Acute nociception in neonatal pigs undergoing tail docking: Influence of docking method and age, evaluation of pain mitigation strategies, and assessment of the potential for longer-term pain

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Nikki J Kells

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ABSTRACT

Tail docking of pigs is performed routinely in many parts of the world to reduce the incidence of unwanted tail biting behaviour. Whilst tail biting can have serious welfare consequences for affected pigs, tail docking may also negatively affect pig welfare as a result of acute pain induced by the procedure itself, as well as through long-term changes in afferent neural inputs from the remaining tail stump. The aims of this thesis were to examine the influences of docking method and piglet age on acute nociceptive responses to tail docking; to evaluate the efficacy of selected anti-nociceptive strategies in mitigating acute nociceptive responses to tail docking; to determine whether docking method affects subsequent neural morphology of the healed tail stump. The minimal anaesthesia model (MAM), which involves analysis of electroencephalographic (EEG) data, was used to evaluate acute nociceptive responses and to ascertain the efficacy of anti-nociceptive strategies. Histopathological examination of tissue harvested from tail tips was performed to evaluate alterations in neural morphology that might be associated with long-term changes in pain processing.

Comparison of the acute nociceptive responses of 2- and 20-day-old pigs to tail docking revealed little evidence of nociception in the younger age group compared with a typical response in the older pigs. In addition, total EEG power was lower in 2 day-old pigs. These results suggest that there are differences in either neural maturity, and/or in nociceptive processing between the two ages.

Tail docking using cautery iron appears to be less acutely painful to pigs than tail docking using clippers. However, the longer-term pain consequences associated with the two methods need to be assessed before one method is recommended over the other.

Prior application of a topical anaesthetic (EMLA) cream to the tail abolished EEG indicators of acute nociception in pigs tail docked using clippers, whereas prior administration of oral meloxicam had no effect on EEG responses. When no analgesia was used, tail docking using cautery iron ameliorated EEG indicators of nociception, relative to docking using clippers. Thus, prior administration of EMLA cream or the use of cautery iron in place of clippers have the potential to reduce the acute pain during routine tail docking.

Acute EEG responses of pigs to the noxious stimulus of tail docking varied significantly with postnatal age over the first 15 days of life. Docking at 1 day-of-age elicited no EEG evidence of nociception, whilst cortical responsiveness to tail docking increased with postnatal age across the range of 5–15 days. This enhanced responsiveness may be due to the gradual withdrawal of fetal neurosuppressive mechanisms after birth, or rapid postnatal maturation of the cerebral cortex, or a combination of both.

Tail docking using both side clippers and cautery iron resulted in the formation of neuromas, which have been associated with neuropathic pain, in the tail stump. Neither the proportion of tails with neuromas, nor the degree of abnormal nerve proliferation in the tail tip differed between the two docking methods. This suggests no longer-term welfare advantage of one method over the other, at least in terms of the potential for alterations in pain processing following stimulation of tail stump nociceptors.

In terms of best practice guidelines for the performance of tail docking in pigs, this research provides support for current recommendations that tail docking, along with other painful husbandry procedures, be performed within one week of birth. Furthermore, tail docking with cautery induced less acute pain than docking with clippers, whilst both methods cause long-term changes in neural morphology in the tail stump. Docking using cautery may therefore be preferable to docking with clippers. Whilst cautery reduces the acute pain associated with docking relative to clippers, prior application of a topical anaesthetic cream (EMLA) completely abolished acute nociceptive responses to. Prior administration of topical anaesthesia, or the use of a cautery iron in place of clippers, has the potential to improve the welfare of pigs undergoing routine tail docking.

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"Life is not easy for any of us. But what of that? We must have perseverance and above all confidence in ourselves. We must believe that we are gifted for something and that this thing must be attained"

– Marie Curie

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Kells NJ, Beausoleil NJ, Johnson CB, Sutherland MA, Morrison RS, Roe W. Comparison of neural histomorphology in tail tips from pigs docked using clippers or cautery iron. *Animal,* In Press. (Accepted for publication 28 September 2016)

Kells NJ, Beausoleil NJ, Sutherland MA, Morrison RB, and Johnson CB. Electroencephalographic assessment of oral meloxicam, topical anaesthetic cream and cautery iron for mitigating acute pain in pigs (Sus scrofa) undergoing tail docking. *Veterinary Anaesthesia & Analgesia,* In Press. (Accepted for publication 15 February 2017)

Kells NJ, Beausoleil NJ, Sutherland MA, Morrison RB, and Johnson CB. Electroencephalographic responses of anaesthetised pigs (Sus scrofa) to tail docking using clippers or cautery iron, performed at two or twenty days of age. *Veterinary Anaesthesia & Analgesia,* In Press. (Accepted for publication 14 February 2017)

Morrison RS, Kells NJ, Johnson CB, Hemsworth PH. *Assessment of Pain Induced by Tail Docking in Piglets and Strategies to Reduce this Pain*. Final Report APL Project 2012/1018.348, NSW, Australia, 2013

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Kongara K, McIlhone AE, Kells NJ, Johnson CB. Electroencephalographic evaluation of decapitation of the anaesthetized rat. *Laboratory Animals* 48, 15–9, 2014

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Rault JL, Kells N, Johnson C, Dennis R, Sutherland M, Lay DC, Jr. Nitrous oxide as a humane method for piglet euthanasia: Behavior and electroencephalography (EEG). *Physiology & Behavior* 151, 29–37, 2015

CHAPTER 1 General Introduction

The aim of this body of work was to compare the potential for acute and long-term pain experience by piglets undergoing tail docking using either side cutter pliers or hot cautery iron, and to evaluate some commercially viable strategies to mitigate acute nociceptive responses to tail docking. In addition, the influence of piglet age on the development of acute nociceptive responses to tail docking was also explored. Acute nociception was evaluated *in-vivo* using electroencephalographic (EEG) measures recorded under light halothane anaesthesia (minimal anaesthesia model, see 1.5.2 below), whilst the potential for long term pain was investigated via post-mortem histological analysis of tail tissues collected from mature pigs at the time of slaughter.

1.1 THESIS STRUCTURE

Following a general introduction to the topic area, this thesis presents the results of four separate experimental studies, each of which is formatted according to the specific guidelines of the peer-reviewed journal to which it was submitted for publication. Chapters 2–5 and Appendix B have been published or submitted for publication in substantially equivalent, though slightly abridged, formats to those presented in the thesis. The experimental chapters are followed by a general discussion (Chapter 6).

Experiment 1 (presented in Chapter 2) compared the acute nociceptive responses of piglets to the noxious stimulus of tail docking performed using side cutters or hot cautery at 2 or 20 days-of-age. A paper based on this has been accepted for publication in *Veterinary Anaesthesia and Analgesia*. Experiment 2 (presented in Chapter 3) evaluated the efficacy of oral meloxicam, a topical anaesthetic cream or hot cautery in mitigating acute nociceptive responses to tail docking, relative to control pigs docked using side cutters without prior analgesia. A paper based on this has been accepted for publication in *Veterinary Anaesthesia and Analgesia*. In experiment 3 (presented in Chapter 4), the post-natal development of acute nociceptive responses to the noxious stimulus of tail docking using side cutters was examined in piglets aged between 1 and 15 days-of-age. A paper based on this has been submitted for publication in *Animal Welfare*. Experiment 4 (presented in Chapter 5) compared the neural histomorphology, including the incidence of neuromas, in the healed tips of tails collected from mature pigs at slaughter that had been docked using either side cutters or hot cautery. This paper has been accepted for publication in *Animal*. In addition, EEG data collected during experiment 2 were further analysed to evaluate the nociceptive responses of pigs to intra-peritoneal injection of sodium pentobarbital used to perform euthanasia. This is presented in Appendix B. A paper based on this has been submitted for publication in *Animal Welfare*. Appendix A presents a statistical comparison of spectral EEG data recorded from the left and right cerebral cortices in Chapter 3, to investigate whether there is any lateralisation of nociceptive signal processing in pigs.

1.2 TAIL DOCKING

1.2.1 Description

Tail docking involves amputation of the distal portion of the tail. This may be performed using teeth clippers, side cutter pliers, scissors, scalpel blade, or a gas or electrical heated cautery iron (Sutherland and Tucker 2011). The procedure is normally performed within the first week of life, without the provision of analgesia. There is considerable variation among producers with regard to the proportion of tail removed, some of which relates to different national recommendations. The New Zealand Code of Welfare for Pigs (MPI 2010) recommends removal of one third to half of the tail; Canadian recommendations state that the remaining tail stump must be of sufficient length to cover the anus (National Farm Animal Care Council 2014); whilst Australian (CSIRO 2008), American (AVMA 2016) and UK (DEFRA 2003; Council of the European Union 2008) recommendations do not specify docking length. It has been reported that common practice in the US is to retain a stump of sufficient length to cover the vulva in females and an equivalent length in males (Sutherland and Tucker 2011).

1.2.2 Rationale and justification

In modern commercial pig production, tail docking is routinely performed as a means of reducing the incidence of undesirable tail-biting behaviour. The term 'tail-biting' has been used to describe a range of behaviours whereby one pig orally manipulates another's tail. This may range from gentle oral manipulation through to partial or complete amputation of the tail and gouging of the rump (Taylor *et al.* 2010). For the purposes of this thesis, the term tail biting will be used to describe oral manipulation of the tail resulting in any form of lesions or other damage (see section 1.2 for more detail).

It has been proposed that tail biting occurs in two distinct stages; a 'pre-injury stage' before any visual wound is present on the tail and an 'injury stage' where the tail is wounded and bleeding (Fraser and Broom 1990). Behaviour in the pre-injury stage, involving tail manipulation with no apparent injury, is also known as tail-in-mouth (TIM) behaviour (Schrøder-Petersen *et al.* 2004). TIM behaviour is usually tolerated by the recipient and often occurs while both pigs are lying down (Newberry and Wood-Gush 1988; Schrøder-Petersen *et al.* 2004). TIM is thought to be a natural behaviour among pigs, based on its observation in semi-natural conditions (Newberry and Wood-Gush 1988). In contrast, tail biting leading to injury is considered an abnormal, pathological behaviour (EFSA 2007; Sonoda *et al.* 2012). Although reports of tail biting among domesticated pigs date back more than a century (Fraser and Broom 1990) the behaviour is generally associated with modern production systems in which pigs are maintained at high stocking densities with restricted access to resources and without the opportunity to engage in normal foraging or exploratory behaviours (Stafford 2010). Of note, tail-biting behaviour has not been reported in other non-domesticated suid or peccary species, in the wild or in captivity (Taylor *et al.* 2010).

Whilst TIM behaviour typically appears early in life and tends to decline with age (EFSA 2007), tail biting often begins later in life, with reports of the behaviour being more prevalent among grower-finisher pigs (EFSA 2007; Schrøder-Petersen and Simonsen 2001). However, whether these trends are a direct function of piglet age or a reflection of changes in housing and husbandry has not been determined (EFSA 2007).

There is substantial evidence that tail docking reduces the incidence of tail biting among commercially farmed pigs (Penny and Hill 1974; McGlone and Nicholson 1992; Hunter *et al.* 1999; 2001; Sutherland *et al.* 2008; Sutherland *et al.* 2009; Keeling *et al.* 2012; Scollo *et al.* 2015). However, the reported incidence of tail bite lesions among docked pigs varies, likely due to variations in both docking length and

management factors between farms and regions. In an experimental study across four pig farms, Thodberg and co-workers (2010) reported that removing less than three-quarters of the tail (half, quarter, or none) was ineffective in reducing tail biting incidence. In addition, where sufficient healing has occurred severely bitten tails may be indistinguishable from docked tails at slaughter, thus resulting in overestimation of docking prevalence based on slaughterhouse data (Harley *et al.* 2012).

