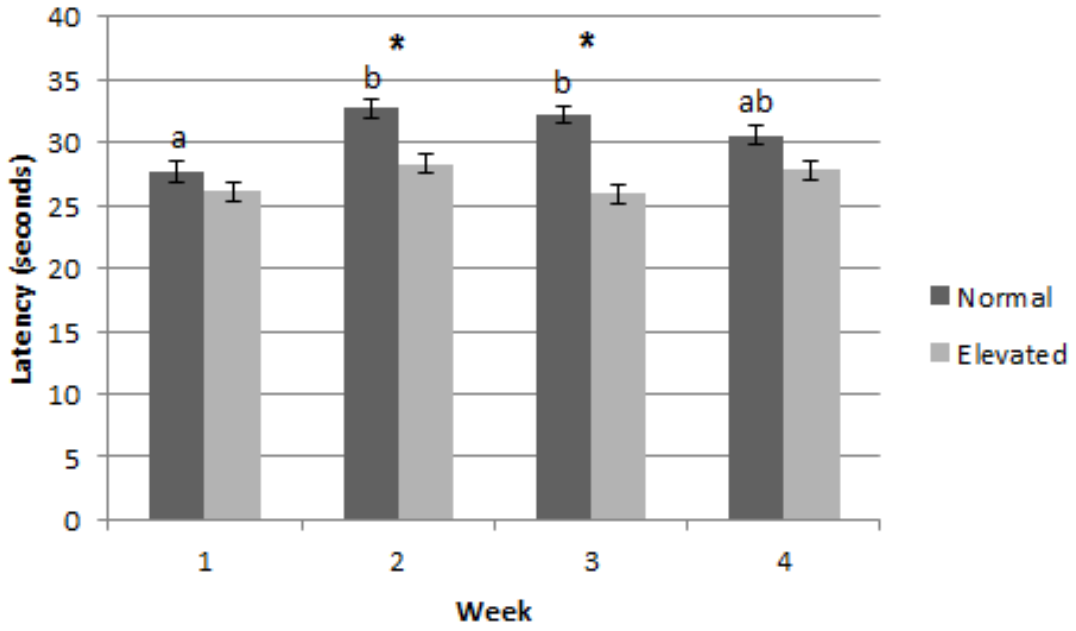


Figure 2.3: Differences in raw mean ± SE between the weeks for latency to respond (seconds) with both normal and elevated baseline conditions. Significant differences between weeks within the normal condition are indicated by the different letters. Significant differences between conditions within week are indicted by asterisk. Differences considered significant at $p<0.05$

2.3.2 Response temperature

The significant effects on response temperature were week of testing, thermode and the interaction between week and condition (Table 2.2). The mean of response temperature for the nociceptive thermal threshold of eleven dogs for all time points was 48.8 ± 0.1^{o}C.

The dogs responded at lower temperatures with the Dallas 3 thermode (48.5 ± 0.17^{o}C) than with Dallas 1 (49.2 ± 0.16 ^{o}C).

A week and condition effect existed. In the normal baseline condition the dogs responded at lower temperatures in week one than all subsequent weeks (Figure 2.4). In the elevated baseline there was no difference among most the weeks, with the exception of week three and four. In week three only, the dogs tended to respond at lower temperatures when the baseline was elevated compared to normal ($t=3.12$, $P=0.06$), there was no significant differences in the other weeks (Figure 2.4).

There was no effect of test nor any interactions between test and other variables on response temperature.

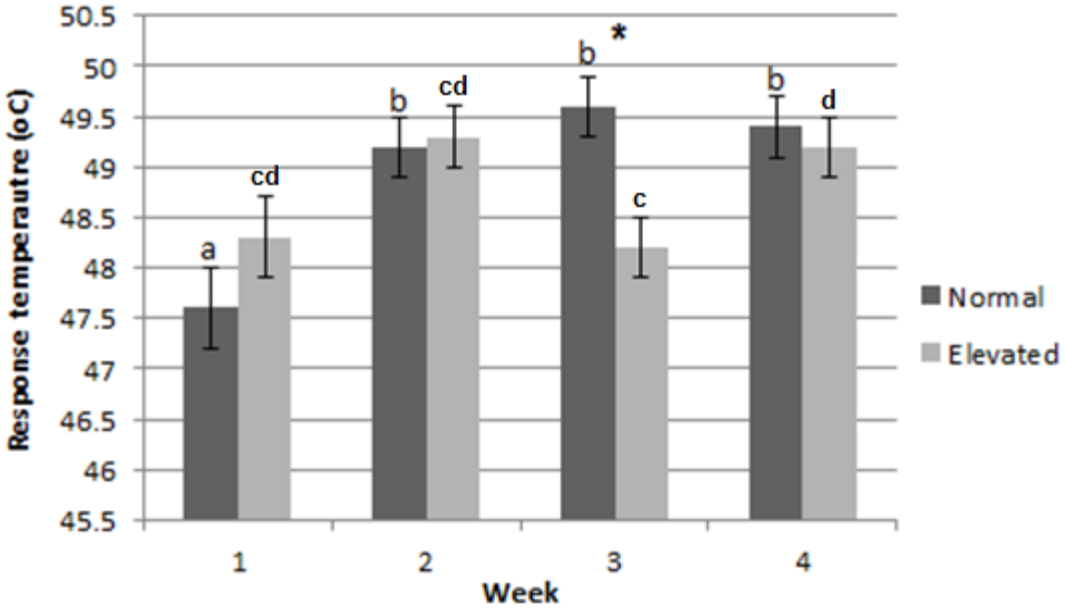


Figure 2.4: Differences in raw mean ± SE between the weeks for response temperature (^{o}C) with both normal and elevated baseline conditions. Significant differences between weeks within the normal condition are indicated by the different letters a and b. Significant differences between weeks within the elevated condition are indicated by the different letters c and d. Significant differences between conditions within week are indicted by asterisk. Differences considered significant at p<0.05

2.3.3 Initial thermode temperature

In week two, the initial thermode temperature was lower than other weeks (Figure 2.5). The normal baseline condition had a significantly lower starting temperature over all four weeks compared to the elevated condition.

In the normal baseline condition, the initial thermode temperature was different between the two thermodes. Dallas three (33.9 ± 0.09 ^{o}C) started at higher temperatures than Dallas one (32.7 ± 0.09 ^{o}C), (t= 9.21, P=<0.0001). There was no difference in initial temperature between thermodes when condition was elevated. Both thermodes had a lower starting temperature in the normal condition compared to the elevated (Dallas three: t= -9.3, P= <0.0001; Dallas one: t = -19.2, P= <0.0001).

There was a test and condition effect, in the normal condition the thermode had a lower start temperature in the first test than the second (t=-3.5, P=0.02) and third (t=-5.5, P=<0.0001). There was no difference between tests for the elevated baseline condition. All three normal condition tests had significantly lower starting temperatures than the elevated condition (Figure 2.6).

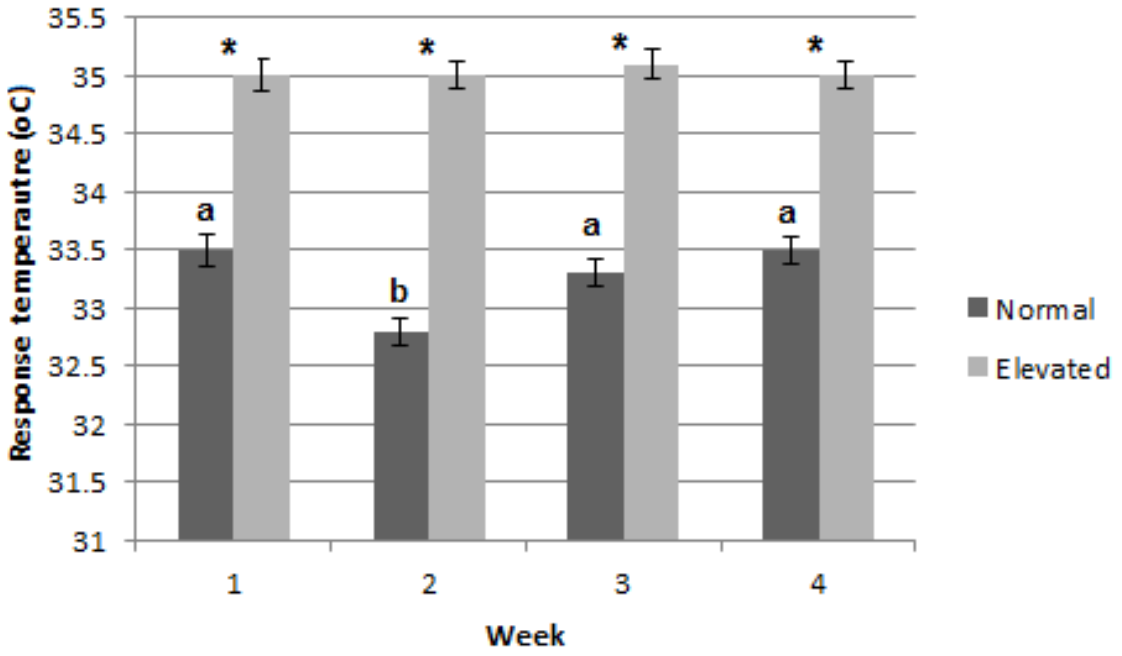


Figure 2.5: Differences in raw mean ± SE between the weeks for initial thermode temperature (^{o}C) with both normal and elevated baseline conditions. Significant differences between weeks within the normal condition are indicated by the different letters. Significant differences between conditions within week are indicted by asterisk. Differences considered significant at $p<0.05$

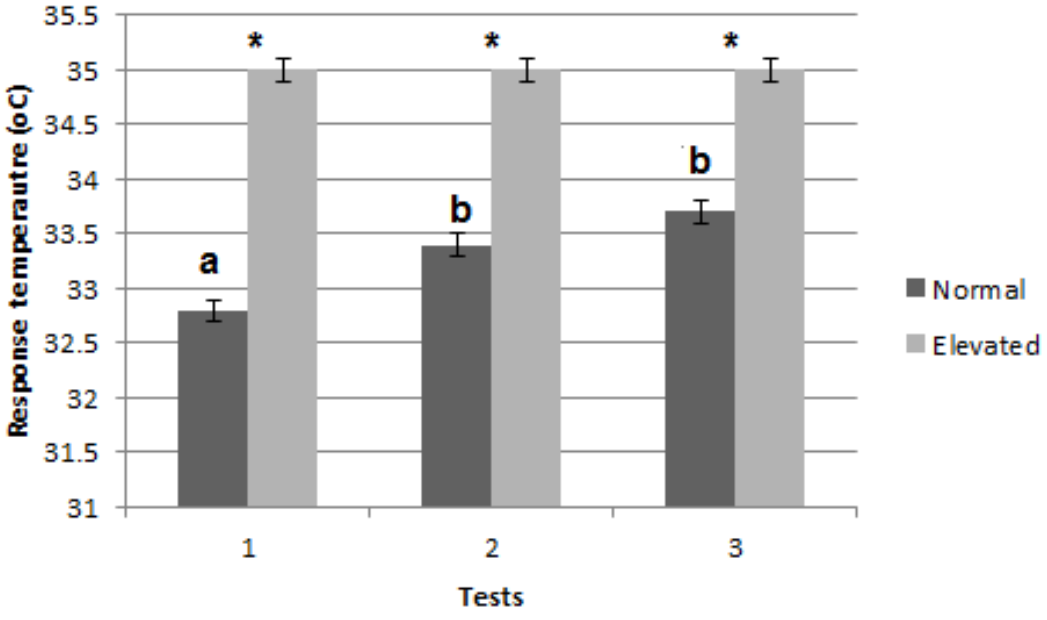


Figure 2.6: Differences in raw mean ± SE between the tests for initial thermode temperature (^{o}C) with both normal and elevated baseline conditions. Significant differences between tests within the normal condition are indicated by the different letters. Significant differences between conditions within tests are indicted by asterisk. Differences considered significant at $p<0.05$

2.3.4 Ambient temperature

The average mean of the ambient temperature for week two (raw mean ^{o}C + SE, 16.1 ± 0.18) was lower than week three (16.9 ± 0.06) and four (16.9 ± 0.23). Ambient temperature were not collected in week one.