Conversely, two studies have shown an increase in tail biting risk among docked pigs (Chambers *et al.* 1995; Moinard *et al.* 2003); however, this may be due to tail docking being performed in response to existing tail biting problems on the farms in question (EFSA 2007). Overall, it is thought that tail docking effects a 2-to-4-fold reduction in tail biting prevalence (Valros and Heinonen 2015).

1.2.3 Legislation and recommendations

Tail docking is routinely performed in many countries, despite the introduction of recommendations and/or legislation in some countries aimed at limiting its routine practice. In New Zealand, the Code of Welfare (Pigs) recommended best practice states, "Other measures to control tail biting should be considered before tail docking is undertaken" (Anonymous 2010). In addition, the minimum standard states that a veterinarian must perform tail docking of pigs over seven days of age, whilst recommended best practice guidelines state that when undertaken to prevent tail biting, tail docking should be carried out within 72 hours of birth and pain relief should be given, irrespective of age.

Similarly, Australia's Model Code of Practice for the Welfare of Pigs states that "Tail docking should be avoided wherever possible" and that "Where tail biting is a problem, all aspects of the environment, feeding and management should be investigated to identify the contributing factors so that remedial action can be taken, e.g. environmental enrichment with straw or other materials that can be manipulated" (CSIRO 2008). It further stipulates that where practiced as a preventive measure, tail docking should be carried out before seven days of age.

In the European Union (EU), EU council directive 2008/120/EC, introduced in December 2008, states "Neither tail-docking nor reduction of corner teeth must be carried out routinely but only where there is evidence that injuries to sows' teats or to other pigs' ears or tails have occurred. Before carrying out these procedures, other measures shall be taken to prevent tail-biting and other vices, taking into account environment and stocking densities. For this reason, inadequate environmental conditions or management systems must be changed" (Council of the European Union 2008). Further, the directive indicates that tail docking should only be performed by a veterinarian or competently trained person and, where practiced after the seventh day of life, under anaesthetic and prolonged analgesia by a veterinarian. EU member states Sweden, Finland and Lithuania have national legislation banning the practice of tail docking, whilst Norway only permits tail docking for medical reasons and requires the provision of anaesthesia and prolonged analgesia regardless of age (EFSA 2007). Denmark allows tail docking to be performed between the second and fourth day of age, in cases where it has been documented that tail biting occurs in the absence of tail docking, and requires provision of prolonged analgesia if performed after four days of age (EFSA 2007).

In the United States, where tail docking is routine practice, no limitations are placed on its routine use (AVMA 2016). In terms of age, AVMA guidelines do not specify a maximum age, but state, "*Tail docking should be performed early and sufficiently prior to weaning such that no open wounds remain at the time of weaning*" (AVMA 2016).

In China, the world's largest pork producer, there are no existing welfare guidelines for pig production, although the country is working toward the development of codes of practice for pigs and other farmed animals[1].

[1] http://www.thepigsite.com/swinenews/40838/china-making-progress-in-welfare-in-pig-production/

1.2.4 Prevalence

Tail docking is routinely performed on commercial pig farms in the United States, New Zealand and Australia where specific legislation banning or limiting its use does not exist. In the EU, the number of pigs subject to tail docking likely varies according to housing system and legislation; however, the results from a survey of experts in all EU countries plus Norway indicates that more than 90% of EU pigs are tail docked (EFSA 2007), despite current legislation. The reason for the continuing high incidence of routine tail docking in the EU is thought to relate to the lack of specific details in EU legislation regarding the alternative steps that producers should take before resorting to tail docking (D'Eath *et al.* 2016), a view that probably holds true for countries such as New Zealand and Australia where it is recommended that tail docking only be performed when other management factors fail to control tail biting. In European countries where tail docking is permitted, undocked pigs are almost exclusively associated with organic pork production, due to specific regulations surrounding organic accreditation (EFSA 2007).

1.2.5 Welfare implications

Acute responses to tail docking, utilising both physiological and behavioural indices, have been widely studied in pigs. Acute physiological responses to tail docking vary according to both docking method and age. In terms of plasma cortisol responses, Prunier *et al.* (2005) reported no differences between pigs docked using cautery and sham-docked or control pigs at 1-day old. Likewise, Sutherland *et al.* (2008) also reported no difference in plasma cortisol concentration between cautery-docked and control-handled pigs at 6 days of age; however, cortisol was higher in pigs docked using cutting pliers than cautery-docked or control-handled pigs 60 minutes after docking. In their comparison of pigs docked using clippers or cautery at 2, 3 or 8 days of age, Marchant-Forde *et al.* (2009) reported no effect of docking method or age on plasma cortisol or ß-endorphin levels from 45 minutes to 2 weeks after docking.

Acute behavioural responses to tail docking have been more extensively investigated. Several pain-related behaviours were found to be more common in tail-docked than control pigs following processing, including jamming the tail between the legs (Noonan *et al.* 1994; Torrey *et al.* 2009), tail wagging (Noonan *et al.* 1994), posterior scooting (dragging the caudal part of the body across the ground; Sutherland *et al.* 2008) and standing with the head lowered (Morrison *et al.* 2013). In addition, tail docked pigs exhibited more escape attempts (Marchant-Forde *et al.* 2009; Morrison *et al.* 2013), produced more vocalisations (Noonan *et al.* 1994; Marchant-Forde *et al.* 2009; Morrison *et al.* 2013) and higher peak vocal frequencies (Marchant-Forde *et al.* 2009) during processing than sham-docked controls. Together, these studies indicate that tail docking causes acute stress and pain, regardless of the method used.

In addition to the accepted acute pain and distress induced by the procedure, there is concern that tail docking might have long-term consequences for pain perception. It has been proposed that part of the efficacy of tail docking in reducing the incidence of tail biting relates to subsequent hypersensitivity at the site of docking, stimulating protective behaviour toward the remaining tail stump (Simonsen *et al.* 1991). Histopathological studies of finishing pigs have revealed the presence of regressive changes in the peripheral nerves at the site of docking, including the presence of traumatic neuromas (Simonsen *et al.* 1991; Herskin *et al.* 2014).

Due to associations between the presence of neural lesions such as neuromas and the development of neuropathic pain following limb amputation and peripheral nerve injury in humans (Lewin-Kowalik *et al.* 2006), the presence of neuromas in docked tails raises the possibility of long term pain sequelae in affected individuals.

To date, there has only been a single set of experimental studies assessing changes in protective behaviour toward the tail following tail docking. Chermat (2006) reported that less than 5% of tail-

docked pigs reacted (avoidance or, less commonly, aggression) to being subject to TIM behaviour by another pig when no lesions were present on the tail; this figure increased to 70% when tail lesions were present. In contrast, more than 50% of pigs reacted to attempted ear-in-mouth behaviour. The author concluded there was no evidence of increased reactivity to tail assault as a consequence of tail docking.

Given the fact that approximately 1.25 billion pigs are slaughtered annually, with more than 85% of these produced in China, the EU and the United States[2], where tail docking is routinely carried out, improving the welfare of pigs undergoing tail docking has the potential to benefit large numbers of animals worldwide.

[2] Statistics obtained from the United States Department of Agriculture www.fas.usda.gov

1.3 TAIL BITING

1.3.1. Prevalence

Attempts to quantify the incidence of tail biting are complicated by the use of different methodologies e.g. carcase examination at slaughter versus clinical herd examination, as well as by variations in the definition and assessment of tail damage between studies. There is little published information regarding on-farm assessment of tail biting prevalence, and that which does exist often relies on farmer reports, rather than specialist assessment. Based on a review of the available literature, the European Food Safety Authority (EFSA) estimated the on-farm prevalence of tail-biting behaviour to be between 30–70%, whereas the prevalence of tail-lesioned pigs on farms ranged from 1–5% (EFSA 2007).

Most estimates of tail biting prevalence in the literature are based on routine monitoring of tail damage at the abattoir, which is commonly performed in many European countries. Meat inspection records from Denmark, Sweden, The Netherlands and the UK indicate tail-lesion prevalence ranges from 0.22 to 4% (EFSA 2007).

A survey of 6 slaughterhouses in the United Kingdom revealed damage to 3% of docked and 9% of undocked tails (Hunter *et al.* 1999). Valros *et al.* (2004) reported the prevalence of tail damage (anything from scratches through to severe wounds or tail amputation) at a single Finnish slaughterhouse as 34.6%. In contrast, in their study of 37,000 carcases at 6 abattoirs in Northern Ireland and the Republic of Ireland, Harley *et al.* (2012) reported detectable tail lesions in 58.1% of all carcases, with 1.03% having severe lesions. Further, they reported that 99% of processed carcasses were tail docked.

However, such studies likely underestimate true tail biting prevalence, due to a combination of severely bitten or infected pigs dying or being euthanased on-farm, and healed mild lesions not being detectable at slaughter (EFSA 2007). Herd prevalence estimates obtained via on-farm clinical examination have been reported as two-to-three times higher than those obtained through carcase inspections at the abattoir (Busch *et al.* 2004; Keeling and Larsen 2004).

Gender also appears to influence tail biting prevalence, with several studies reporting a higher incidence of tail biting among male than female pigs, irrespective of whether males were castrated (e.g. Penny and Hill 1974; Hunter *et al.* 1999; Kritas and Morrison 2007; Harley *et al.* 2012; Teixeira *et al.* 2016).

1.3.2 Economic implications

Attempts have been made to gauge the economic impact of tail biting, due to losses incurred through carcase condemnation, carcase trimming and reduced carcase weight associated with tail lesions. In a study involving 37,000 pigs at six abattoirs, losses due to carcase condemnation were estimated to equate to an average of €0.37 per pig (Harley *et al.* 2012). However, when losses due to carcase trimming and reductions in carcase weight attributable to tail lesions were also considered, it was estimated that tail biting accounted for a 43% loss of profit margin per pig (Harley *et al.* 2014), highlighting the magnitude of the economic impact of this undesirable behaviour.

1.3.3 Welfare implications

Tail biting is considered a welfare issue, both for the bitten pig and for the biter. Welfare may be compromised in the bitten pig due to pain and distress caused by the biting itself, as well as subsequent inflammation and infection. As it is not considered a natural behaviour, the performance of tail biting is thought to reflect frustration and is thus considered indicative of poor welfare in the instigator (EFSA 2007).

Tail biting results in direct physical damage to the tail of the bitten pig, the severity of which may range from barely detectable puncture wounds through to partial or complete tail amputation. In severe cases the rump of the bitten pig may also be affected. Direct trauma can result in pain, inflammation and severe blood loss. Further, both the initial biter as well as other pigs in the pen may be attracted to the blood, leading to an increase in the frequency and severity of tail biting (Fraser 1987). Open wounds on the tail or rump predispose affected pigs to infection in bitten and adjacent tissues and to osteomyelitis in the caudal vertebrae (Schrøder-Petersen and Simonsen 2001). Infection can subsequently spread via venous or lymph drainage, leading to secondary infection in the lungs or, less commonly, the kidneys (Schrøder-Petersen and Simonsen 2001).