2.4 Discussion

The first aim of this study was to see whether changing the initial temperature of the thermode had any impact on the thermal nociceptive thresholds of dogs. There was a significant difference between the normal baseline condition and the elevated baseline condition, with the initial thermode temperature being lower in the normal condition. The results suggest that testing nociceptive thresholds is more reliable when using the constant elevated baseline rather than the normal baseline. In the normal condition both response temperature and latency to respond was greatly influenced by the week of testing and choice of thermode than the elevated condition.

The second aim was to evaluate the repeatability of responses of dogs using a new remotely operated thermal nociceptive device. I found that both latency to respond and response temperature was repeatable within a test session but that there was some effect of week of testing, particularly when the normal baseline thermode temperature was used.

2.4.1 Repeatability

2.4.1.1 Test

Latency to respond and response temperature were repeatable within a daily session of six tests. Test only affected the repeatability of the thermode starting temperature and only in the normal starting condition. In the normal baseline condition the initial thermode temperature was lower in the first test than the second and third test. This could be because the thermode was not warmed up by contact on the dog's skin for long enough before the first test or did not cool down entirely between tests. Alternatively this could be the result of Stress Induced Analgesia (SIA) which has been found to influence pain thresholds and may impact the behavioural responses (Butler and Finn, 2009). However, failure to start a same

thermode temperature didn't appear to affect latency to respond or response temperature across daily test sessions.

Previous research has only used the normal condition, the natural starting temperature of the thermode. Within these studies repeatability testing was consistent within a 24 hour period (Dixon et al., 2002; Love et al., 2008; Hoffmann et al., 2012). This current study is in agreement with previous research as the response of the dogs in the normal condition was repeatable within a daily test session.

2.4.1.2 Week

There was a week by condition effect on latency to respond, response temperature and initial thermode temperature. In the normal condition in week one, dogs had lower threshold values for both latency and response temperatures compared to most other weeks. A possible reason could be due to SIA. Habituation was used to familiarise the dogs to researcher before testing, however over the four weeks of testing the dogs continued to show less behavioural signs of stress. So it could be that following week one the dogs were more comfortable with the testing procedure.

Other TNT studies often incorporate analgesic treatment after a control test with a 24 hour period and so it can be difficult to assess week repeatability due to this added variable. However, Hoffmann et al., (2012) found no significant difference between the placebo groups over the six months period, with a mean response temperature of 39.7°C ± 0.4°C. This is lower when compared to the mean of the harrier hounds. This could be due to differences in site of testing, where the foreleg was used in this study and the thorax by Hoffmann et al. (2012); or environmental differences, with the dogs' background and housing conditions different between the two studies.

The effect of desensitization at the site of testing has been found to influence repeatability in dogs. However, it does not appear that dogs in this experiment learned or were desensitized to the thermal stimulus, as the responses values did not decrease over the weeks of testing.

2.4.2 The variation between the two testing conditions

In both responses there was variation between the two conditions. Dogs responded quicker in weeks two and three when the baseline was elevated compared to the normal condition. A possible reason for this may be due to the initial temperature of the thermode. However, the initial thermode temperature was only lower in week two; in week three there was no significant difference compared to the other weeks. In response temperature the elevated baseline was lower only in week three compared to the normal condition; there was no difference in the other weeks. It is unclear why it was only in week three that there was a significant difference between the testing conditions in both response temperature and latency to response.

Using the elevated condition was more reliable than the normal condition. Within the normal condition, dogs responded more quickly in week one than week two and three and responded at lower temperatures in week one than in all subsequent weeks. The cause of this is believed to be the result of stress, as the dogs might not have been accustomed to the testing procedure yet and the normal condition was the first tested. Whereas, in the elevated condition the only significant difference was between week three and week four and this was only in response temperature. It is unclear why this may have occurred. Thus the elevated condition was more repeatable within the four weeks.

The normal condition was also influenced by thermode type in both initial thermode temperate and latency to respond. Dogs were quicker to reach their response with the Dallas three thermode compared to Dallas one. This reason is most likely due to Dallas three thermode starting at higher temperatures than Dallas one. There was no difference between thermodes in the elevated condition. The normal condition had a lower initial thermode temperature for the first test than second and third. This suggests that thermode might not have cooled down entirely between tests. This pattern was not observed in the elevated condition.

The reason the elevated condition was more reliable could be due to it having a constant initial thermode temperature or starting at a higher temperature than the normal baseline. Previous research has only used the normal baseline condition, however it did not appear to affect the repeatability of testing (Dixon et al., 2002; Taylor et al., 2007; Love et al., 2008; Hoffmann et al., 2012). The results gathered

from this study show that there could be variance in the normal condition and it would make sense in future research to adopt the elevated condition.

2.4.3 Differences between latency and response

In latency to respond, the response was quicker in week two and three in the elevated condition than normal. The reason the normal condition is lower could be due to the skin temperature starting at a lower temperature compared to the elevated condition. Thus it took longer for the skin and therefore the thermal nociceptors to reach the threshold temperature and elicit a behavioural response. In response temperature this is not seen; the only tendency towards a difference between the elevated and normal baseline was seen in week three. This indicates that response temperature was less influenced by the initial thermode temperature.

In agreement with the current findings, studies in thermal threshold testing often use the skin temperature at which a behavioural response occurs rather than latency to respond due to significantly less variation (Dixon et al., 2002; Love et al., 2011; Hoffmann et al., 2012; Poller et al., 2013). Love et al. (2011), states that using latency to respond is disfavoured as it includes inaccuracies associated with timing. This can be significant when the latency to respond is short as there is a delay between the response of the animal and the reaction of the observer to stop timing.

2.4.4 Ambient temperature

The thermodes had a lower starting temperature in week two in the normal condition than in the other weeks. The reason for this may be ambient temperature as it was lower on average in week two than in week three and four. However, this is only a degree in difference, so it should not influence initial thermode temperature. Also, unfortunately no measures of ambient temperature were made in week one. This was due to a delay in acquiring a device that could measure the ambient temperature accurately. Due to this no statistical analyses was done comparing the ambient temperatures.

Variations in ambient temperature could have an effect on skin temperature as it can result in cutaneous vasodilation and vasoconstriction and impact the dissipation of heat during thermal nociceptive tests (Love et al., 2011). It seems that nociceptors require more energy to get an action potential reaction in the cold, thus it takes

longer for the withdrawal behaviour to occur. Poller et al. (2013) found that in horses, the temperature of the skin after response was significantly lower at warm ambient temperatures (>20 ^{o}C) compared to cold (<10 ^{o}C). However, there are studies that show ambient temperature has no effect on nociceptive thresholds (Lichtman et al., 1993; Whay, 1998).

2.4.5 Limitations

2.4.5.1 Thermodes

The main limitation with the device was the difference in performance and results between the two thermodes. In the normal baseline condition the natural starting temperature of the thermodes was different. Dallas three started at higher temperatures than Dallas one. This could be the reason why in the normal condition, the dogs responded quicker and at lower temperatures with Dallas three. It was also found after the study that the rate of heating between the thermodes was different. For future studies the thermodes should be calibrated to ensure they provide the same linear rate of heating.

It was believed from previous research (Gieseg, unpublished data ; Hoffmann et al., 2012) that a cut off temperature of 55 degrees would negate burning. However, on two of the dogs there were burn marks. The marks were checked and caused no future problems in the dogs; however the fact that the device could possible cause burning is a welfare issue.

The anatomical region of testing in the current study was different compared to previous work on cats and dogs. Most studies attached the thermode around the thorax (Dixon et al., 2002; Hoffmann et al., 2012), whereas this study used the foreleg. Testing on the foreleg has been validated in larger animal studies, such as horses (Love et al., 2011), sheep (Stubsjøen et al., 2009) and has been used on dogs (Gieseg, unpublished data). The reason for the use of the foreleg in the current study was due to the design of the device which had the controller around the thorax of the dogs.

2.4.5.2 Stress

I attempted to keep stress to a minimum. The dogs were tested in a place that they were familiar with and close to their shelters. Along with this the dogs were tested in pairs. Poller et al. (2013) stated that in nociceptive threshold testing it is important for individual horses not to be separated from the herd or surrounding environment. Doing so may lead to abnormal behaviour and altered responses. The dogs were also able to freely move around with the device on them. The only time stress may have been a factor was when the Velcro strap connecting the thermode to the skin loosened. The researcher then had to intervene and fix the problem. The close proximity of the researcher while the experiment was underway might have brought upon stress. However in this study I spent two weeks familiarizing the dogs to myself before testing.

2.4.5.3 Order effect

There was a possible order effect with the initial thermode temperature conditions. During testing three normal tests were done followed by three elevated tests. It is possible that the differences between the two conditions were actually be due to an order effect, i.e. dogs responded more quickly in test four to six each day than in tests one to three, suggesting sensitisation after repeated testing. However, this would only be in weeks three and four. Ideally it would have been better to have alternated between normal baseline tests and elevated baseline tests. In week three there was a tendency that response temperature got lower over repeated testing.

2.4.5.4 Study animals

An improvement on this study would be balancing the male to female ratio of canines. Three males and eight females were used, not enough to be able to compare sexes. Along with this, increasing the number of dogs used would help to provide a clearer indication whether individual differences in pain sensitivity exist. Several females were in heat over during the experiment, it would be best to keep the dogs consistent so either have even numbers of females on heat or none at all.

2.5 Conclusions

This study demonstrates that the initial temperature of the thermode can influence the latency of dogs to respond, but has less of an effect on the temperature at which the dogs responded. Using a constant elevated baseline as the initial thermode temperature appears to be more reliable than using the normal baseline temperature as this was influenced by week and thermode type. There is no consistent effect of initial temperature on response and latency on temperature.

This study used a new thermal threshold device. A main factor that affected the results was the two thermodes used. The thermodes did not start at the same temperature in the normal condition and their rate of heating was different. For future studies the thermodes should be calibrated to ensure they produced the same starting temperature and rate of heating.

The fact that week had an effect on the latency to respond and response temperature when normal baseline condition was used highlights the importance of repeated testing for nociceptive thresholds. Overall the response of dogs to this thermal nociceptive testing device was repeatable within daily test sessions and was more repeatable over weeks when a consistent starting temperature was used.