Several abattoir-based studies have identified significant associations between tail lesions and carcase damage. For example, 78% of carcases in Norway identified as having bitten tails also showed signs of lung infection (Hagen and Skulberg 1960). Teixeira *et al.* (2016) reported pleurisy in 13.7% of Irish pigs with tail lesions and pneumonia in 10.4% of pigs with tail lesions, and a significant overall association between tail lesions and carcase condemnations for pleurisy, pneumonia and pleuropneumonia. In a study of almost 75,000 baconer carcases at a Northern Ireland abattoir, Huey and co-workers (1996) found tail biting to be the cause of abscesses in 20% of carcases with a single abscess and in 62% of carcases with multiple abscesses. More recently, significant associations between tail biting and pleuritis and tail biting and abscess were reported in a study of approximately 14,000 carcases (Kritas and Morrison 2007). In addition, this study reported that severely tail-bitten carcases were 3.12 times more likely to require trimming than those without tail lesions.

In addition to infection-related sequelae, severely bitten pigs are also reported to exhibit reduced weight gain, relative to unbitten pigs, further impacting both pig welfare and economic returns (Schrøder-Petersen and Simonsen 2001; Harley *et al.* 2014).

1.3.4 Risk factors and alternative preventive strategies

The aetiology of tail biting has been extensively studied, with researchers agreeing that the behaviour is of multifactorial origin (Hunter *et al.* 2001; Moinard *et al.* 2003; EFSA 2007; Sonoda *et al.* 2012; Taylor *et al.* 2012). This is further supported by the unpredictable nature of tail-biting outbreaks, along with failures to experimentally induce the behaviour (Van Putten 1969; Ewbank 1973).

At the request of the European Commission, the EFSA Panel on Animal Health and Welfare produced a Scientific Opinion on the risks associated with tail biting in pigs and possible means to reduce the need for tail docking as a control measure (EFSA 2007). The panel, through an extensive review of the scientific literature up to and including 2007, identified the hazards associated with tail biting among pigs, then, through a process of expert elicitation, conducted a semi-quantitative risk assessment. The major risk factors for tail biting among docked and undocked pigs, as identified through this process, are shown in Table 1.1.

Overall, the absence of straw or a compatible particulate substrate, the amount and form of straw (where provided), the presence of slatted floors and a barren environment were the greatest risk factors. Competition for feed and/or inadequate feed intake, inadequate dietary sodium, deficiency in essential dietary amino acids, sudden change in diet composition, heat stress, cold stress, high air speed (artificial ventilation), and poor health status were also identified as hazards for tail biting (EFSA 2007). It was also noted that, although not empirically supported, anecdotal evidence and industry opinion suggests that mixing of pigs may trigger tail biting behaviour.

Table 1.1 *Major risk factors for tail biting among docked and undocked pigs, as identified by the European Food Safety Authority's Panel on Animal Health and Welfare (EFSA 2007).*

Docked pigs	**Undocked pigs**
Lack of straw and absence of adequate enrichment; No particulate rooting substrate, no destructible toy	Lack of long straw
Lack of long straw	Castration in males
Lack of straws combined with 100% slatted floor	Absence of bedding after previously having had bedding since weaning
	Genotype with high lean tissue growth rate (low fatness)
	Presence of tail bitten and tail biting animals (i.e. biters or bitten pigs not removed)

Using a Husbandry Advisory Tool developed to assess risk factors for tail biting in finisher pigs, Taylor *et al.* (2012) identified atmosphere/environment (e.g. thermal comfort, air quality, presence of wet or fouled bedding), lack of provision of enrichment, inadequate provision of food and drink, and health factors as being the key risk factors for tail biting. Further, they discriminated risks between different housing systems, noting that atmosphere and environmental factors contributed the most risk in straw units, whereas enrichment limitations were the key risk in no-straw units (Taylor *et al.* 2012). The authors also found that offering a financial incentive to farmers to adopt management practices to reduce tail biting risk resulted in a significant reduction in on-farm risk scores over the period of the study. Thus, financial incentives may provide a possible route to reducing the need for tail docking in commercially farmed pigs.

Data from two major abattoirs in Finland, where tail docking is banned, indicated a tail biting prevalence of 2.3% among some 1.6 million pigs at slaughter (Valros and Heinonen 2015). Even though abattoir-based data may underestimate tail biting prevalence, these data suggest that management strategies adopted by Finnish farmers, including reduced stocking density, daily provision of manipulable materials and provision of adequate feeding space, can successfully reduce tail biting incidence among commercially farmed pigs (Valros and Heinonen 2015).

Of note, a phone survey of 520 Dutch pig farms (487 conventional, 33 organic) found that farmers considered climate factors to be the main risk factor for tail biting, with conventional farmers rating enrichment as being less effective in the control of tail biting (Bracke *et al.* 2013). This highlights the need for the effective transfer of scientific knowledge to farm managers.

Despite clear evidence of associations between environmental factors such as flooring, provision of straw, or stocking density and tail biting occurrence, the behaviour has nonetheless been reported in improved environments i.e. those providing rooting or foraging material and increased space (Hunter *et al.* 2001; EFSA 2007). This serves to further emphasise the interactions between multiple variables in the development of this behaviour, and highlights the need for a case-by-case multifactorial approach to managing and eliminating tail biting among commercially farmed pigs.

1.3.5 The future of tail docking

In gauging the relative welfare impacts of tail biting versus tail docking, the repeated pain, distress and risk of infection associated with tail biting outweighs the acute pain associated with tail docking (D'Eath *et al.* 2016). However, the number of affected animals needs to be taken into account. Despite the potential severity of tail biting sequelae, the number of animals affected may be relatively few, whilst tail docking involves 100% of the herd. In addition, the performance of tail docking does not eliminate tail biting, despite reducing its incidence (Hunter *et al.* 1999; Sutherland *et al.* 2009). Therefore, moves toward alternatives to tail docking to control tail biting are being investigated. This, along with legislation restricting the performance of tail docking as a routine measure, will likely see the practice phased out in future years.

However, due to the lack of a single 'quick fix' measure, the need to determine the underlying motivation on a farm-by-farm basis, the lack of enforcement of current legislation regarding routine tail docking, and the costs associated with implementing changes to the rearing environment, tail docking remains the most widely practiced method for controlling tail biting on commercial pig farms (Nannoni *et al.* 2014). Without changes to current housing and management systems, a complete ban on tail docking would likely result in an increase in tail-biting behaviour (D'Eath *et al.* 2016). Therefore, in the short term at least, tail docking is likely to remain a common practice on commercial pig farms. With this in mind, it is imperative to ensure that the method used to dock tails causes the least possible pain and distress, thus minimising the welfare impact on pigs.

1.4 PAIN

1.4.1 Definition

Pain has been defined as "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (International Association for the Study of Pain 2015). Several different types of pain have been defined; those relevant to this body of work are described below.

1.4.2 Nociceptive pain

Nociceptive pain occurs through activation of high threshold peripheral sensory neurons, known as nociceptors, in response to internally or externally generated mechanical, chemical or thermal noxious stimuli (Kehlet *et al.* 2006). It functions as an early warning system, signalling the presence, location and duration of a noxious stimulus, stimulating protective behavioural responses. Nociceptive pain only continues for the duration of the nociceptive stimulus, fading once the peripheral driving force is removed (Kehlet *et al.* 2006; Costigan *et al.* 2009). In the context of tail docking, nociceptive pain may occur through activation of mechanosensitive nociceptors during tail tissue severance and, in the case of cautery, through activation of thermosensitive nociceptors during application of the heated blade. Thus, nociceptive pain during tail docking is of relatively short duration.

1.4.3 Inflammatory pain

In contrast, inflammatory pain may persist for a longer duration. Inflammatory pain, occurring in response to tissue damage and subsequent inflammatory responses, is directed toward addressing the consequences of tissue damage. The release of sensitising inflammatory mediators leads to a reduction in nociceptive thresholds in damaged and adjacent tissues through a process of peripheral sensitisation (Costigan *et al.* 2009). Central sensitisation may also occur, whereby an increase in excitability of neurons in the central nervous system (CNS) leads to magnification of responses to normal sensory inputs (Costigan *et al.* 2009). Normally innocuous stimuli may be perceived as painful, whilst responses to noxious stimuli are enhanced and prolonged. Such responses may outlast the primary tissue injury by hours or days, but usually cease after resolution of the tissue injury (Kehlet *et al.* 2006; Costigan *et al.* 2009).

1.4.4 Neuropathic pain

Peripheral neuropathic pain arises from lesions to the peripheral nervous system (PNS), leading to multiple pathophysiological changes in both the PNS and CNS (Costigan *et al.* 2009). The resultant sensory hypersensitivity typically persists for long periods, well beyond resolution of the initial etiological cause (Costigan *et al.* 2009). Whilst nerve injury provides the impetus for reactive changes that lead to abnormal neural function, such lesions alone are not sufficient to cause neuropathic pain; other factors such as age, gender, injury site, and genetic and psychosocial factors are thought to play a role in the development of chronic pain conditions in people (Kehlet *et al.* 2006; Costigan *et al.* 2009).

In their systematic review of epidemiological studies of neuropathic pain in the general population published between 1966 and 2012, van Hecke *et al.* (2014) reported incidence rates between 0.9 and 17.9%, depending on the methodology and population studied. They further estimated the population prevalence of chronic pain with neuropathic characteristics as being 6.9–10%. The prevalence of postoperative chronic pain, with or without neuropathic characteristics, has also been investigated, with reports ranging from 32–48% (Torrance *et al.* 2006; Bouhassira *et al.* 2008; de Moraes Vieira *et al.* 2012).

1.5 PAIN ASSESSMENT IN ANIMALS

The ability to quantify the degree of pain experienced by animals is integral to animal welfare assessment (Barnett 1997) and to the provision of effective pain management (Murrell and Johnson 2006). This is of particular importance to animal husbandry, where painful procedures such as tail docking and castration are routinely performed without the provision of anaesthesia or analgesia. In human medicine, pain assessment typically relies on self-report to determine the character, intensity and duration of perceived pain. Pain assessment in animals, however, is complicated by the lack of a common language, meaning we must rely upon subjective measures to infer pain perception.

Traditionally, pain assessment in animals has relied upon changes in physiological, endocrine or behavioural indices, each of which has limitations. Physiological indices used to assess pain typically relate to activation of the sympathetic-adreno-medullary axis, such as changes in heart rate or arterial blood pressure, or the hypothalamic-pituitary-adrenal axis, such as changes in plasma cortisol concentration (Mellor *et al.* 2000). Whilst these measures are useful in species that exhibit limited behavioural evidence of pain, or when behavioural expression of pain is limited due to restraint, they are however non-specific, occurring in response to non-painful stimuli or in response to the act of sampling itself (Weary *et al.* 2006).

Behavioural measures, such as the expression of pain-specific behaviours or vocalisations, or an increase or decline in the frequency of expression of other specific behaviours, have been widely used in pain assessment. Such measures are both species and individual-specific, requiring extensive knowledge of the species or individuals under investigation. In addition, some behaviours associated with pain, such as escape attempts or vocalisation, may also arise in response to non-painful stressors, confounding pain assessment (Barnett 1997). More recently, the EEG has been used as a pain assessment tool in a range of mammalian species.