3. Breed differences in pain sensitivity in dogs

Harrier hounds huddled up during experiment

3.1 Introduction

Pain is defined as a negative experience that affects the animal's welfare (Stafford, 2006). It includes physiological and behavioural responses to a noxious stimulus that can cause damage to the tissues. Pain may allow an animal to identify potentially damaging stimuli and thus learn to avoid them in the future (Weary et al., 2006). All dogs will experience pain in their lifetime. However it is difficult to assess pain in dogs because they are unable to give detailed information about their pain, as humans can. A problem in assessing and alleviating pain in animals is the variability in pain sensitivity within a species (Mathews et al., 2014). The studies that have investigated individual differences in pain sensitivity have used rodents. Eleven inbred strains of mice were tested on twelve separate measures of nociception (Mogil et al., 1999). All nociceptive assays displayed moderate-to-high levels of heritability ($h2$ = 0.30–0.76). No studies have looked at variability in baseline nociceptive thresholds amongst dog breeds.

A breed is a group of animals that have common ancestors and certain distinguishable traits, often developed by deliberate selection and maintained by controlled breeding (Fleischer et al., 2008). Over a thousand dog breeds have been identified (Mehrkam and Wynne, 2014). The physical differences between dog breeds are apparent. But there are also physiological differences (Fleischer et al., 2008) and there may be differences in pain sensitivity between breeds. Anecdotally it is believed that there are differences in pain perception between dog breeds. For example, the New Zealand huntaway, a farm working breed, is thought to be less sensitive to pain than other breeds, in other words they are believed to have a high pain threshold.

Nociceptive threshold tests (NTT) are a standard model for testing pain sensitivity in animals (Le Bars et al., 2001). Pain sensitivity is the point at which a noxious stimulus is recognised by the animal (Fox, 2013). In NTT a thermal, chemical, mechanical or electrical stimulus is applied and the latency to response or strength of the stimulus when a withdrawal response behaviour occurs is recorded. NTTs are preferred over behavioural and physiological assessments of pain sensitivity because the stimulus intensity and duration can be controlled (Arendt-Nielsen and Yarnitsky, 2009); the responses shown can be quantitatively assessed and

compared over time; and the animals assessed do not have to be injured i.e. nociceptors can be briefly activated without tissue damage. As such, studies using measurements of NTT primarily focus on providing a better understanding of the inherent responsiveness of the pain processing system and the effectiveness of analgesics (Bergadano et al., 2006).

The advantages of using heat as a stimulus are that it is considered a natural stimulus and that it uniquely activates heat-sensitive nociceptors (Arendt-Nielsen and Chen, 2003). Thermodes can be controlled very precisely, and the rate of heating is adjustable (Love et a., 2011). Measurement of TNT in dogs has been assessed using thermodes (Hoffmann et al., 2012). TNT assessment involves positioning a thermode and temperature sensor onto a shaved area of the skin. The thermode is heated at a constant rate and the temperature and which a withdrawal behaviour is shown and/or latency to show the response are measured. Thermodes were used by Dixon et al. (2002) for the evaluation of analgesics in cats. Hoffmann et al. (2012) used thermodes to measure TNT in dogs and looked at the reproducibility of the responses and the antinociceptive properties of various analgesics. The previous chapter adopted this method and found that thermodes were a suitable method for assessing TNT in dogs.

The following research looked at whether there was any difference in thermal pain sensitivity among dog breeds by measuring TNTs. This research follows the previous one expect the study will use three dog breeds instead of one. The three dog breeds used are greyhounds, harrier hounds and huntaways. Both greyhounds and harrier hounds have backgrounds as sporting dogs, and huntaways are famous for being used for farming.

3.2 Materials and Methods

3.2.1 Animal Ethics Approval

The procedures were approved by the Massey University Animal Ethics Committee (Protocol 14/77).

3.2.2 Animals and experimental conditions

Ten individuals in each of three breeds of dogs, greyhounds, harrier hounds and huntaways, were used (total n=30) (Table 3.1). They were chosen as a convenience sample, as they were the only ones available to us as that had sufficient numbers. The harrier hounds were the same dogs from the first study. The experiment was conducted between the 17th November and the 12th December 2014.

The experiment was carried out in a windowless room (2.3m x 3.08m) located on the ninth floor of the Institute of Veterinary, Animal and Biomedical sciences tower on the Palmerston North campus. This room was temperature controlled, the ambient temperature was maintained between 19°C and 21°C throughout the experiment. Two dog mats, a bowl of water for the dogs and a desk and seat for the researcher were setup in the room.

Dogs from each breed were transported to Massey University from their respective homes. Harrier hounds were sourced from and were transported a distance of 0.9km. The huntaways came from a different source, which were much further away, as did the greyhounds (Table 3.1). They were returned to their respective residences after each session.

Three bitches, Royal (harrier hound), Siren and Wanda (greyhounds) came into oestrus during the study; Royal in weeks one and two, Siren in week two and Wanda in week two and three

3.2.2.1 Habituation

Before bringing any of the dogs to Massey University the researcher first went out to the source site of the dogs to meet them. In the week before the trials began all but one of the dogs (Troy: who had not been bought by the owner yet), were habituated to the testing room, the procedure, and the researcher. During this habituation session a pair of dogs that came from the same breed and source and were familiar with one another were brought into the room and allowed to roam around and become familiar with it. Each pair of dogs had one session of habituation over this week. Once the dogs settled down, and sat or lay down, a 25 x 25 mm area was shaved on the medial surface of their left foreleg, distal to the elbow joint and

proximal to the carpal joint. The heating device and a thermode were put on each dog and the dogs were left to adjust to the attachments.

Then, a trial run of the test was performed to ensure the dogs were familiar with the heating process. The session took approximately one hour. If a dog was nervous or shy, it took longer to complete the session as it was very important to be patient and make sure that the dog was comfortable.

3.2.3 Experimental design

During the trial, six tests were performed on each dog on one day each week for four consecutive weeks. The dogs were tested in the same pairs as during habituation. Three pairs, each of a different breed, were tested each day with the dogs being tested on the same day of the week each week. Each day a randomly generated sequence determined the order of testing for the three pairs of dogs. Testing alternated between each dog in the pair, with a five minute gap between each dog. For example, dog A was tested first, after five minutes dog B would be tested on, after another five minutes the test would go back to dog A, this was repeated so each dog had six tests completed. There was a ten minute gap between each individual dog on a particular day to allow the thermode to cool.

3.2.4 Thermal nociceptive threshold testing device

The device used in this study is described in chapter two but several adjustments were made for this study. Initially Dallas 1 and Dallas 3 were being used as the two thermodes. However, in the first week the Dallas 3 thermode malfunctioned and was replaced with a Dallas 2 thermode for the rest of the experiment.

Table 3.1: Dogs used in the study, their breed, sex, neuter status, age, source and the distance travelled each session between source and Massey University

Dog name	Breed	Sex	Neuter Status (Y/N)	Approximate age (years)	Source	Distance travelled (kilometres)
Quick	Harrier	M	Y	2	Massey University Nutritional Unit	0.9 km
Nemo	Harrier	M	Y	3	Massey University Nutritional Unit	0.9 km
Gloman	Harrier	M	Y	5	Massey University Nutritional Unit	0.9 km
Royal	Harrier	F	N	2	Massey University Nutritional Unit	0.9 km
Neat	Harrier	F	N	3	Massey University Nutritional Unit	0.9 km
Jade	Harrier	F	N	4	Massey University Nutritional Unit	0.9 km
Golly	Harrier	F	N	5	Massey University Nutritional Unit	0.9 km
Fay	Harrier	F	N	6	Massey University Nutritional Unit	0.9 km
Dawn	Harrier	F	N	8	Massey University Nutritional Unit	0.9 km
Chrissy	Harrier	F	N	9	Massey University Nutritional Unit	0.9 km
Noodler	Huntaway	F	N	<1	Estendart Colony	19.4 km
Omo	Huntaway	F	N	3	Estendart Colony	19.4 km
Frieda	Huntaway	F	N	4	Estendart Colony	19.4 km
Rua	Huntaway	F	N	5	Estendart Colony	19.4 km
Tete	Huntaway	F	N	5	Estendart Colony	19.4 km
Tahi	Huntaway	F	N	5	Estendart Colony	19.4 km
Patake	Huntaway	F	N	5	Estendart Colony	19.4 km
Lucy	Huntaway	F	N	6	Estendart Colony	19.4 km
Meg	Huntaway	F	N	8	Pet owner 1	44.3km
Bella	Huntaway	F	N	8	Pet owner 1	44.3km
Milo	Greyhound	M	Y	2	Pet owner 1	44.3km
Siren	Greyhound	F	N	3	Pet owner 1	44.3km
Troy	Greyhound	M	N	3	Pet owner 1	44.3km
Wanda	Greyhound	F	N	5	Pet owner 1	44.3km
Com	Greyhound	M	Y	6	Pet owner 1	44.3km
Domino	Greyhound	M	Y	8	Pet owner 1	44.3km
Blue	Greyhound	M	Y	9	Pet owner 1	44.3km
Holly	Greyhound	F	N	9	Pet owner 1	44.3km
Opal	Greyhound	F	N	4	Pet owner 2	27.9km
Max	Greyhound	M	Y	6	Pet owner 2	27.9km

All the thermodes consists of a probe containing a heating element and a temperature sensor which are held to a shaved area on the body. After the first study (chapter 2), the two thermodes were modified to ensure they produced the same surface temperature. This was done by calibrating the thermodes with a separate Dallas DS18B20 digital temperature sensor. The sensor was attached to the heating surface of the thermode with a thin smear of heat-sink compound. Heating data for the surface of the thermode was recorded at 0.5 second intervals. Simple linear regression plots of the average of three heatings for each thermode were graphed using Microsoft Excel. Simple linear regression plots were graphed of the average of three runs for each thermode using Microsoft Excel.

The calculated linear regression equation between 40°C and 55°C was calculated and incorporated into the software so the reported thermode temperature matched the surface temperature. The correction factor calibration was done before and after the whole trial. A new command was also added to Coolterm that allowed the researcher to choose the thermode that was being used to select the appropriate correction factor. This was an improvement over the pilot study where the controller did not recognise the different thermodes.

3.2.5 Experimental procedure

The six dogs that were being used on a particular day were brought to Massey University in the morning. They were kept in kennels when they were not being used. Only the harrier hounds were familiar with the Massey University kennels. However, the other two breeds were used to being kennelled at their residence. Testing started at 09:00 and ended at 16:00 with each pair of dogs being in the test room for 1 to 1.5 h. Following the order that was randomly generated, the first pair would be taken up to the testing room by elevator. Here the dogs were given a ten minutes to adjust to the room and then an area of skin on the dog’s front left foreleg measuring approximately 25 x 25 mm was clipped each test day and then washed with swabs and water to remove debris.

If there were any injuries or burn marks on the testing area the testing would be done on the right leg. The thermode was placed onto the clipped skin area and the wire wrapped around the harness and connected to the controller. Both controllers on

each dog were turned on and connected via Bluetooth to a laptop. Two coolterm programmes would be run simultaneously, with each one linked to a separate controller. Within each pair the dogs switched thermodes each week, with their starting one being randomly assigned.