1.5.1 The EEG as an indicator of cerebral cortical activity

It is nowadays widely accepted that the cerebral cortex and associated structures play an integral role in pain perception (Talbot *et al.* 1991; Jones *et al.* 1992; Treede *et al.* 1999; Schnitzler and Ploner 2000). The EEG is the summated electrical activity of populations of neurones in the cerebral cortex, recorded through electrodes placed at specific locations on the head (Murrell and Johnson 2006). The frequency of the EEG is proportional to the metabolic activity in the cerebral cortex, which can alter in response to changes in oxygen availability, blood flow, temperature and pharmacological agents including anaesthetics (Boveroux *et al.* 2008). Thus, changes in the frequency spectrum of the EEG mirror changes in the electrical activity of cortical neurons, providing a real-time reflection of cortical activity.

The EEG frequency spectrum is derived from the raw EEG following Fast Fourier Transformation (FFT), a complex mathematical process that transforms the raw EEG signal from the time domain to the frequency domain. A frequency spectrum, illustrating the contribution of each frequency to the power of the EEG waveform, is generated for each consecutive epoch of transformed data (Figure 1.1). The summary variables median frequency (F50), 95% spectral edge frequency (F95) and total power (P_{TOT}) are derived from the frequency spectrum. The F50 is the frequency (Hz) below which 50% of the total EEG power is located; the F95 is the frequency below which 95% of the total EEG power is located; the total power is the total area under the frequency spectrum curve (Figure 1.1).

During states of sleep or anaesthesia, the mammalian EEG typically exhibits a pattern of low frequency, high amplitude activity (Simons *et al.* 1989; Otto 2008), referred to as synchronisation as it occurs when large groups of neurones depolarise at the same frequency. In contrast, during states of arousal or consciousness EEG activity is characterised by high frequency, low amplitude waveforms, referred to as

desynchronisation (Simons *et al.* 1989; Murrell and Johnson 2006; Otto 2008), where neuronal activity is less synchronous.

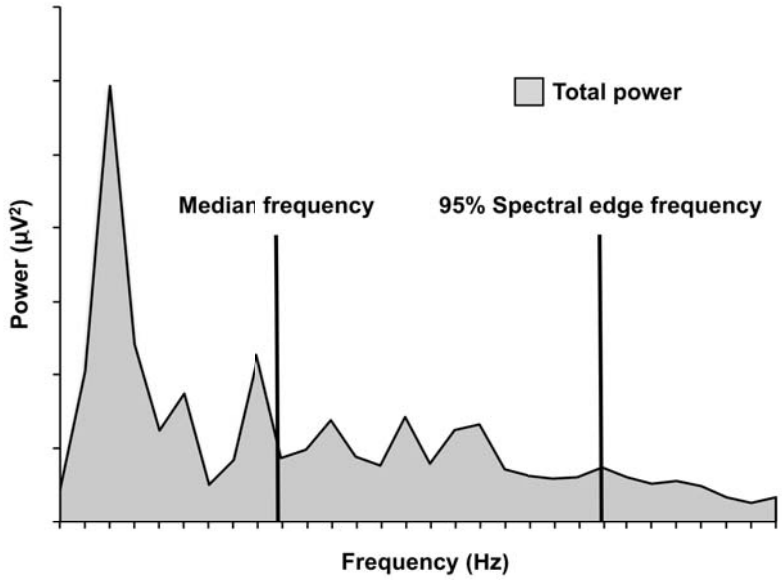

Figure 1.1 *Example of a frequency spectrum from a 1-second EEG epoch recorded from a 20-day-old pig, illustrating how the median frequency (frequency below which 50% of the total EEG power is located), 95% spectral edge frequency (frequency below which 95% of the total EEG power is located) and total power (area under the curve) are derived.*

1.5.2 Minimal anaesthesia model

The minimal anaesthesia model (MAM), developed by Murrell and Johnson (Murrell *et al.* 2003; Murrell *et al.* 2005; Murrell and Johnson 2006) has been used to assess acute nociceptive responses and/or analgesic efficacy in a range of mammals including horses (Murrell *et al.* 2003; Murrell *et al.* 2005), sheep (Johnson *et al.* 2005b; Johnson *et al.* 2009), deer (Johnson *et al.* 2005a), cattle (Gibson *et al.* 2007; Gibson *et al.* 2009a; Gibson *et al.* 2009c; Gibson *et al.* 2009b), dogs (Kongara *et al.* 2010), cats (Kongara *et al.* 2014b), rats (Murrell *et al.* 2007; Diesch *et al.* 2009; Kongara *et al.* 2014a), tammar wallabies (Diesch *et al.* 2010) and pigs (Haga and Ranheim 2005).

Briefly, the procedure involves anaesthetising the animal using halothane delivered in oxygen and, following induction and instrumentation, maintaining the animal at a minimal plane of anaesthesia, normally at or near the halothane minimal alveolar concentration (MAC) for the species in question. The MAC of an inhalant anaesthetic is defined as the concentration that prevents gross purposeful movement in 50% of subjects when a supramaximal noxious stimulus is applied (Eger *et al.* 1965). Using the MAM, once a stable plane of anaesthesia is obtained, baseline EEG data are recorded, after which the noxious stimulus is applied. At the conclusion of data recording, EEG data collected during and immediately after noxious stimulation can then be compared with baseline (pre-stimulus) EEG.

Halothane is the anaesthetic of choice in this model as it causes less cortical depression at concentrations sufficient to provide a surgical plane of anaesthesia than other inhalant agents (Johnson and Taylor 1998; Murrell *et al.* 2008) and is not believed to possess antinociceptive properties (England and Jones 1992). Other more modern inhalant anaesthetic agents, such as isoflurane, desflurane, sevoflurane and methoxyflurane, may cause considerable depression of cortical function, even at MAC

(Murrell *et al.* 2008). As well as reducing the amplitude of the raw EEG signal, burst suppression, involving periods of isoelectric activity interspersed with active EEG, or complete EEG suppression leading to an isoelectric trace, may occur, rendering the resultant EEG data unsuitable for spectral analyses.

Using the MAM, the application of a noxious stimulus typically elicits EEG desynchronisation, as a result of activation of cortical neurones involved in pain processing. This is characterised by an increase in F50 and decrease in P_{TOT} of the EEG (Murrell and Johnson 2006). Desynchronisation in response to noxious stimulation has been observed in horses (Murrell *et al.* 2003), sheep (Johnson *et al.* 2005b), cattle (Gibson *et al.* 2007), pigs (Haga and Ranheim 2005), cats (Kongara *et al.* 2014b), dogs (Kongara *et al.* 2010) and rats (Kongara *et al.* 2014a).

Although the cerebral cortex responds to noxious stimulation under light halothane anaesthesia, the animals are nonetheless unconscious and therefore unable to perceive any pain, making this an ethical pain assessment tool. In addition, the provision of general anaesthesia minimises the influence of extraneous variables such as handling and restraint that may confound responses in conscious animals, as well as largely immobilising the animal, thus preventing contamination of the EEG signal with artefacts arising from skeletal muscle activity.

Given that tail docking requires the animal to be handled and restrained, and is believed to be acutely painful, the MAM provides an ideal tool for assessing and comparing acute nociceptive responses of piglets to this procedure.

1.6 RESEARCH OBJECTIVES

The objectives of this research were:

- To use the MAM to evaluate the acute pain associated with two common methods for tail docking of pigs (cold clipping versus hot cautery iron).
- To use the MAM to evaluate the efficacy of selected analgesic strategies in reducing acute nociception during tail docking.
- To use the MAM to investigate the effects of piglet age at the time of docking on acute nociceptive responses.
- To investigate the potential for long-term pain as a consequence of tail docking using clippers or cautery, based on examination of tail histomorphology.

The results of this research, along with the existing body of literature, will assist in the development of best-practice guidelines for tail docking of commercially raised pigs.

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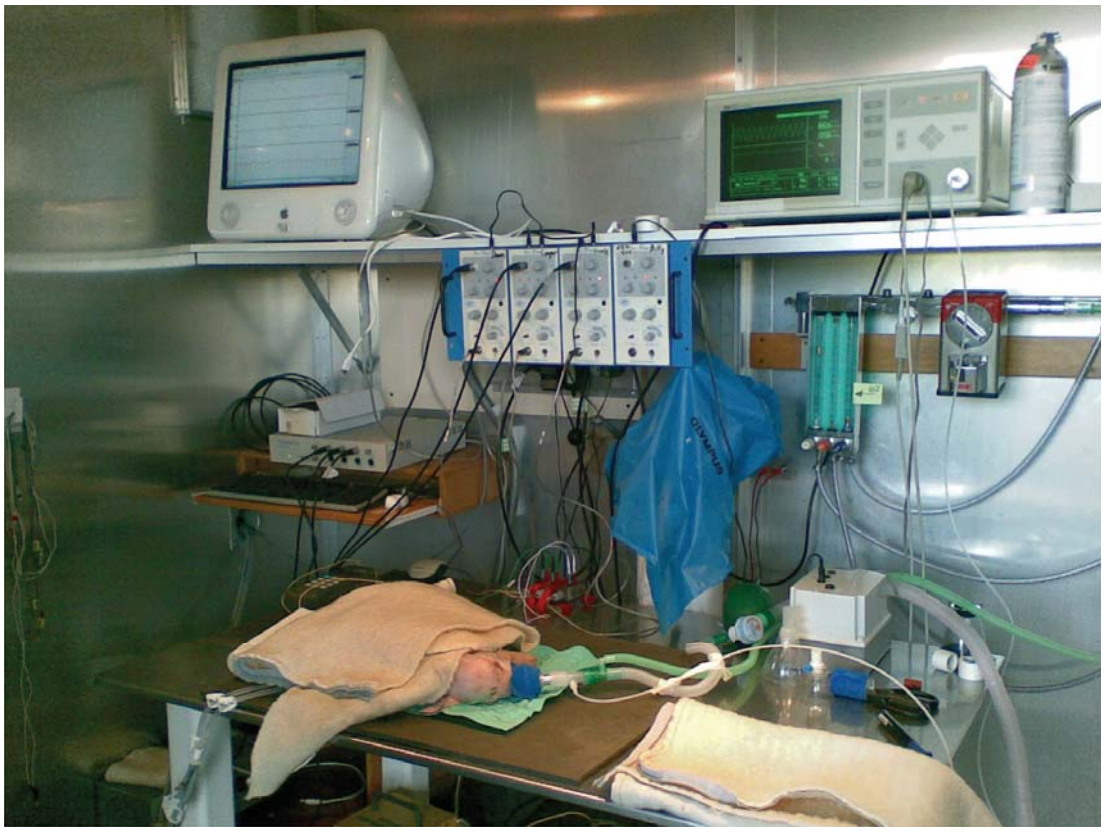
CHAPTER 2 Electroencephalographic responses of anaesthetised pigs (*Sus scrofa*) to tail docking using clippers or cautery iron, performed at two or twenty days of age



Kells NJ, Beausoleil NJ, Chambers JP, Sutherland MA, Morrison RB, Johnson CB. Electroencephalographic responses of anaesthetised pigs (*Sus scrofa*) to tail docking using clippers or cautery iron, performed at two or twenty days of age.

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2.1 ABSTRACT

Objective: To compare electroencephalographic (EEG) responses of pigs to tail docking using clippers or cautery iron, performed at 2 or 20 days-of-age.

Study design: Prospective, randomised controlled experimental study

Animals Forty Large white x Landrace entire male pigs aged either 2 (*n* = 20) or 20 (*n* = 20) days were randomly assigned to undergo tail docking using either clippers or cautery iron.