TNTs were determined by measuring the latency to respond and the temperature at which the dog responded (response temperature). A dog was determined to have reached its TNT when it showed a pain response behaviour, moving the leg with the attached thermode and/or turning its head towards the thermode. Testing was stopped once one of these behaviours was observed. Alternatively if no obvious behavioural indication of nociception was shown before the thermode reached 55°C, the test was terminated and the thermode turned off automatically.

The method of testing was the same as the first study, with the following exception: the thermode was not heated because ambient temperature was tightly controlled before testing started and was set at skin temperature i.e. normal baseline condition

3.2.6 Statistical analysis

Raw data were explored graphically to see if any trends existed within the data. The breeds were compared to see if there was any difference between the three. The distribution of the latency and response temperature residuals were tested for normality and were found not to be normally distributed. A Blom's transformation (Blom, 1958) was performed using the PROC RANK procedure in SAS® 9.4 (SAS Institute Inc. Cary, NC, USA) to normalise the data. The latency and response temperature were analysed using a generalized linear mixed model analysis in SAS® 9.4 The model included the fixed effects of thermode, breed, week, test, starting temperature and their interactions, and dog as a random effect and breed as a grouping effect. Significant findings were considered at $p<0.05$. When a significant effect was found post hoc analyses were done using a manual correction for multiple comparisons. After initial analysis it was found that there was a significant effect of test on both variables. Post hoc analysis showed that test 1 was significantly different from all other tests. Thus, the analysis was also run excluding the first test in each week with a new manual correction. The tables and graphs in results section show raw results and means (± standard error) as it is not possible to back-transform from Bloms transformation.

3.3 Results

In week one, no data were recorded for one huntaway (Rua) and one greyhound (Troy). The huntaway did not have a large enough area of fur shaved to provide accurate results; and the greyhound had not been purchased by the owner. One latency to respond data point was missing (Max week one, test six). 37 out of the 708 (5%) tests had to be redone due to the thermode losing contact pressure.

3.3.1 Latency to Respond

The significant effects on latency to respond are shown in Table 3.2. The main finding is that there was a breed effect. Huntaways ($\bar{x}$ = 36.63, SE= 0.35 seconds) had significantly longer latencies to the response than both greyhounds ($\bar{x}$ = 34.58, SE= 0.37 seconds; t=-2.12, P=0.04) and harrier hounds ($\bar{x}$ = 32.59, SE= 0.31 seconds; t=-3.40, P=0.002). Greyhounds and harrier hounds were not significantly different (t= 0.73, P= 0.47).

There was a significant test effect. The dogs took longer to respond in test one than in all subsequent tests (Figure 3.2). There was a significant difference between weeks, where week two had the dogs responding significantly quicker than all subsequent weeks (week one: t = 4.18, P= <0.0001; week three: t= -4.19, P= <0.0001; week four: t= -2.78, P= 0.007).

There were no significant second order interactions between the variables. The type of thermode used had no effect on the latency at which the response occurred (Table 3.2). The initial thermode temperature was significantly different for latency to respond (Table 3.2).

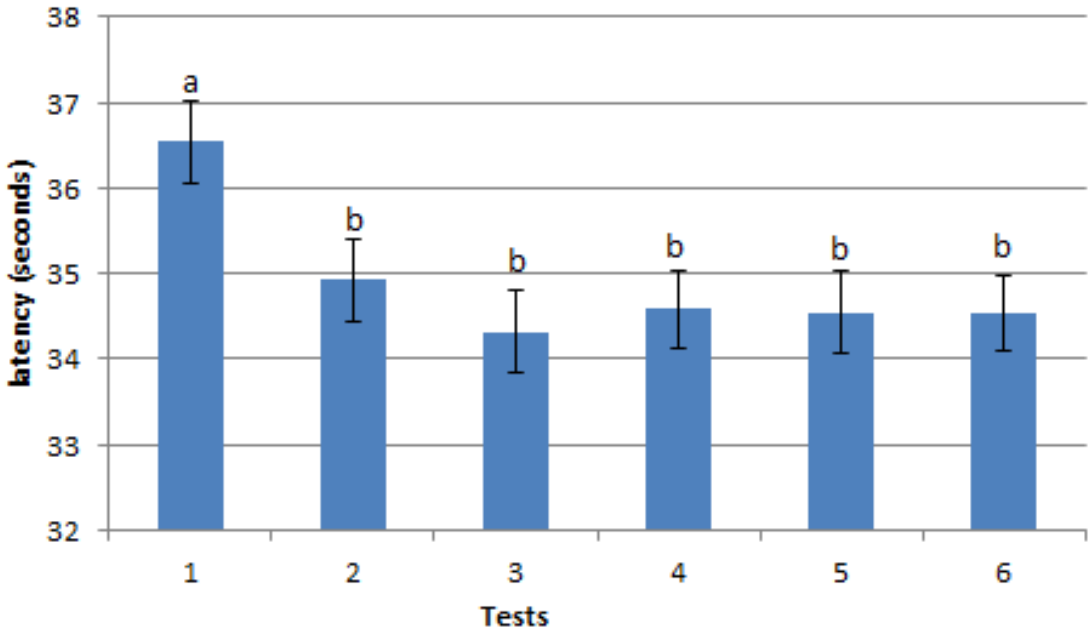


Figure 3.2: Test effect on latency to respond (seconds) (raw mean ± SE). Significant differences are indicated by different letters. Differences considered significant at p<0.05

3.3.2 Response temperature

For the temperature at which the dogs responded there were overall breed, test and week effects (Table 3.2). Huntaways ($\bar{x}$ = 49.84, SE= 0.17 ^{o}C) responded at a higher temperature than greyhounds ($\bar{x}$ = 48.66, SE= 0.16 ^{o}C; t= -2.17, P= 0.04) and harrier hounds ($\bar{x}$ = 48.37, SE= 0.14 ^{o}C; t= -3.32, P= 0.003). There was no difference between harrier hounds and greyhounds (t= 0.57, P= 0.57).

Test number had a similar pattern of effect to latency to respond, where dogs responded at a significantly higher temperature in test one than all the other tests (Figure 3.3). Likewise with the week effect, in which in week two the dogs had lower response temperatures compared to the other weeks (week one: t = 3.84, P= <0.0002; week three: t= -4.34, P= <0.0001; week four: t= -2.75, P= 0.008).

Table 3.2: Results of statistical analysis for latency to respond (seconds) and response temperature (^{o}C), using Bloms transformed data.

Responses	Breed		Week		Test		Thermode		Initial thermode Temperature	
	F (2,27)	P	F (3,79)	P	F (5,135)	P	F (2,37)	P	F (3,27)	P
Latency to respond	6.01	0.0069	8.31	<0.0001	2.77	0.02	0.81	0.45	99.18	<0.0001
Response temperature	5.92	0.0074	8.00	0.0001	3.34	0.007	1.17	0.32	1.11	0.29

Table 3.3: Results of statistical analysis for latency to respond (seconds) and response temperature (^{o}C), using Bloms transformed data **after the first test has been removed**.

Responses	Breed		Week		Test		Thermode		Initial thermode Temperature	
	F (2,27)	P	F (3,79)	P	F (5,135)	P	F (2,37)	P	F (3,27)	P
Latency to respond	6.30	0.0057	5.66	0.0014	0.48	0.75	0.72	0.49	74.81	<0.0001
Response temperature	6.28	0.0057	5.07	0.0029	0.34	0.85	1.50	0.24	2.93	0.08

Unlike latency to respond, there was no significant difference between the initial thermode temperatures and response temperature (Table 3.2). There were no significant second order interactions between the variables. The type of thermode used had no effect on the temperature at which the response occurred (Table 3.2).

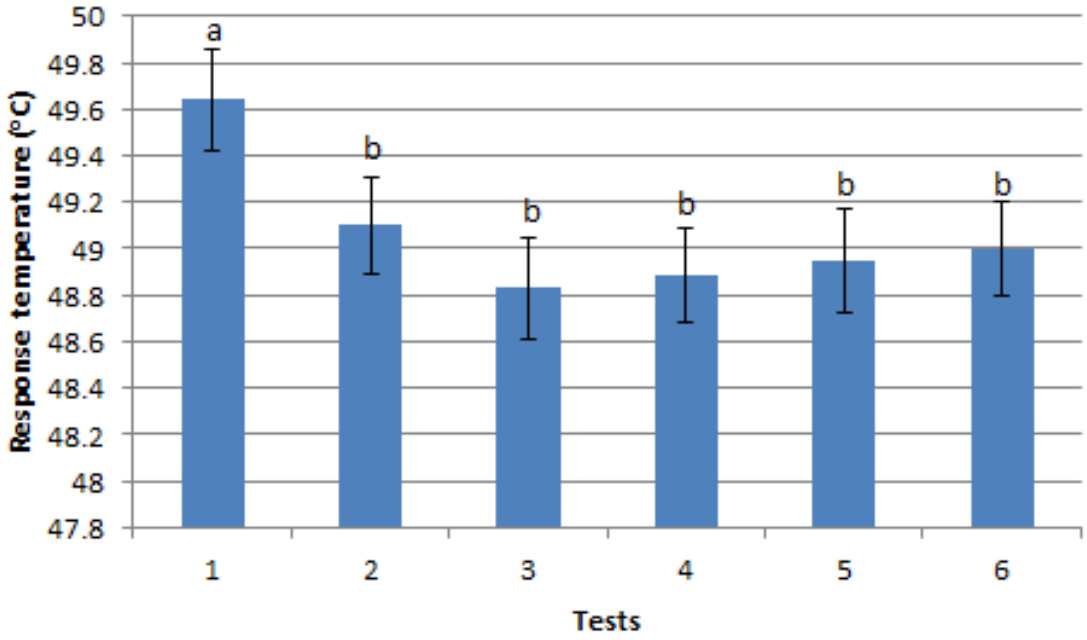


Figure 3.3: Test effect on response temperature (^{o}C) (raw mean ± SE). Significant differences are indicated by different letters. Differences considered significant at $p<0.05$.

3.3.3 Analysis with first test removed

Both latency to respond and response temperature were significantly different in daily test number one than in the other tests. It was a clear outlier compared to the rest of the data and as such was removed and the data re-analysed. Week two was not removed as it was unclear why there was a drop in the thresholds.

The test effect no longer existed in either latency to respond or response temperature once the first test was removed (Table 3.3). The breed effect, however, is still present for both responses. There is no significant difference between huntaways and greyhounds in latency to respond, unlike what was seen before the correction. Figure 3.4 shows the difference between the three breeds for latency to respond, however it does not correctly display that there is no significant difference between the greyhounds and the two other breeds. This could be due to the graph showing the raw data instead of the transformed data. Huntaways still responded at

significantly higher temperatures compared to greyhounds and harrier hounds (Figure 3.5). Harrier hounds and greyhounds were not significantly different for both responses.