Methods: Anaesthesia was induced and maintained with halothane delivered in oxygen. Following instrumentation, end-tidal halothane was stabilised at 1.0 ± 0.5% and EEG recording commenced. After a 5-minute baseline period, tail docking was performed and recording continued for a further 10 minutes. Electroencephalographic data were subjected to Fast Fourier transformation, yielding the summary variables median frequency (F50), 95% spectral edge frequency (F95) and total power (P_{TOT}). Variables recoded during baseline were compared with those calculated at consecutive 15-second intervals following tail docking.

Results: Following tail docking, F50 decreased briefly but significantly in 2-day-olds, whilst 20-day-olds exhibited a sustained increase in F50. Immediately after tail docking, F50 was lower overall in 2- than 20-day-old pigs. Spectral edge frequency increased after docking in 20-day-olds docked using clippers, but did not change in 20-day-olds docked using cautery iron, or in 2-day-olds docked using either method. Overall, F95 was lower in 2- than 20-day-old pigs from 30–60 seconds after docking. Total power decreased significantly after docking in 20-day-old pigs, but did not change in 2-day-old pigs. Overall, P_{TOT} was lower in 2- than 20-day-old pigs during baseline and after tail docking.

Conclusions and clinical relevance: These data suggest that tail docking using clippers is more acutely painful than docking using cautery iron, and that docking within the first days of birth may be less acutely painful than docking at a later age.

2.2 INTRODUCTION

Tail biting among commercially farmed pigs can pose a serious welfare threat to affected animals, as well as having economic consequences for pork producers. Although management strategies have been advocated to reduce the incidence of tail biting, tail docking remains the most widely adopted on-farm preventive strategy (Schrøder-Petersen & Simonsen 2001; Moinard *et al.* 2003). Two tail docking methods are commonly employed: cutting using side clippers, or cutting and concurrent cautery using a heated cautery iron. Commercially, tail docking of pigs is performed without analgesia and is therefore thought to be acutely painful. From a welfare perspective, it would be valuable to determine whether acute pain responses differ according to docking method. Due to its subjective nature, pain in animals cannot be directly measured; instead it must be inferred. Traditionally, behavioural and physiological measures have been used to assess nociception in animals; however, these are subject to influence from non-painful stressors, including handling, restraint and the act of sampling itself.

Similarities between EEG responses to nociception in conscious and anaesthetised animals (Ong *et al.* 1997; Murrell *et al.* 2003) and those of conscious humans exposed to painful stimuli (Chen *et al.* 1989) led to the

development of the minimal anaesthesia model. Using this model, changes in the EEG spectra under light anaesthesia have been shown to reliably indicate nociception in horses (Murrell *et al.* 2003), sheep (Johnson *et al.* 2005a), cattle (Gibson *et al.* 2007), deer (Johnson *et al.* 2005b) and pigs (Haga & Ranheim 2005). In sheep, noxious stimuli of graded intensity were found to elicit graded responses in both EEG and behaviour (Ong *et al.* 1997), whilst in human patients the magnitude of EEG responses correlated well with reports of perceived pain intensity (Chen *et al.* 1989), indicating that EEG responses have both qualitative and quantitative application in pain assessment.

The objective of the current study was to compare EEG responses of anaesthetised pigs undergoing tail docking using either side-cutter clippers or heated cautery iron at 2 or 20 days-of-age, in order to assess their acute nociceptive responses. Whilst tail docking is normally carried out within 2 days of birth, the reported literature indicates that EEG has previously only been recorded from 20-day-old pigs. Additionally, prior research has shown that EEG may be absent, or reduced in power, in neonatal mammals relative to their adult counterparts. Therefore both 2- and 20-day-old pigs were included in the study. We hypothesised that any differences in acute nociceptive responses as a function of docking method would be indicated by differences in the magnitude and/or duration of EEG responses.

2.3 MATERIALS AND METHODS

The study was conducted with approval from the Massey University Animal Ethics Committee (MUAEC). All procedures were undertaken in accordance with the MUAEC code of ethical conduct for the use of live animals for research, testing and teaching. Forty healthy commercial white line (Large white x Landrace) male pigs were sourced from a commercial pig farm on the day of testing and housed in a temperature-controlled (30°C) indoor facility with *ad libitum* access to water. Pigs had not previously undergone any potentially painful husbandry procedures (e.g. castration, teeth clipping, tail docking). Pigs were randomly assigned to one of four groups (n = 10 per group): tail docking using clippers at 2 days old (2 CLIP), tail docking using clippers at 20 days old (20 CLIP), tail docking using cautery iron at 2 days old (2 CAUT), or tail docking using cautery iron at 20 days old (20 CAUT). The decision to include n = 10 per group was based upon previous studies using this methodology, whereby statistically significant results were obtained using group sizes of 10 (Murrell *et al.* 2005; Gibson *et al.* 2007; Gibson *et al.* 2009), nine (Johnson *et al.* 2005b), eight (Kongara *et al.* 2010; Kongara *et al.* 2014), or five (Johnson *et al.* 2009). Treatment order was randomly allocated prior to study commencement. As pigs were sourced directly from commercial premises, there was limited control over selection of individuals or litter distribution. As a result, two day-old pigs (n = 20) were sourced from 13 litters in total; nine of these had pigs allocated to CLIP only, seven to CAUT only, with three having pigs allocated to both CLIP and CAUT. Twenty day-old pigs (n = 20) were sourced from 11 litters; nine had pigs allocated to CLIP only, seven to CAUT only, and two had pigs allocated to both CLIP and CAUT. Experiments were conducted over a period of 16 test days, with 1–4 pigs tested per day.

Anaesthesia

An established minimal anaesthesia model (MAM) was followed. Anaesthesia was induced using 4% halothane (Halothane-Vet; Merial NZ Limited, Manukau City, NZ) vaporised in oxygen (4 L minute^{-1}). Once adequate depth of anaesthesia was achieved (absence of palpebral reflex and no withdrawal response to toe pinch), electrode placement was undertaken. Following instrumentation, halothane delivery was adjusted to maintain an end-tidal concentration of 0.95–1.05% for the remainder of anaesthesia. End tidal halothane and CO_2 concentrations and respiration rate were monitored throughout using an anaesthetic

agent monitor (Hewlett Packard M1025B, Hewlett Packard, Hamburg, Germany). Rectal temperature was monitored using a digital thermometer (Q 1437; Dick Smith Electronics, New Zealand) and body temperature maintained at approximately 39°C with the aid of a circulating warm-water heating pad (T pump; Gaymar Industries Inc., NY, USA).

EEG recording

Subcutaneous 27-gauge stainless steel needle electrodes (Viasys Healthcare, Surrey, England) were positioned to record EEG and electrocardiograph (ECG) activity. A five-electrode montage was used to record EEG from both the left and right cerebral hemispheres, with inverting electrodes placed parallel to the midline over the left and right frontal bone zygomatic processes, non-inverting electrodes over the left and right mastoid processes and a ground electrode placed caudal to the occipital process (see Murrell & Johnson 2006). ECG was recorded using a base-apex configuration.

Both EEG and ECG signals were fed via breakout boxes to separate amplifiers (Iso-Dam isolated biological amplifier, World Precision Instruments Inc.). The signals were amplified with a gain of 1000 and a band-pass of 1.0–500Hz and digitised at a rate of 1 kHz (Powerlab 4/20, ADInstruments Ltd, Colorado Springs, Co). The digitised signals were recorded on an Apple Macintosh personal computer for off-line analysis at the conclusion of the experiment.

Experimental procedure

Once end tidal halothane tension was stable at 0.95–1.05% 5 minutes of baseline EEG was recorded. Tail docking was then carried out according to group assignment and recording continued for a further 10 minutes. Pigs in the CLIP groups had their tails amputated using a pair of clean, disinfected side-clippers. Pigs in the CAUT groups had their tails amputated using a clean, disinfected gas operated cautery iron (Stericut Tail Docker, Cotran Corp., Portsmouth RI, USA). In all cases the tail was docked approximately 2 cm from the base, with care taken to sever between adjacent vertebrae. A disinfectant was applied to the tail wound at the completion of the recording period. Following recovery from anaesthesia all pigs were housed in an indoor temperature-controlled recovery pen before relocation to a private farm the same day.

Data analysis

Raw EEG recordings were inspected manually and any artefacts such over-scale, under-scale, nystagmus or other muscular activity was excluded from subsequent analysis. The total power (P_{TOT}), median frequency (F50) and spectral edge frequency (F95) were calculated for consecutive 1-second epochs, using purpose-written software (Spectral Analyser, CB Johnson, Massey University, Palmerston North, NZ, 2002).

For the purposes of statistical analysis, EEG data from the last 60 seconds of baseline and for consecutive 15-second blocks (up to 180 seconds) after tail docking were compared. A single mean value for each EEG variable was calculated for each time-period in each individual, generating a total of 13 data points per pig per variable (1 before and 12 after tail docking).

Statistical analyses

Electroencephalographic data from each group of pigs were subjected to factorial analysis of variance using a generalised linear model in SAS version 9.3.1 (SAS Institute Inc., Cary NC, USA, 2012). The model incorporated age, method and time as fixed effects, pig as a random effect and time as a repeated measure. Where significant effects or interactions were identified (p <0.05), Bonferroni post-hoc tests were performed to identify differences.

2.4 RESULTS

EEG data were successfully collected from all 40 pigs. Movement responses to tail docking occurred in 19 (48%) pigs, with resultant EMG artefacts excluded from subsequent data analyses. Movement responses to docking were observed in both the CLIP (*n* =9) and CAUT (*n* =10) treatments, and in both 2 (*n* =7) and 20 (*n* =12) day-old pigs. $PE'CO_2$ did not exceed 57 mmHg in any animal at any time during anaesthesia. Body temperatures recorded during anaesthesia ranged between 37.7 and 39.9°C.

Statistical analyses revealed no overall effect of treatment (docking method), and no age by treatment interaction on any EEG variable (Table 2.1). There was, however, a significant treatment by time effect on F50 and a significant age by treatment by time effect on F95 (Table 2.1). For F50, pigs docked using CLIP demonstrated an increase, relative to baseline, over the period 60–90 seconds after docking, whereas those docked using CAUT showed no change (Figure 2.1). Mean F50 did not differ at any individual time point between the CLIP and CAUT treatments.

Whilst P_{TOT} of the pig EEG was significantly lower than baseline 15 (p <0.001), 30 (p <0.001), and 45 (p =0.0372) seconds after docking (ages and docking methods combined), P_{TOT} did not differ over time between docking methods (Table 2.1).