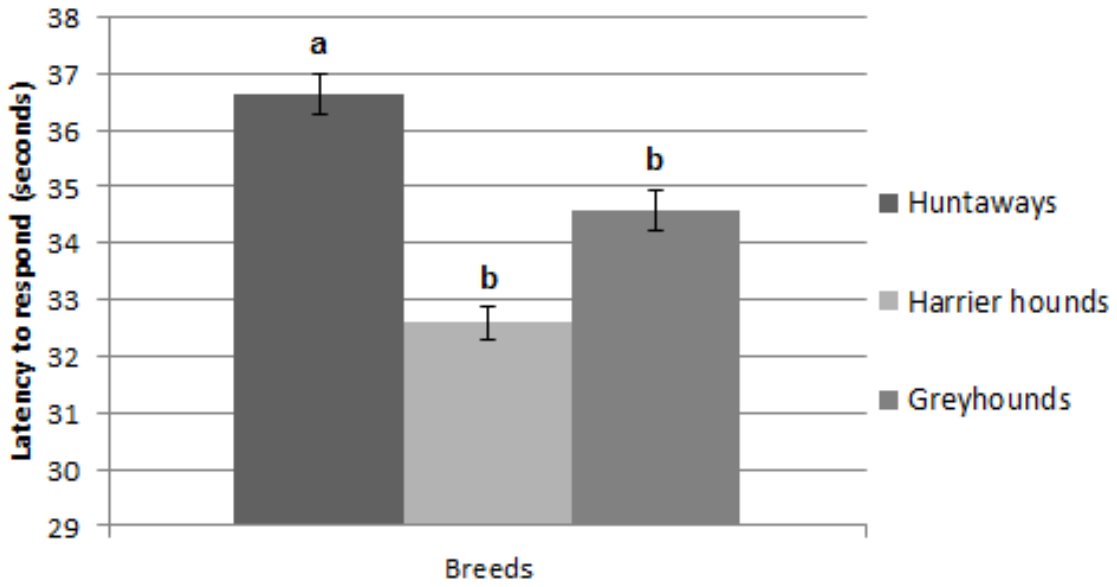


Figure 3.4: Raw mean ± SE for the breeds for latency to respond (seconds), after the 1st test has been removed. Significant differences between breeds are indicated by the different letters. Differences considered significant at $p<0.05$.

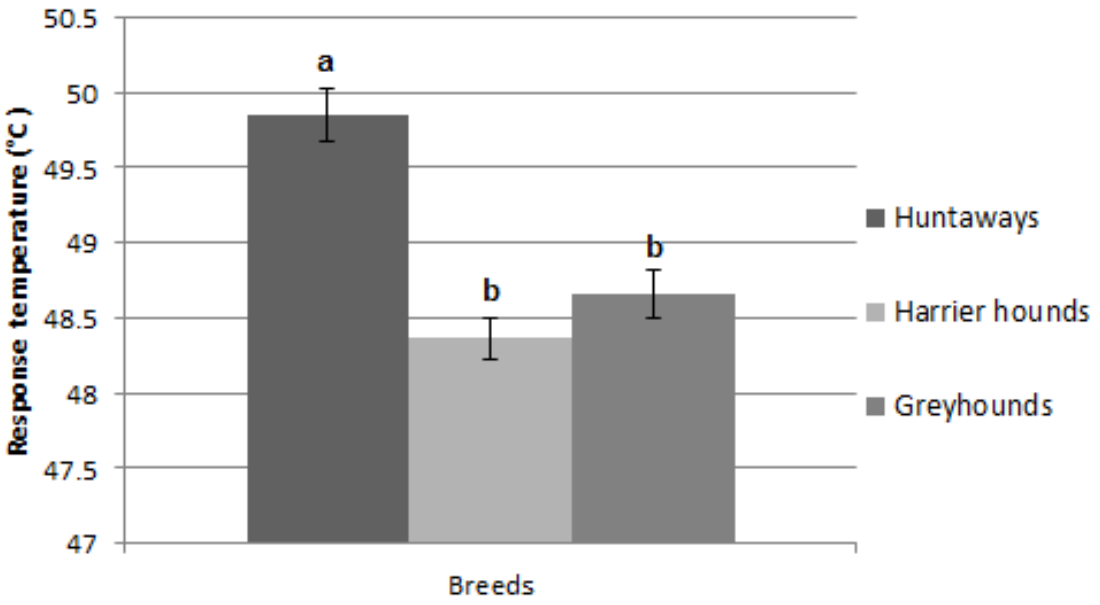


Figure 3.5: Raw mean ± SE for the breeds for response temperature (°C), after the first test has been removed. Significant differences between breeds are indicated by the different letters. Differences considered significant at $p<0.05$.

There was still a week effect in both responses, where in week two the dogs responded quicker and had a lower response temperature than the other weeks.

There were still no thermode effect and no significant second order interactions between the variables (Table 3.3). The initial thermode temperature had a significant effect on latency to respond, with no effect on response temperature.

3.4 Discussion

The aim of this study was to see whether selected dog breeds had differed in terms of their thermal pain sensitivity. I found that there was a difference between the breeds. Huntaways significantly took longer to respond than harrier hounds and responded at higher temperatures than both harrier hounds and greyhounds. This is the first scientific evidence of breed differences in pain sensitivity in dogs.

3.4.1 Possible reasons for breed difference

3.4.1.1 Physiological difference between dog breeds

The difference in TNTs between the dog breeds tested suggests a physiological difference in the transduction and for transmission of nociceptive signals. The breeding of dogs for particular traits and functions has shaped their physiological makeups. Several studies have shown differences in the physiology of different dog breeds. For example, differences in the blood leukocytes subsets exists between police Labrador retrievers and German shepherds were found (Villaescusa et al., 2012). Many breeds also have certain genetically-based diseases, with there being over 370 inherited disorders identified in purebred populations (Fleischer et al., 2008). It is observed that breed heritability has an effect on pain sensitivity in rodent populations (Mogil, 1999). In humans there is a difference in pain sensitivity between ethnic groups. African-Americans had lower thermal pain tolerances and rated thermal stimuli as more unpleasant than Caucasians (Edwards et al., 2001). Thus, like the examples above, there could be physiological reasons why dog breeds show disparities in pain sensitivity.

It is unclear at the moment why a physiological difference may underpin the difference in observed thermal pain thresholds in these particular breeds. Historically the breeds have been maintained under different geographical and culture

conditions, which may affect the evolution of the dog's genes and physiology (Lark and Chase, 2012). Harrier hounds are descended from greyhounds (New Zealand Kennel Club, n.d.), which could be why the harrier hounds and greyhounds have similar pain threshold results. Huntaways have a wider, more recent genetic background, with their exact origin unknown. They are selected for their performance and ability to work tirelessly moving stock everyday (New Zealand Kennel Club, n.d.). The pain processing system could have been altered by selection. The breeding of huntaways to be herding dogs may have influenced pain threshold levels. Farmers may have preferred their huntaways to resist pain for a better efficiency when working. However, it is clear that more research on the mechanisms behind these differences in pain thresholds needs to be performed.

The average of nociceptive thermal thresholds of six beagles tested by Hoffmann et al. (2012) trial was 39.7°C ± 0.4°C. This was far lower than the averages found in these three breeds. Harrier hounds and beagles are genetically close and have similar breeding histories, the difference between the two averages could be due to physiological differences, environmental conditions or experiment procedures, as the thermode was tested on different areas of the body.

3.4.1.2 Environment

Another possible explanation for the observed difference between huntaways and the other breeds is difference in environmental factors. The test environment was kept consistent as possible; the dogs were all tested in same location with same person and same procedures. Nonetheless there were some key differences among the three breeds including: source, husbandry, transport (harriers <1km, huntaways approximately 20km and greyhounds > 25km).

The huntaways had never left their colony before and were reluctant to travel in van to Massey University. Am electric shaver was used to remove the fur on the testing site on the foreleg. Both huntaways and harrier hounds responded with fear behaviour and activity tried to avoid it. It is possible that due to this Stress Induced Analgesia (SIA) was induced and that is the potential reason for the breed effect. Huntaways could have had a higher SIA than the other two breeds.

3.4.1.3 Stress-Induced analgesia

Stress induced analgesia (SIA) has been shown to inhibit pain signals and produce opiate-like effects (Butler and Finn, 2009), thus increasing thresholds and decreasing pain sensitivity. Stress can also cause vasoconstriction in the extremities of animals and thus lead to a reduction in skin temperature. This could affect research TNTs as it could influence the dissipation of heat (Love et al., 2011).

SIA therefore, could be believed to have influenced the breed effect. If the breeds had different levels of stress then this could be what the results represent. The huntaways were observed to be more stressed compared to the other breeds. They showed obvious behavioural cues of stress when travelling and when the shaver was used. If SIA was having an effect then we would expect to see that over time there would be a decrease in latency and response temperature as the huntaways adjusted and become acclimatised to the situation. However there is no decrease over weeks or over the six tests. Any fluctuation over time happened in the other breeds as well, for example all breeds had an unexplained drop in week two. Therefore, it appears that SIA cannot be attributed to being the reason why there was a breed difference in pain thresholds.

More evidence of this is shown where two huntaways came from the same source as the majority of the greyhounds, in which they were visibly less stressed than the other huntaways, as they were used to travelling away from their source site. Their results were consistent of the other huntaways rather than greyhounds. This demonstrates that these dogs followed the pain sensitivity levels of their breed rather than their source.

SIA could have affected test one, during which there was a significantly higher latency to respond and response temperature compared to the other tests. This is the main reason why test one was removed and the data was re-analysed.

3.4.2 Repeatability

TNT has been proven to be a reliable indicator of pain sensitivity in cats (Dixon et al., 2002; Steagall et al., 2007; Taylor et al., 2007; Robertson et al., 2009) and in horses (Love et al., 2008; Love et al., 2012).

3.4.2.1 Test effect

The repeatability of the dogs' responses was affected by the daily test number. Dogs had a higher response in test number one compared to the other tests for both response temperature and latency to respond. It is possible that SIA was the cause of this due to insufficient time to settle and recover from shaving. This was not seen in Dixon et al (2002) as their testing with cats was repeatable within 4^{o}C over a 24 hour period. Removing test one, removed the test effect and the data became more and resembled the data of Dixon's et al (2002). Thus more time appeared to be needed to allow the dogs to settle before testing commenced.

3.4.2.2 Week effect

The repeatability of the dogs' responses was affected by week of testing. All three dog breeds responded quicker and had lower response temperatures in week two than the other weeks. It is unclear why this may have happened.

3.4.2.3 Initial thermode temperature

In the first study it was found using a constant elevated baseline was the more reliable method for testing nociceptive thermal thresholds. In this study instead of raising the temperature of the thermode to 35^{o}C before every test, a temperature controlled room was used in hope that it would make the initial thermode temperature consistent between the three breeds. However, this was not the case as in latency to respond the initial thermode temperature was significantly different. Thus, a consistent initial thermode temperature should be used in future studies.

3.4.2.4 Thermodes

Unlike the pilot study, there was no difference between the results obtained with three thermodes. The thermodes were calibrated by using a digital temperature sensor and then calculating a linear regression equation so all three thermodes produced the same surface temperature and rate of heating

3.4.3 Behavioural responses

Identifying the point at which the animals react to noxious stimulation is one of the most important aspects of threshold testing. The behaviour responses used in this study were similar to previous studies using thermal stimuli on dogs (Hoffmann et al., 2012). The common endpoint used for all tests were related to the thermode. This included a movement of the leg with the thermode in towards the body or a head turn towards the thermode. Dogs are not the only species to show a range of response behaviours to a noxious stimulus. The power setting of a CO_2 laser affected the nature of response in calves (Veissier et al., 2000). At a high power setting the calf was more likely to react by kicking rather than moving the foot. This indicates that it is important to describe and take note of the range of behaviours shown when a noxious stimulus is applied. It is not wise to rely on a single reflexive response (Veissier et al., 2000).

There are times when such behaviours may not represent pain. Animals may react before pain is felt, often due to the activation of other fibres (Le Bars et al., 2001). Pinpointing the exact moment pain has been experienced can be difficult. The dogs, in this study, had different reactions when the thermode was heating up, before the pain threshold was reached. Some dogs would consistently reposition the leg that was being tested and others would get up and change seating position. I took precautions to counter this effect. There was a habituation period, in which the researcher observed the individual responses of the dogs and learned the behaviour most likely to indicate a pain response for each.

Coleman et al. (2014) reported that learning can confound the assessment of pain sensitivity when using a mechanical stimulus. The response to the noxious stimulus can decrease over time. It does not appear that the dogs in this experiment learned or were desensitized to the thermal stimulus, as the weeks were consistent, except for week two. The difference could be that mechanical stimuli are often noisy and allow the dogs to learn when the noxious stimulus is being applied.

3.4.4 Differences between response variables

The first study showed that response temperature may be a more reliable variable for evaluating thermal pain sensitivity as latency to respond was more affected by

the initial temperature of the thermode. This study agrees, with the initial thermode temperature only affecting latency to respond. In addition, response temperature also had a lower variance than latency. These results indicate that the use of response temperature is the most reliable method for assessing TNT.

3.4.5 Limitations

3.4.5.1 Device

The biggest equipment problem in this study was maintaining an adequate pressure of the thermode onto the skin. This study only used a Velcro strap and a small rectangular foam to keep the thermode in contact with the skin. In total 37 out of the 708 tests had to be repeated due to the thermode losing contact pressure. It was very easy to fix and only needed the researcher to tighten the Velcro strap. However, this did mean that interference with the subject occurred, although most dogs appeared relaxed during this adjustment. There are better ways to produce a consistent contact to between the thermode and the skin. Using equipment such as a pressure bladder used by Dixon et al (2002) would have maintained the pressure of the thermode onto the skin.

3.4.5.2 Burning

Initially it was hoped that the thermode would not cause any burning as previous research found 55^{o}C to be an optimum cut off point to negate burning. However, in this study, seven huntaways, two greyhounds and four harriers had burn marks that arose during testing. This could cause hypersensitivity in the testing area to the thermal stimulus. To avoid this, I moved the thermode to the right leg or to a different area on the leg if any burning marks were found. These marks were checked by a veterinarian, and they disappeared a week after testing. However, this is a serious drawback to the piece of equipment and shows that using a cut off of 55^{o}C does not limit burning.

3.4.5.3 Initial Temperature of the thermode

The initial temperature of the thermode influenced the dog's latency to respond. A possible reason was that the thermode was not sufficiently warmed up or did not cool efficiently between tests. Using a practice test to warm the thermode or using

the elevated baseline condition mentioned in study one would improve the repeatability.

3.4.5.4 Stress

An attempt was made to keep stress at a minimum when measuring pain thresholds, due to its ability to confound results. From observation in this study most the dogs tolerated the testing procedure well. However the most stressful part of the testing was using the electric clippers to shave off fur at the testing site on the leg. The huntaways and harrier hounds actively tried to avoid being shaved, while the greyhounds were fine. It seemed it was the noise of the shaver that scared the dogs and using one that is quieter would have helped lower stress before any testing began. The huntaways were the only breed that had not ever been transported before. They were visibly stressed during transport compared to the other two breeds which were used to leaving their source sites. A longer habituation time might help overcome these issues.

3.4.5.5 Study Animals

The study only used three breeds, which were all working breeds. However, these were the only ones available for the study and it was fortunate that there was at least ten of each breed. An improvement on this study would be evening out the male to female ratio of canines. 9 males and 21 females were used in this study, thus this made it hard to compare pain threshold levels between sexes. No male huntaways were used, because we were only allowed to use the females. This limits the huntaways as we cannot test a sex difference. Increasing the number of dogs, with a mix between the sexes and having more breeds used would help to provide a clearer indication whether breed differences in pain exists.

3.5 Conclusions

The result of this study provides knowledge on the differences of nociceptive thresholds within species. It was demonstrated that the breed of the canine influences pain sensitivity as measured in the study. Huntaways had higher TNTs than greyhounds and harrier hounds. This is the first scientific evidence of breed differences in pain sensitivity in dogs. There was an effect of the test number within a daily test session with test number one significantly different compared to the other

five tests. The possible reason for this may be due to stress induced analgesia, thus this test was removed from the analysis. This topic warrants further exploration into why differences between breeds can lead to variation in pain thresholds. For veterinarians the research is beneficial as, given the side-effects of commonly used analgesics in dogs, a greater understanding of variations in pain sensitivity would contribute to a rational approach to pain management

4. General Discussion

4.1 Overview of results

The overall objective of this thesis was to assess the repeatability of a new thermal threshold device and determine if there was any difference in thermal nociceptive thresholds between three dog breeds. Huntaways took significantly longer to respond than harrier hounds and responded at higher temperatures than both harrier hounds and greyhounds, suggesting that huntaways have a higher threshold than the other two breeds.

Initial temperature of the thermode had an effect on TNT. It was observed that the initial temperature had stronger effect on latency to respond than response temperature. The results suggest that it is more reliable to use the constant elevated initial thermode temperature for testing nociceptive thresholds than using the natural starting temperature, most likely due to the influence of the ambient temperature.

The measures in both studies were influenced by either the week of testing or by daily test number. Due to effects of stress induced analgesia (SIA) there is a need for proper habituation to various aspects of procedure, particular travel and shaving. This highlights the inconsistences in NTT and shows the need for repeat testing.

There was variation in the behavioural responses to the heated thermode. The behaviour taken to indicate TNT was either moving the leg with the attached thermode and/or turning towards the thermode. However, during the period when the thermode warmed the dogs behaved in a range of ways.

4.2 Method considerations

4.2.1 Thermodes

Three thermodes were prepared for these experiments. It was discovered in study one that the natural starting temperatures of the thermodes were different. Along with this it was found that the rate of heating was also different between the thermodes. The thermodes were calibrated before the second study to make sure they provide the same linear rate of heating and started at the same temperature.

In the second study there was a difficulty in applying consistent pressure of the thermode onto the skin, which could have influenced the rate of skin heating and thus time to nociception activation. Loss of contact between the thermode and the skin would prolong the latency and make it seem like the dog responded at higher temperatures than would have been with better contact. In both studies a Velcro strap and a small rectangular foam were used to keep the thermode adjacent to the skin. In total 37 out of the 708 tests had to be redone due to the thermode losing contact pressure. A solution to correct this problem is to use a pressure bladder used by Dixon et al (2002). Along with this the temperature measured is the thermode surface temperature and thus may not equal the skin temperature.

An issue with thermal threshold testing is that the stimulus may cause burning. This could cause hyper sensitivity in the testing area to the thermal stimulus and is a welfare concern. Dixon et al. (2015) set out to refine thermodes that prevented burns in cats and humans. The results showed that a thermode with a reduced thermal mass and even heating across the skin contact area was the most suitable, with no skin damage being evident. Incorporating this into more studies using a thermal stimulus for nociceptive testing will help avoid burning and the problem of hyper sensitivity to the testing area.

4.2.2 Study Animals

The number of dogs that were used in both studies was also limited. An improvement in each study would be to increase the amount of dogs used and to have an even sex ratio or just use a single sex. There is a practical limitation in acquiring dogs, permission and ability to get proper numbers affect the amount of dogs used in this study.

4.2.3 Behaviour Responses

Identifying the point at which pain is felt is one of the most important aspects of threshold testing. The habituation period in both studies was used to identify the individual responses of each dog. A dog was considered to have reached its TNT when it showed response behaviour (moving the leg with the attached thermode and/or turning its head towards the thermode).

Several behaviours were observed before the pain threshold was reached, when thermode heating was occurring. Animals reacting before the threshold did so probably due to the activation of other fibres. However, these behaviours were not recorded and no comparisons were done between the behaviours. In future experiments these data should be collected as it may provide insight into breed differences in behavioural responses to pain sensitivity.

4.2.4 Repeatability of results over time

Study one was repeatable within a daily test session. However, in the normal condition, the dogs were quicker to respond and responded at lower temperatures in week one than in the following weeks. The suggested reason for this was SIA and a possible order effect as the normal tests were done first and then the elevated ones.

Study two had a test and week effect. For an unknown reason, in week two the dogs responded at lower temperatures and quicker than in the other weeks. For the test effect, the dogs had significantly higher responses for latency and temperature in daily test number one compared to the other tests i.e. it was less sensitive. This was believed to be the result of SIA, as the dogs did not have enough time to settle before testing commenced.

There was no apparent habituation to the stimulus, as there was no decrease from week one to week four or from tests one to test six on individual days in either study. This agrees with Hoffmann et al. (2012) in which sham testing showed that the dogs did not show any pattern of anticipating the stimulus.

4.2.5 Differences between initial thermode conditions

For the initial thermode temperature the results were less repeatable with the normal baseline condition in study one which ambient temperature was quite variable. In this condition both the weeks and thermode type affected the starting temperature and response of latency and temperature. When elevated baseline was used there were no differences over the four weeks and between the thermodes in the dogs responses. This suggests that fluctuations in starting temperatures over the weeks, tests and thermodes in the normal condition influenced the measured responses and that a constant start thermode temperature is recommended to accurately measure TNTs.

4.2.6 Differences between response temperature and latency to respond

From both studies it is apparent that using the response temperature is better than latency to respond. The first study found the latency to respond was more strongly influenced by the initial temperature of the thermode than was response temperature. In the second study response temperature had a lower variance than latency and was not influenced by the starting temperature. This indicates that response temperature may be a more reliable variable for measuring thermal pain sensitivity.

Latency has already been criticised in other research due to the inaccuracies associated with timing (Love et al., 2011). Studies in thermal threshold testing now usually use the response temperature at which a behavioural response occurs rather than latency to respond as there is also significantly less variation in it (Dixon et al., 2002; Love et al., 2011; Hoffmann et al., 2012; Poller et al., 2013).

4.3 Future research

4.3.1 Dogs

The pain sensitivity of other breeds needs to be investigated. This study only used three working breeds. Using other breeds can help provide a clearer indication of breed differences in pain sensitivity. Breed strain difference in pain thresholds in other species should also be investigated.

It is unclear why physiologically there might be a difference in nociceptive thresholds in dogs. Research on the mechanism behind these differences needs to be performed.

4.3.2 Behaviour

The behaviour during heating varied between the dogs. Research has been done in calves where the behaviour responses to the thermal stimulus varied (Veissier et al., 2000). The calves were more likely to respond by kicking than by simply moving the foot. Future studies in NTT should record the behaviour of the animal during and at the endpoint of testing. Then the data can be looked at to see if any significant differences exist.