Table 2.1 *The effects of age, time, treatment and their interactions on the median frequency (F50), spectral edge frequency (F95) and total power (P_{TOT}) of the pig electroencephalogram following tail docking using side clippers (n =20) or cautery iron (n =20), performed at 2 or 20 days-of-age.*

	F50, Hz		**F95, Hz**		**P_{TOT}, μV^2**	
Variable	***F***	**Pr > *F***	***F***	**Pr > *F***	***F***	**Pr > *F***
Age	6.50	0.0158	6.35	0.0169	26.91	<0.0001
Treatment	1.50	0.2293	2.14	0.1534	1.69	0.2024
Age x Treatment	2.37	0.1338	0.46	0.5020	0.59	0.4467
Time	7.06	<0.0001	5.51	<0.0001	9.29	<0.0001
Age x Time	3.23	0.0002	4.08	<0.0001	5.18	<0.0001
Treatment x Time	1.73	0.0578	2.61	0.0023	0.67	0.7793
Age x Treatment x Time	1.25	0.2458	1.97	0.0257	1.00	0.4496

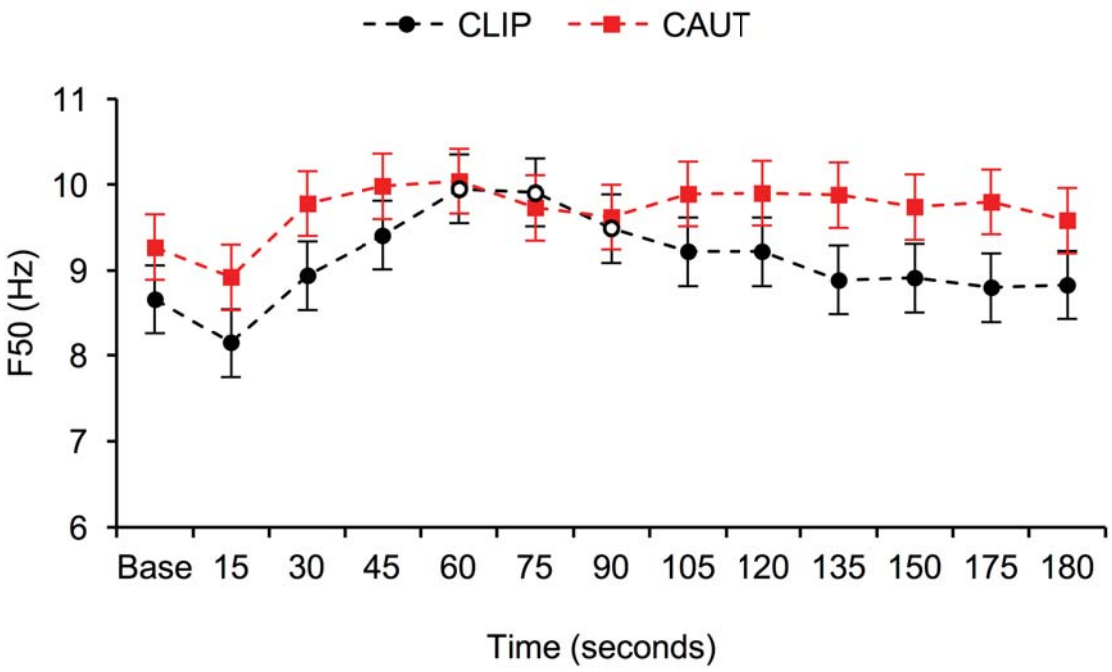

Figure 2.1 *Comparison of the mean (±SEM) median frequency (F50) of the pig electroencephalogram at consecutive 15-second intervals following tail docking with either clippers (CLIP, black circle; n =20) or cautery iron (CAUT, red square; n =20). Baseline (Base) represents the mean of the 60 seconds immediately preceding tail docking. Open symbols indicate post-docking time points at which the mean differed from baseline within each treatment (adjusted p <0.05)*

There were significant age x time interaction effects on F50 and P_{TOT} (Table 2.1). In 2-day-old pigs, F50 showed a transient decrease from baseline immediately after docking, whereas in 20-day-old pigs there was a delayed and more sustained increase in F50 after docking (Table 2.2). F50 was lower in 2- than 20-day-old pigs from 15–45 and at 120 seconds after docking (Table 2.2).

P_{TOT} did not differ from baseline in pigs docked at 2 days-of-age, whereas 20-day-old pigs demonstrated a decrease in P_{TOT} from 15–60 seconds after docking (Table 2.2). Baseline P_{TOT} was lower in 2- than 20-day-old pigs, and remained lower between 15–30 and 75–180 seconds after docking (Table 2.2).

Mean F95 differed between ages within docking treatment. Pigs docked at 2 days-of-age showed no change in F95, whether docked by CLIP or CAUT (Figure 2.2). In contrast, pigs docked at 20 days-of-age demonstrated an increase in F95 in response to docking by CLIP, but not by CAUT (Figure 2.2). In addition, F95 was higher in 20-day-old pigs than 2-day-old pigs docked using clippers from 30–60 seconds after docking. Mean F95 did not differ between docking methods within age groups (adjusted p >0.05).

Table 2.2 *Comparison of the mean (SEM) median frequency (F50), spectral edge frequency (F95) and total power (P_{TOT}) of the pig electroencephalogram at consecutive 15-second intervals in 2-day-old and 20-day-old pigs after tail docking using side clippers (n =10 per age) or cautery iron (n =10 per age). Data are shown as mean values over consecutive 15-second intervals after docking, with baseline representing the mean of the 60 seconds immediately preceding tail docking.*

	F50, Hz		F95, Hz		P_{TOT}, μV^2	
Time	**2-day-old**	**20-day-old**	**2-day-old**	**20-day-old**	**2-day-old**	**20-day-old**
Baseline	8.60 (0.39)	9.33 (0.38)	25.22 (0.23)	25.73 (0.22)	23.15 (1.72)^{x}	36.13 (1.78)^{y}
15	*7.57 (0.39)^{x}	9.50 (0.39)^{y}	25.51 (0.23)	*26.35 (0.23)	20.69 (1.72)^{x}	*30.27 (1.80)^{y}
30	8.18 (0.39)^{x}	*10.53 (0.38)^{y}	25.17 (0.23)^{x}	*26.53 (0.22)^{y}	22.45 (1.72)^{x}	*30.60 (1.78)^{y}
45	8.78 (0.39)^{x}	*10.61 (0.38)^{y}	25.24 (0.23)^{x}	*26.49 (0.22)^{y}	23.54 (1.72)	*31.41 (1.78)
60	9.21 (0.39)	*10.77 (0.38)	25.21 (0.23)^{x}	*26.36 (0.22)^{y}	24.21 (1.72)	*32.01 (1.78)
75	9.02 (0.39)	*10.61 (0.38)	25.21 (0.23)^{x}	*26.21 (0.22)^{y}	24.08 (1.72)^{x}	33.52 (1.78)^{y}
90	9.06 (0.39)	10.06 (0.38)	25.21 (0.23)	25.97 (0.22)	24.21 (1.72)^{x}	35.33 (1.78)^{y}
105	8.83 (0.39)	*10.28 (0.38)	25.26 (0.23)	26.05 (0.22)	23.76 (1.72)^{x}	33.78 (1.78)^{y}
120	8.67 (0.39)^{x}	*10.45 (0.38)^{y}	25.25 (0.23)	26.08 (0.22)	23.83 (1.72)^{x}	34.68 (1.78)^{y}
135	8.62 (0.39)	10.14 (0.38)	25.25 (0.23)	26.02 (0.22)	23.53 (1.72)^{x}	35.76 (1.78)^{y}
150	8.84 (0.39)	9.80 (0.38)	25.19 (0.23)	25.98 (0.22)	23.39 (1.72)^{x}	36.42 (1.78)^{y}
165	8.86 (0.39)	9.74 (0.38)	25.19 (0.23)	25.86 (0.22)	23.40 (1.72)^{x}	37.24 (1.78)^{y}
180	8.78 (0.39)	9.64 (0.38)	25.17 (0.23)	25.85 (0.22)	23.50 (1.72)^{x}	36.95 (1.78)^{y}

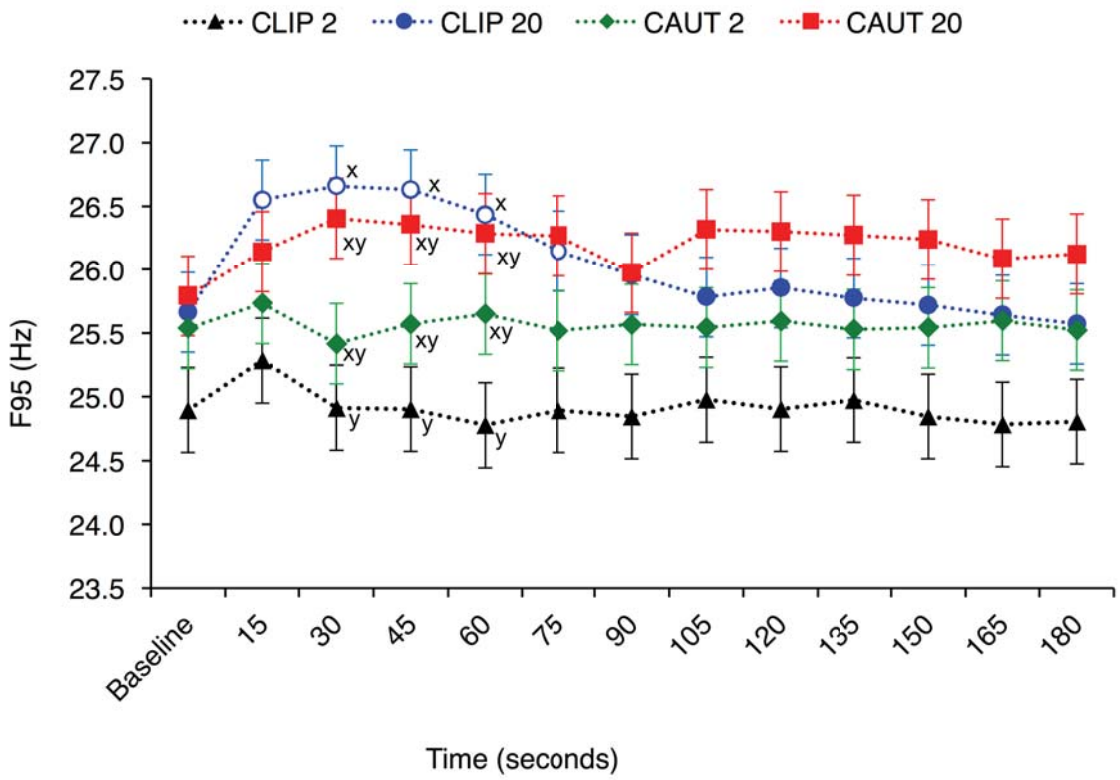

Figure 2.2 *Comparison of the changes in mean (SEM) 95% spectral edge frequency (F95) of the pig electroencephalogram following tail docking by side clippers (CLIP) at 2 (black triangle; n =10) and 20 (blue circle; n =10) days-of-age, and cautery iron (CAUT), in pigs aged 2 (green diamond; n =10) and 20 (red square; n =10) days-of-age. Data are shown as mean (SEM) values for consecutive 15-second intervals after docking, with Baseline representing the mean of the 60 seconds immediately preceding tail docking. Open symbols indicate post-docking time points at which the mean differed from baseline within treatment (adjusted p <0.05). Superscript characters indicate time points at which means differed between treatments (adjusted p <0.05).*

2.5 DISCUSSION

The objective of the current study was to compare acute EEG responses of anaesthetised pigs to tail docking using either side cutter clippers or heated cautery iron, performed at 2 and 20 day-of-age.

The usual mammalian EEG response to noxious stimulation is desynchronisation, characterised by a shift toward lower amplitude, higher frequency activity (Otto 2008), resulting in increases in F50 and F95, and a decrease in P_{TOT} (Johnson *et al.* 2012). However, paradoxical arousal, or synchronisation, characterised by a shift toward higher amplitude, lower frequency activity and corresponding decreases in F50 and F95, has also been reported following noxious stimulation. In a study of isoflurane-anaesthetised sheep undergoing orthopaedic surgery, both synchronisation and desynchronisation of the EEG were observed, with responses differing according to depth of anaesthesia and stimulation intensity (Otto & Mally 2003). In a study of EEG responses to skin incision in anaesthetised people, adult patients demonstrated desynchronisation following skin incision, whereas EEG synchronisation predominated in infants and children <8 years, suggesting an age-dependent effect (Oshima *et al.* 1981).