The habituation period that was used in these studies for the researcher to identify the individual responses should be increased. In future there should be several weeks of practising to learn individual responses and get appropriate familiarisation with the dogs. This means during the experiment the researcher will know exactly what to look for with individual animals. Habituation to travel, shaving, and handling should be increased to reduce the effects of SIA. It would also be ideal to use dogs which have all been raised and housed similarly to reduce effects of these measurements

4.3.3 Analgesics

Given the side-effects of commonly used analgesics in dogs (Lascelles et al., 1994), a greater understanding of the variations in pain sensitivity would contribute to a rational approach to pain management. Previously, little research had been done on individual treatment of pain. The current research suggests that pain relief should take into account the breed of dog. Following on from this study, research should explore breed differences in response to analgesics. An increase in research in this area can provide more reliable data on the breed differences in pain. With this the pain management guidelines can evolve to take into account breed differences if necessary.

4.4 Summary

In summary this thesis has provided preliminary evidence of differences in pain thresholds between dog breeds. Huntaways had higher threshold indicator of lower thermal pain sensitivity compared to the other breeds tested. This thesis also supplies data on how the initial temperature of the thermode can affect the measured thresholds. Using a constant and elevated initial thermode temperature appeared to be a more reliable method for NTT. The results provide reliable information on a new thermal threshold device. Future work should compare more breeds of dog and the incorporation of analgesics to further characterize breed differences in pain sensitivity.

5. References

Almeida, T.F., Roizenblatt, S., Tufik, S., 2004. Afferent pain pathways: a neuroanatomical review. Brain Research 1000, 40-56.

Arendt-Nielsen, L., Chen, A.C., 2003. Lasers and other thermal stimulators for activation of skin nociceptors in humans. Neurophysiologie Clinique/Clinical Neurophysiology 33, 259-268.

Arendt-Nielsen, L., Yarnitsky, D., 2009. Experimental and clinical applications of quantitative sensory testing applied to skin, muscles and viscera. The Journal of Pain 10, 556-572.

Asp, H.E., Fikse, W.F., Nilsson, K., Strandberg, E., 2015. Breed differences in everyday behaviour of dogs. Applied Animal Behaviour Science 169, 69-77.

Bannon, A.W., Malmberg, A.B., 2007. Models of nociception: hot-plate, tail-flick, and formalin tests in rodents. Current Protocols in Neuroscience 41, 891-896.

Baranauskas, G., Nistri, A., 1998. Sensitization of pain pathways in the spinal cord: cellular mechanisms. Progress in Neurobiology 54, 349-365.

Bergadano, A., Andersen, O.K., Arendt-Nielsen, L., Schatzmann, U., Spadavecchia, C., 2006. Quantitative assessment of nociceptive processes in conscious dogs by use of the nociceptive withdrawal reflex. American Journal of Veterinary Research 67, 882-889.

Bergadano, A., Andersen, O.K., Arendt-Nielsen, L., Spadavecchia, C., 2007. Noninvasive assessment of the facilitation of the nociceptive withdrawal reflex by repeated electrical stimulations in conscious dogs. American journal of veterinary research 68, 899-907.

Blom, G., 1958. Statistical estimates and transformed beta-variables.

Bridgestock, C., Rae, C.P., 2013. Anatomy, physiology and pharmacology of pain. Anaesthesia & Intensive Care Medicine 14, 480-483.

Briley, J.D., Williams, M.D., Freire, M., Griffith, E.H., Lascelles, B.D.X., 2014. Feasibility and repeatability of cold and mechanical quantitative sensory testing in normal dogs. The Veterinary Journal 199, 245-250.

Broom, D., 2001. Evolution of pain. Vlaams Diergeneeskundig Tijdschrift 70, 17-21.

Butler, R.K., Finn, D.P., 2009. Stress-induced analgesia. Progress in Neurobiology 88, 184-202.

Campbell, J.N., Meyer, R.A., 2006. Mechanisms of neuropathic pain. Neuron 52, 77-92.

Carregaro, A.B., Luna, S.P., Mataqueiro, M.I., de Queiroz-Neto, A., 2007. Effects of buprenorphine on nociception and spontaneous locomotor activity in horses. American Journal of Veterinary Research 68, 246-250.

Cervero, F., 2012. Understanding Pain: Exploring the Perception of Pain. Mit Press.

Chambers, J., Waterman, A., Livingston, A., 1994. Further development of equipment to measure nociceptive thresholds in large animals. Veterinary Anaesthesia and Analgesia 21, 66-72.

Chen, A.C., Dworkin, S.F., Haug, J., Gehrig, J., 1989. Topographic brain measures of human pain and pain responsivity. Pain 37, 129-141.

Chesler, E.J., Wilson, S.G., Lariviere, W.R., Rodriguez-Zas, S.L., Mogil, J.S., 2002. Identification and ranking of genetic and laboratory environment factors influencing a behavioral trait, thermal nociception, via computational analysis of a large data archive. Neuroscience & Biobehavioral Reviews 26, 907-923.

Colborn, D., Thompson Jr, D., Roth, T., Capehart, J., White, K., 1991. Responses of cortisol and prolactin to sexual excitement and stress in stallions and geldings. Journal of Animal Science 69, 2556-2562.

Cole, E., 2002. Pain management: classifying, understanding, and treating pain. Hospital Physician, 23.

Coleman, K.D., Schmiedt, C.W., Kirkby, K.A., Coleman, A.E., Robertson, S.A., Hash, J., Lascelles, B.D.X., 2014. Learning confounds algometric assessment of mechanical thresholds in normal dogs. Veterinary Surgery 43, 361-367.

Crabbe, J.C., Wahlsten, D., Dudek, B.C., 1999. Genetics of mouse behavior: interactions with laboratory environment. Science 284, 1670-1672.

Criado, A., 2010. Recognition and alleviation of pain in laboratory animals. Laboratory Animals 44, 380-380.

Davila, D., Keeshen, T.P., Evans, R.B., Conzemius, M.G., 2013. Comparison of the analgesic efficacy of perioperative firocoxib and tramadol administration in dogs undergoing tibial plateau leveling osteotomy. Journal of the American Veterinary Medical Association 243, 225-231.

Dixon, M., Robertson, S., Taylor, P., 2002. A thermal threshold testing device for evaluation of analgesics in cats. Research in Veterinary Science 72, 205-210.

Dixon, M., Taylor, P., Slingsby, L., Hoffmann, M., Kästner, S., Murrell, J., 2010. A small, silent, low friction, linear actuator for mechanical nociceptive testing in veterinary research. Laboratory Animals 44, 247-253.

Dixon, M., Taylor, P., Slingsby, L., Murrell, J., 2015. Refinement of a thermal threshold probe to prevent burns. Laboratory animals, 0023677215577313.

Edwards, C.L., Fillingim, R.B., Keefe, F., 2001. Race, ethnicity and pain. Pain 94, 133-137.

Farnworth, M.J., Beausoleil, N.J., Adams, N.J., Barrett, L.A., Stevenson, M., Thomas, D.G., Waterland, M.R., Waran, N.K., Stafford, K.J., 2013. Validating the use of a carbon dioxide laser for assessing nociceptive thresholds in adult domestic cats (*Felis catus*). Applied Animal Behaviour Science 143, 104-109.

Firth, A.M., Haldane, S.L., 1999. Development of a scale to evaluate postoperative pain in dogs. Journal of the American Veterinary Medical Association 214, 651-659.

Fleischer, S., Sharkey, M., Mealey, K., Ostrander, E.A., Martinez, M., 2008. Pharmacogenetic and metabolic differences between dog breeds: their impact on canine medicine and the use of the dog as a preclinical animal model. AAPS J 10, 110-119.

Fox, S.M., 2013. Pain management in small animal medicine. CRC Press, Boca Raton, FL, USA.

Gardy-Godillot, M., Durand, D., Dalle, M., Bauchart, D., 1989. Diurnal pattern of plasma cortisol in preruminant calves fasted or fed different milk proteins. Journal of Dairy Science 72, 1842-1846.

Guesgen, M.J., Beausoleil, N.J., Minot, E.O., Stewart, M., Jones, G., Stafford, K.J., 2011. The effects of age and sex on pain sensitivity in young lambs. Applied Animal Behaviour Science 135, 51-56.

Hansen, B.D., 2003. Assessment of pain in dogs: veterinary clinical studies. Ilar Journal 44, 197-205.

Hekman, J.P., Karas, A.Z., Dreschel, N.A., 2012. Salivary cortisol concentrations and behavior in a population of healthy dogs hospitalized for elective procedures. Applied Animal Behaviour Science 141, 149-157.

Herrero, J.F., Laird, J.M., Lopez-Garcia, J.A., 2000. Wind-up of spinal cord neurones and pain sensation: much ado about something? Progress in Neurobiology 61, 169-203.

Hielm-Björkman, A., 2013. Recognition and assessment of chronic pain in dogs. Wiley-Blackwell, Iowa State University Press.

Hoffmann, M.V., Kästner, S.B.R., Kietzmann, M., Kramer, S., 2012. Contact heat thermal threshold testing in beagle dogs: baseline reproducibility and the effect of acepromazine, levomethadone and fenpipramide. BMC Veterinary Research 8, 206.

Hole, K., Tjølsen, A., 1993. The tail-flick and formalin tests in rodents: changes in skin temperature as a confounding factor. Pain 53, 247-254.

Holton, L., Reid, J., Scott, E., Pawson, P., Nolan, A., 2001. Development of a behaviour-based scale to measure acute pain in dogs. The Veterinary Record 148, 525-531.

Imbe, H., Iwai-Liao, Y., Senba, E., 2005. Stress-induced hyperalgesia: animal models and putative mechanisms. Frontiers in Bioscience: A Journal and Virtual Library 11, 2179-2192.

ISAP, 1994. ISAP taxonomy.

Kaelin, C., Barsh, G., 2012. Molecular genetics of coat colour, texture and length in the dog, in: Ostrander, E., Ruvinsky, A. (Eds.), The genetics of the dog, CABI, Cambridge, MA, USA.

Lark, K., Chase, K., 2012. Complex traits in the dog, in: Ostrander, E., Ruvinsky, A. (Eds.), The genetics of the dog, CABI, Cambridge, MA, USA.

Lascelles, B., Butterworth, S., Waterman, A., 1994. Postoperative analgesic and sedative effects of carprofen and pethidine in dogs. The Veterinary Record 134, 187-191.

Le Bars, D., Gozariu, M., Cadden, S.W., 2001. Animal models of nociception. Pharmacological Reviews 53, 597-652.

Lichtman, A.H., Smith, F.L., Martin, B.R., 1993. Evidence that the antinociceptive tail-flick response is produced independently from changes in either tail-skin temperature or core temperature. Pain 55, 283-295.

Lineberry, C., 1981. Laboratory animals in pain research. Methods in Animal Experimentation 6, 237-311.

Love, E., Taylor, P., Murrell, J., Dixon, M., Whay, H., Waterman-Pearson, A., 2008. Modification of a feline thermal threshold testing system for use in horses. Veterinary Anaesthesia and Analgesia 35, 3-14.

Love, E., Taylor, P., Murrell, J., Whay, H., 2012. Effects of acepromazine, butorphanol and buprenorphine on thermal and mechanical nociceptive thresholds in horses. Equine Veterinary Journal 44, 221-225.

Love, E.J., Murrell, J., Whay, H.R., 2011. Thermal and mechanical nociceptive threshold testing in horses: a review. Veterinary Anaesthesia and Analgesia 38, 3-14.

Mathews, K., Kronen, P.W., Lascelles, D., Nolan, A., Robertson, S., Steagall, P.V., Wright, B., Yamashita, K., 2014. Guidelines for recognition, assessment and treatment of pain. Journal of Small Animal Practice 55, 10-68.

McMullan, S., Simpson, D.A., Lumb, B.M., 2004. A reliable method for the preferential activation of C-or A-fibre heat nociceptors. Journal of Neuroscience Methods 138, 133-139.

Mehrkam, L.R., Wynne, C.D.L., 2014. Behavioral differences among breeds of domestic dogs (*Canis lupus familiaris*): Current status of the science. Applied Animal Behaviour Science 155, 12-27.

Meintjes, R.A., 2012. An overview of the physiology of pain for the veterinarian. The Veterinary Journal 193, 344-348.

Mellor, D., Cook, C., Stafford, K., 2000. Quantifying some responses to pain as a stressor. The Biology of Animal Stress: Basic Principles and Implications for Welfare, 171-198.

Melzack, R., Wall, P.D., 1999. Textbook of pain. Churchill Livingstone.

Millan, M.J., 1999. The induction of pain: an integrative review. Progress in Neurobiology 57, 1-164.

Millette, V.M., Steagall, P.V., Duke-Novakovski, T., Livingston, A.J., 2008. Effects of meperidine or saline on thermal, mechanical and electrical nociceptive thresholds in cats. Veterinary Anaesthesia and Analgesia 35, 543-547.

Minto, B.W., Rodrigues, L.C., Steagall, P.V., Monteiro, E.R., Brandão, C.V., 2013. Assessment of postoperative pain after unilateral mastectomy using two different surgical techniques in dogs. Acta Veterinaria Scandinavica 55, 60.

Mogil, J.S., 1999. The genetic mediation of individual differences in sensitivity to pain and its inhibition. Proceedings of the National Academy of Sciences 96, 7744-7751.

Mogil, J.S., Wilson, S.G., Bon, K., Lee, S.E., Chung, K., Raber, P., Pieper, J.O., Hain, H.S., Belknap, J.K., Hubert, L., 1999. Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. Pain 80, 67-82.

Molony, V., Kent, J., 1997. Assessment of acute pain in farm animals using behavioral and physiological measurements. Journal of Animal Science 75, 266-272.

Moore, S., Hettlich, B., Waln, A., 2013. The use of an electronic von Frey device for evaluation of sensory threshold in neurologically normal dogs and those with acute spinal cord injury. The Veterinary Journal 197, 216-219.

Morgaz, J., Navarrete, R., Muñoz-Rascón, P., Domínguez, J., Fernández-Sarmiento, J., Gómez-Villamandos, R., Granados, M., 2013. Postoperative analgesic effects of dexketoprofen, buprenorphine and tramadol in dogs undergoing ovariohysterectomy. Research in Veterinary Science 95, 278-282.

Morton, C.M., Reid, J., Scott, E.M., Holton, L.L., Nolan, A.M., 2005. Application of a scaling model to establish and validate an interval level pain scale for assessment of acute pain in dogs. American Journal of Veterinary Research 66, 2154-2166.

Nielsen, C.S., Stubhaug, A., Price, D.D., Vassend, O., Czajkowski, N., Harris, J.R., 2008. Individual differences in pain sensitivity: genetic and environmental contributions. Pain 136, 21-29.

New Zealand Kennel Club. (n.d.) NZ Huntaway. Retrieved from http://www.nzkc.org.nz /breed_info/br535.html

Pascoe, P., 2012. Anesthesia and pain management. Saunders Elsevier, Edinburgh.

Perez, T.E., Grubb, T.L., Greene, S.A., Meyer, S., Valdez, N., Bingman, J., Farnsworth, R., 2013. Effects of intratesticular injection of bupivacaine and epidural administration of morphine in dogs undergoing castration. Journal of the American Veterinary Medical Association 242, 631-642.

Poller, C., Hopster, K., Rohn, K., Kästner, S.B., 2013. Evaluation of contact heat thermal threshold testing for standardized assessment of cutaneous nociception in horses-comparison of different locations and environmental conditions. BMC Veterinary Research 9, 4.

Queiroz-Neto, A., Zamur, G., Gonçalves, S., Carregaro, A., Mataqueiro, M., Harkins, J., Tobin, T., 1998. Characterization of the antinociceptive and sedative effect of amitraz in horses. Journal of Veterinary Pharmacology and Therapeutics 21, 400-405.

Rauser, P., Janalik, P., Markova, M., Fichtel, T., 2013. Early analgesia after periodontal treatment in dogs: a comparison of three analgesic protocols. Veterinarni Medicina 58, 312-317.

Reddi, D., Curran, N., Stephens, R., 2013. An introduction to pain pathways and mechanisms. British Journal of Hospital Medicine 74, 188-191.

Reid, J., Nolan, A., Hughes, J., Lascelles, D., Pawson, P., Scott, E., 2007. Development of the short-form Glasgow Composite Measure Pain Scale (CMPS-SF) and derivation of an analgesic intervention score. Animal Welfare 16, 97-104.

Robertson, S., Sanchez, L., Merritt, A., Doherty, T., 2005. Effect of systemic lidocaine on visceral and somatic nociception in conscious horses. Equine Veterinary Journal 37, 122-127.

Robertson, S., Taylor, P., Lascelles, B., Dixon, M., 2003. Changes in thermal threshold response in eight cats after administration of buprenorphine, butorphanol and morphine. The Veterinary Record 153, 462-465.

Robertson, S.A., Wegner, K., Lascelles, B.D.X., 2009. Antinociceptive and side-effects of hydromorphone after subcutaneous administration in cats. Journal of Feline Medicine and Surgery 11, 76-81.

Schaible, H.-G., 2007. Peripheral and central mechanisms of pain generation, Analgesia, Springer, pp. 3-28.

Sharkey, M., 2013. The challenges of assessing osteoarthritis and postoperative pain in dogs. The AAPS journal 15, 598-607.

Sheffield, D., Biles, P.L., Orom, H., Maixner, W., Sheps, D.S., 2000. Race and sex differences in cutaneous pain perception. Psychosomatic Medicine 62, 517-523.

Shir, Y., Seltzer, Z.e., 2001. Heat hyperalgesia following partial sciatic ligation in rats: interacting nature and nurture. Neuroreport 12, 809-813.

Slingsby, L.S., Taylor, P.M., Monroe, T., 2009. Thermal antinociception after dexmedetomidine administration in cats: a comparison between intramuscular and oral transmucosal administration. Journal of Feline Medicine and Surgery 11, 829-834.

Sneddon, L.U., Elwood, R.W., Adamo, S.A., Leach, M.C., 2014. Defining and assessing animal pain. Animal Behaviour 97, 201-212.

Stafford, K., Mellor, D., Gregory, N., 2002. Advances in animal welfare in New Zealand. New Zealand Veterinary Journal 50, 17-21.

Stafford, K.J., 2006. The Welfare of Dogs. Springer Science & Business Media, Netherlands.

Stamford, J., 1995. Descending control of pain. British Journal of Anaesthesia 75, 217-227.

Steagall, P.V.M., Taylor, P.M., Brondani, J.T., Luna, S.P.L., Dixon, M.J., Ferreira, T.H., 2007. Effects of buprenorphine, carprofen and saline on thermal and mechanical nociceptive thresholds in cats. Veterinary Anaesthesia and Analgesia 34, 344-350.

Steeds, C.E., 2013. The anatomy and physiology of pain. Surgery (Oxford) 31, 49-53.

Stubsjøen, S.M., Flø, A.S., Moe, R.O., Janczak, A.M., Skjerve, E., Valle, P.S., Zanella, A.J., 2009. Exploring non-invasive methods to assess pain in sheep. Physiology & behavior 98, 640-648.

Svensson, P., Rosenberg, B., Beydoun, A., Morrow, T., Casey, K., 1997. Comparative psychophysical characteristics of cutaneous CO2 laser and contact heat stimulation. Somatosensory & Motor Research 14, 113-118.

Taylor, P.M., Robertson, S.A., Dixon, M.J., 2007. Evaluation of the use of thermal thresholds to investigate NSAID analgesia in a model of inflammatory pain in cats. Journal of Feline Medicine & Surgery 9, 313-318.

Turk, D.C., Okifuji, A., 2001. Pain terms and taxonomies of pain. 3rd ed ed. Lippincott Williams & Wilkins, Philadelphia, PA.

Veissier, I., Rushen, J., Colwell, D., de Passillé, A.M., 2000. A laser-based method for measuring thermal nociception of cattle. Applied Animal Behaviour Science 66, 289-304.

Villaescusa, A., García-Sancho, M., Delgado, A.M., Tesouro, M.Á., Rodríguez-Franco, F., Sainz, Á., 2012. Immunophenotypic evaluation of working labrador retrievers and german shepherd dogs living in the same environment. The Veterinary Journal 193, 602-605.

Viñuela-Fernández, I., Jones, E., Welsh, E.M., Fleetwood-Walker, S.M., 2007. Pain mechanisms and their implication for the management of pain in farm and companion animals. The Veterinary Journal 174, 227-239.

Vítková, J., Loučka, M., Boček, J., Vaculín, Š., 2015. The effect of acclimatization and ambient temperature on heat withdrawal threshold in rats. European Journal of Pain 19, 21-27.

Weary, D.M., Niel, L., Flower, F.C., Fraser, D., 2006. Identifying and preventing pain in animals. Applied Animal Behaviour Science 100, 64-76.

Weber, G., Morton, J., Keates, H., 2012. Postoperative pain and perioperative analgesic administration in dogs: practices, attitudes and beliefs of Queensland veterinarians. Australian Veterinary Journal 90, 186-193.

Wegner, K., Horais, K.A., Tozier, N.A., Rathbun, M.L., Shtaerman, Y., Yaksh, T.L., 2008. Development of a canine nociceptive thermal escape model. Journal of Neuroscience Methods, 88.

Whay, H.R., 1998. The perception and relief of pain associated with lameness in dairy cattle, University of Bristol.

White, K.L., 2013. Recognition and Assessment of Acute Pain in the Dog, Pain Management in Veterinary Practice, John Wiley & Sons, Ltd, pp. 199-207.

Wiseman-Orr, M.L., Scott, E.M., Reid, J., Nolan, A.M., 2006. Validation of a structured questionnaire as an instrument to measure chronic pain in dogs on the basis of effects on health-related quality of life. American Journal of Veterinary Research 67, 1826-1836.

Woodrow, K.M., Friedman, G.D., Siegelaub, A., Collen, M.F., 1972. Pain tolerance: differences according to age, sex and race. Psychosomatic Medicine 34, 548-556.

Woolf, C.J., 2011. Central sensitization: implications for the diagnosis and treatment of pain. Pain 152, 2-15.

Zatzick, D.F., Dimsdale, J.E., 1990. Cultural variations in response to painful stimuli. Psychosomatic Medicine 52, 544-557.