The lack of controlled ventilation in the present study resulted in individual differences in $PaCO_2$ during EEG recording. Increases in $PaCO_2$ have been associated with EEG suppression (Pollock *et al.* 1949; Stein & Pollock 1949; Caspers *et al.* 1979); therefore, variations in $PaCO_2$ may have influenced baseline EEG measures between pigs. However, as each pig acted as its own control, with post-docking EEG measures being compared with pre-docking baseline values, any individual differences in baseline EEG as a function of $PaCO_2$ will have been accounted for.

In the present study, the pattern of EEG responses of pigs subjected to tail docking differed according to both age and docking method.

Overall, pigs docked at 2 days-of-age demonstrated very little EEG response to tail docking. Whilst there was a transient reduction in F50, there was no significant change in F95 or P_{TOT}. In contrast, pigs docked at 20 days-of-age demonstrated a significant and sustained increase in F50 and decrease in P_{TOT} after docking, consistent with the expected response to a nociceptive stimulus. In addition, F50 and P_{TOT} were significantly lower in 2 than 20-day-old pigs after tail docking, and F95 was lower in the younger animals docked using side cutter clippers.

The less marked responses observed in 2-day-old pigs suggest that tail docking at this age is less acutely painful to pigs than docking at 20 days-of-age. These results provide some support for the current New Zealand requirement that tail docking of pigs without analgesia be performed within the first week of life and the current recommended best practice to dock within 72 hours of birth (NAWAC, 2010). However, whilst these data provide some support for tail docking within days of birth, they do not provide information on nociceptive responses of pigs aged between 2 and 20 days. It would be valuable to examine the ontogeny of EEG responses to tail docking across this age range, to determine whether currently recommended age ranges for performance of tail docking without analgesia are justified. For example, further information is required to evaluate the validity of the current U.S. requirement to perform docking without analgesia within the first 14 days of life (California Veterinary Medical Association 2011).

The observed age differences in pigs are consistent with previously reported differences in EEG responses to noxious stimuli as a function of postnatal age in mammals. In related studies involving rats and wallabies, both of which are neurologically immature at birth, EEG responses to noxious stimuli were absent in the early postnatal period, with responses developing and subsequently increasing in magnitude as postnatal

age increased (Diesch *et al.* 2009a, b). In both studies, the increase in responsiveness to stimulation with age was accompanied by increased EEG power in all frequencies. The authors postulated this was due to anatomical and functional maturation of the cerebral cortex and/or to differences in the maturation of peripheral and central nociceptive pathways as a function of age (Diesch *et al.* 2009a,b). A recent study investigating the development of EEG responses to thermal noxious stimulation in the rat demonstrated significant increases in theta power activity after stimulation in adults that were absent in juveniles (Devonshire *et al.* 2015), further supporting the theory of post-natal neural maturation.

In the present study, younger pigs had lower baseline total power than older pigs and exhibited no change in P_{TOT} after tail docking. Although pigs are neurologically mature at birth compared to rats and wallabies, it is nevertheless plausible that further maturation of the cerebral cortex and/or nociceptive pathways occurs in the early postnatal period. In support of this idea, cerebrocortical responses to castration in lambs, which are extremely neurologically mature at birth, differed between 12 and 29 days of age (Johnson *et al.* 2005a), suggesting maturational alterations in nociceptive mechanisms may continue beyond the very early post-natal period in this species. Such postnatal maturation could account for both the smaller EEG responses to tail docking and the lower EEG power evident in 2-day-old pigs in the present study.

Aside from maturational effects, it is possible that EEG responses to tail docking in 2-day-old pigs were modulated through the lingering presence of in-utero neuro-suppressive agents. In mammals, a number of circulating peptide and endocrine factors act to maintain the fetus in a permanent sleep-like state, and are thought to be responsible for the observed absence of fetal responses to noxious and nociceptive stimuli (Mellor *et al.* 2005). In mammals born neurologically mature, the withdrawal of neuro-suppression, along with concurrent neuro-activation, leads to the onset of consciousness shortly after birth (Mellor *et al.* 2005; Mellor & Diesch 2006). However, in sheep plasma concentrations of the neuro-suppressive agents pregnanolone and allopregnanolone were found to be significant up to 3 days after birth (Nguyen *et al.* 2003), leading to the suggestion that these chemicals may continue to exert some cerebral effects after birth, albeit insufficient to suppress consciousness and concurrent EEG activation (Mellor & Diesch 2006). Whether significant concentrations of these agents are present in the plasma of pigs at birth has not been investigated.

Additionally, differences in EEG responses to a nociceptive stimulus can arise through differences in the magnitude of the noxious stimulus itself. In the present study, although the stimulus of tail docking by clippers or cautery iron was identical between pigs, differences in the magnitude of responses between ages could arise through variation in the size and type of tissue being transected during tail removal (Johnson *et al.* 2005a; Mellor & Diesch 2006), although such differences are likely to be relatively small across the age range examined.

In addition to the effects of pig age, EEG responses to tail docking were also influenced by the method employed. Overall, docking using clippers tended to elicit an increase in F50, and elicited a sustained increase in F95 in 20-day-old pigs. In contrast, use of a cautery iron elicited no such changes, suggesting that docking using cautery is less acutely painful to pigs than docking using clippers. That the increase in F95 observed after tail docking using clippers was specific to 20 day-old pigs provides further support for age-related differences in nociceptive processing among young pigs.

The greater EEG response observed to docking with clippers is consistent with two previous studies comparing acute physiological stress responses of pigs to tail docking, which demonstrated higher plasma cortisol concentrations in pigs docked using clippers than those docked using cautery 30 minutes after docking in day-old pigs (Morrison *et al.* 2013) or 60 minutes after docking in 6-day-old pigs (Sutherland *et al.* 2008) A similar study involving 2-, 3- and 8-day-old pigs failed to find any difference in plasma cortisol levels

between pigs docked using clippers or cautery in samples taken 45 minutes after docking (Marchant-Forde *et al.* 2009). In the latter study, it was speculated that cortisol concentrations might have peaked prior to the initial 45-minute sampling point, thus explaining the lack of any treatment-related differences.

Both methods of tail docking involve using a blade to sever the tail between adjacent vertebrae. However, when the blade is heated it simultaneously cauterises the tail tissues, and the heat is believed to cause the destruction of local nociceptors, reducing the animal's ability to perceive noxious stimulation (Lester *et al.* 1991; Sutherland *et al.* 2009). The smaller EEG responses recorded following cautery docking in the present study are likely to have resulted from nociceptor destruction during tissue cauterisation.

Given that only male pigs were tested in the current model, caution should be applied to the extrapolation of results to female pigs. However, a systematic review of 10 years of clinical pain research revealed no consistent pattern of sex differences in human pain sensitivity (Racine *et al.* 20012), suggesting there are similarities in pain processing between the sexes in mammals.

In considering the welfare consequences of different docking methods it must be borne in mind that EEG measures provide an indication of acute nociception only. Before recommendations can be made about the appropriate age at which to carry out tail docking, or the most appropriate method to use, long-term welfare outcomes must also be investigated. Eicher and co-workers (2006) reported increases in thermal sensitivity of the ventral tail in dairy heifers that had been tail-docked, relative to undocked controls. In contrast, Sandercock and co-workers (2011) reported no differences in thresholds to mechanical or noxious cold stimulation of the tail root between docked and undocked pigs. The authors did not test nociceptive thresholds at the tail tip or at the site of docking. The formation of neuromas secondary to tail docking has previously been observed in pigs docked by emasculator (Simonsen *et al.* 1991) and, more recently, in pigs docked using cautery iron (Herskin *et al.* 2014). Neuromas are swellings or thickenings caused by abnormal regeneration of nerve fibres secondary to nerve transection (Lewin-Kowalik *et al.* 2006). Neuroma development has been linked with neuropathic pain conditions such as reduced pain threshold, spontaneous pain, and allodynia (pain in response to benign stimuli) (Breward & Gentle 1985; Bennett & Xie 1988; Gentle *et al.* 1990; Jensen & Nikolajsen 1999). Whilst not all neuromas induce neuropathic pain (Costigan *et al.* 2009), the potential for heightened pain perception or spontaneous pain because of tail docking is nonetheless a welfare concern. To date, there is no data comparing the incidence of neuroma formation as a function of docking method or age.

2.6 CONCLUSIONS

Pigs docked at 2 days-of-age demonstrated little evidence of nociception compared with pigs docked at 20 days-of-age and exhibited lower EEG power overall, suggesting differences in neural maturity and/or processing of nociceptive signals between ages. Whether or how this relates to pain perception in conscious pigs requires further investigation. Tail docking using cautery iron appears to be less acutely painful to pigs than tail docking using clippers; however further study is required to determine whether there are differences in long-term welfare outcomes between the two methods.

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MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Nicola Jean Kells

Name/Title of Principal Supervisor: Dr Ngaio Beausoleil

Name of Published Research Output and full reference:

Kells NJ, Beausoleil NJ, Chambers JP, Sutherland MA, Morrison RB, Johnson CB.
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In which Chapter is the Published Work: Chapter 2

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Nikki had a primary role in study design, data collection, statistical analysis, interpretation, writing of the paper and responding to reviewers' comments, with guidance from supervisors.

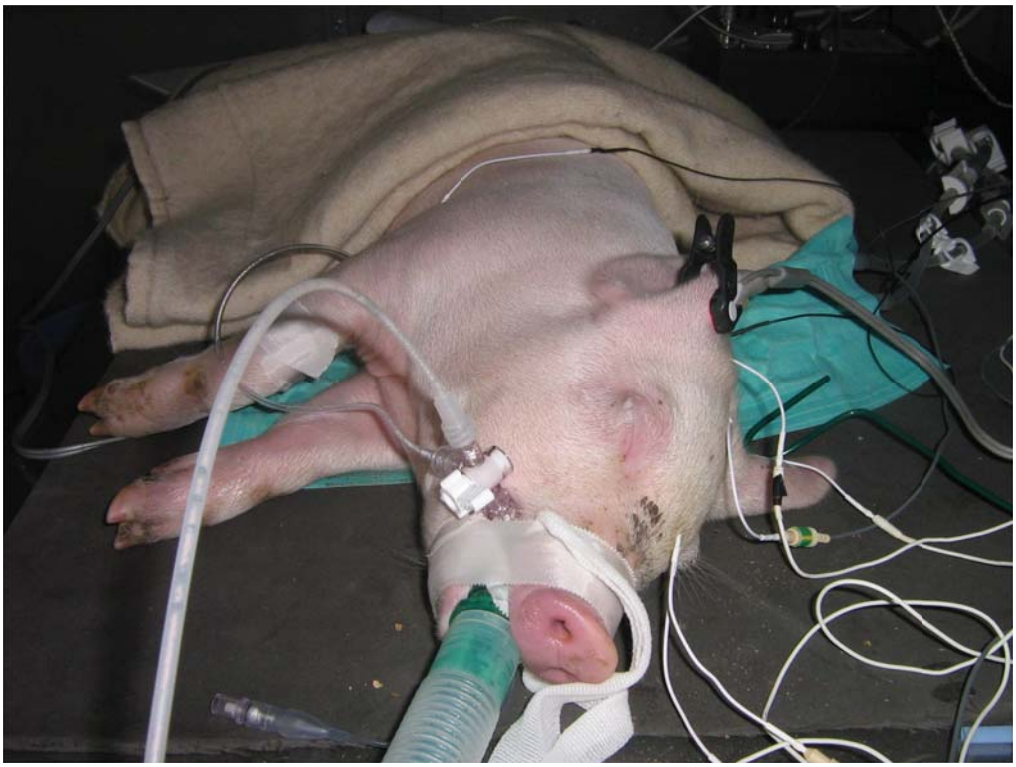
Nikki Kells Digitally signed by Nikki Kells Date: 2017.05.12 09:08:43 +12'00'
Candidate's Signature

12/05/2017
Date

Ngaio Beausoleil Digitally signed by Ngaio Beausoleil Date: 2017.05.15 11:41:05 +12'00'
Principal Supervisor's signature

15/05/2017
Date

CHAPTER 3 Electroencephalographic assessment of oral meloxicam, a topical anaesthetic cream (EMLA) and cautery iron for mitigating acute pain in pigs (*Sus scrofa*) undergoing tail docking



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3.1 ABSTRACT

Objective: To evaluate the analgesic efficacy of oral meloxicam, EMLA topical anaesthetic cream, and hot cautery iron in mitigating acute nociceptive responses to tail docking in pigs.

Study design: Prospective, randomised, controlled experimental study.

Animals: Forty healthy Large White x Landrace pigs aged 21 (SD 1.2) days, weighing 6.1 (SD 0.95) kg, were randomly assigned to one of four tail docking treatments (*n* =10 per treatment): CONTROL: docked using clippers with no analgesia; MEL: docked using clippers after prior administration of oral meloxicam; EMLA: docked using clippers after prior application of topical anaesthetic cream; CAUT: docked using cautery iron with no analgesia.

Methods: Anaesthesia was induced and maintained with halothane delivered in oxygen. Following induction, end-tidal halothane was stabilised at 0.95–1.05% and electroencephalograph (EEG) recording commenced. After 5 minutes of baseline data collection, tail docking was performed and recording continued for a further 10 minutes. The EEG summary variables median frequency (F50), 95% spectral edge frequency (F95) and total power (P_{TOT}) were calculated for the baseline period and for consecutive 30-second intervals following tail docking.

Results: Following docking, F50 increased and P_{TOT} decreased significantly in CONTROL and MEL pigs. EMLA pigs exhibited no change in any variable, whilst CAUT pigs exhibited a reduction in P_{TOT} but no change in F50. Following docking F50 was significantly higher in control pigs than in EMLA pigs, and P_{TOT} was significantly lower in control pigs than in EMLA or CAUT pigs.

Conclusions and clinical relevance: Prior application of EMLA cream abolished EEG indicators of nociception in pigs that were tail docked using clippers. Tail docking using cautery iron without analgesia ameliorated EEG indicators of nociception, relative to docking using clippers without analgesia. Prior administration of EMLA cream or the use of cautery iron in place of clippers have the potential to reduce the acute pain experienced by pigs undergoing tail docking.

3.2 INTRODUCTION

Tail docking of piglets is commonly performed worldwide to reduce the incidence of tail-biting behaviour (Hunter *et al.* 2001; Sutherland *et al.* 2009), which can have serious welfare consequences for affected pigs as well as economic consequences for pig producers (Schrøder-Petersen & Simonsen 2001; Kritas & Morrison 2007). The procedure involves amputation of the distal one-to-two thirds of the tail, using cutting pliers (clippers), scissors, scalpel, or cautery iron (Sutherland and Tucker, 2011). This is usually performed within 7 days of birth, without provision of analgesia.

There is substantial evidence that tail docking of pigs causes acute pain, even when performed at a young age. For example, plasma cortisol was higher in clipper-docked pigs than control-handled pigs at 6 days-of-age (Sutherland *et al.* 2008). Piglets tail docked between 1 and 6 days-of-age exhibited behavioural evidence of acute pain that was not seen in handled control pigs (Noonan *et al.* 1994; Sutherland *et al.* 2008), and vocalised more (Noonan *et al.* 1994; Marchant-Forde *et al.* 2009) and with higher peak vocal frequencies (Marchant-Forde *et al.* 2009) than control-handled pigs. Given such evidence, future regulations may deem provision of analgesia mandatory for tail docking of pigs of all ages. In New Zealand, best practice guidelines already advocate the provision of analgesia for any elective husbandry procedure, irrespective of the age at

which it is carried out (Anonymous 2010), whilst in the European Union provision of anaesthesia and analgesia is mandatory when tail docking is performed beyond 7 days-of-age (Council of the European Union 2008).

The cerebral cortex plays an important role in the conscious perception of pain (Talbot *et al.* 1991; Jones *et al.* 1992; Treede *et al.* 1999). The electroencephalogram (EEG) depicts the electrical activity of the cerebral cortex, and changes in the power spectrum of the EEG have been shown to mirror changes in cortical activity associated with the cognitive perception of pain (Bromm 1984; Chen *et al.* 1989). Further, in humans, the magnitude of changes in the EEG spectrum correlate well with reports of perceived pain intensity (Chen *et al.* 1989).

Changes in the EEG spectra under light general anaesthesia, using the minimal anaesthesia model (MAM), have been shown to reliably indicate nociception in a range of mammals, including sheep (Johnson *et al.* 2005a), cattle (Gibson *et al.* 2007) and pigs (Haga & Ranheim 2005). The model has been successfully used to evaluate analgesic efficacy in deer (Johnson *et al.* 2005b), pigs (Haga & Ranheim 2005), horses (Murrell *et al.* 2003), dogs (Kongara *et al.* 2010) and cats (Kongara *et al.* 2014).

The objective of the current study was to use the MAM to evaluate the efficacy of oral meloxicam, a topical anaesthetic cream, and use of a hot cautery iron in mitigating acute nociceptive responses to tail docking in pigs. A previous study conducted using this model demonstrated that tail docking of piglets using side clippers without analgesia elicits a characteristic nociceptive response (Kells *et al.* 2013). We hypothesised that all three strategies would mitigate acute nociceptive responses relative to tail docking using clippers without analgesia, but that the extent of this mitigation might differ due to the different anti-nociceptive mechanisms involved.

3.3 MATERIALS AND METHODS

This study was conducted with approval from the Massey University Animal Ethics Committee (MUAEC). All procedures were undertaken in accordance with the MUAEC code of ethical conduct for the use of live animals for research, testing and teaching.

Forty (26 male, 14 female) commercial white line (Large white x Landrace) pigs aged 21 ± 0.18 (mean ± SEM) days, weighing 6.1 ± 0.15 kg, sourced from 22 litters (*n* =1 or 2 pigs per litter), were obtained from a commercial pig farm on the day of testing and housed in a temperature-controlled (30°C) indoor facility on deep straw litter with *ad libitum* access to water. Pigs were randomly assigned to receive one of four treatments (*n* =10 per treatment): CONTROL: tail docked using clippers without prior analgesia; MEL: tail docked using clippers (Bahco 2101G side cutter pliers, SNA Europe) following a mean interval of 92 (min =74, max =103) minutes after oral administration of 0.4 mg/kg meloxicam (Metacam 1.5 mg/mL oral suspension, Boehringer Ingelheim NZ Ltd., Manukau, NZ); EMLA: tail docked using clippers following a mean interval of 99 (min =86, max =119) minutes after application of approximately 2 g of topical anaesthetic cream (EMLA cream, 2.5% lignocaine 2.5% prilocaine, AstraZeneca, NSW, Australia) to the base of the tail; CAUT: tail docked using hot cautery iron (Stericut® Tail Docker, Cotran Corp., Portsmouth, RI, USA) without prior analgesia. Pigs in the EMLA treatment had an occluding dressing (Parafilm M®, MicroAnalytix Pty Ltd, Auckland, NZ) applied to the base of the tail following application of EMLA cream, to ensure the product remained on the tail until the time of docking.

Treatment order was randomly allocated across days prior to study commencement. Each treatment was allocated a number and a random number generator was used to determine the order in which treatments

(4 treatments, 10 replicates) were applied. Experiments were conducted on 10 separate test days over a 4-week period, with 4 pigs tested per day.

Anaesthesia

An established minimal anaesthesia model was followed (Murrell and Johnson, 2006). Pigs were anaesthetised with 4% halothane (Halothane-Vet; Merial NZ Limited, Manukau City, NZ) vaporised in oxygen (4 L minute^{-1}) delivered via facemask. Halothane delivery was maintained at 4% during induction and instrumentation, then reduced to achieve an end tidal concentration of 0.95–1.05% during the data acquisition period. Halothane was delivered via facemask throughout. End tidal halothane (F_E´Halo) and CO_2 tensions, SpO_2 and respiration rate were monitored throughout using a side stream anaesthetic monitor at a sampling rate of 200 mL min^{-1} (Hewlett Packard M1025B, Hewlett Packard, Hamburg, Germany). Rectal temperature was monitored using a digital thermometer (Q 1437; Dick Smith Electronics, New Zealand) and maintained at 38–40°C with the aid of a circulating warm-water heating blanket (T pump; Gaymar Industries Inc., NY, USA).

Electrophysiology

Subcutaneous 27-gauge stainless steel needle electrodes (Viasys Healthcare, Surrey, England) were positioned to record EEG and electrocardiograph (ECG) activity. A five-electrode montage was used to record EEG from the left and right cerebral hemispheres, with inverting electrodes placed parallel to the midline over the left and right frontal bone zygomatic processes, non-inverting electrodes over the left and right mastoid processes and a ground electrode placed caudal to the occipital process (see Murrell & Johnson 2006). ECG was recorded using a base-apex configuration.

Both EEG and ECG signals were fed via breakout boxes to separate amplifiers (Iso-Dam isolated biological amplifier, World Precision Instruments Inc.). The signals were amplified with a gain of 1000 and a band-pass of 1.0–500Hz and digitised at a rate of 1 kHz (Powerlab 4/20, ADInstruments Ltd, Colorado Springs, Co). The digitised signals were recorded on an Apple Macintosh personal computer for off-line analysis at the conclusion of the experiment.

Experimental procedure

Once F_E´Halo was stable at 0.95–1.05% 5 minutes of baseline EEG was recorded. Tail docking was then carried out according to group assignment and recording continued for a further 10 minutes. Pigs in the CONTROL, MEL and EMLA groups had their tails amputated using a pair of clean, disinfected side-clippers. Pigs in the CAUT group had their tails amputated using a clean, disinfected gas operated cautery iron (Stericut® Tail Docker). In all cases the tail was docked approximately 2 cm from the base, with care taken to sever between adjacent vertebrae. At the completion of the recording period, a disinfectant was applied to the tail wound and 2 mg/kg carprofen (Rimadyl, Pfizer NZ, Auckland, New Zealand) administered subcutaneously to pigs in the CONTROL, CAUT and EMLA groups. Following recovery from anaesthesia pigs were housed in an indoor temperature-controlled (30°C) recovery pen and offered creep feed and lamb milk replacement (AnLamb, NZAgBiz, Hamilton, NZ), in addition to fresh water. All pigs were rehomed to private farms later the same day.

Data analysis

Raw EEG recordings were inspected manually and any artefacts, such as over-scale, under-scale, nystagmus or other muscular activity, excluded from subsequent analysis. The total power (P_{TOT}), median frequency (F50) and 95% spectral edge frequency (F95) were calculated for consecutive 1-second epochs, using

purpose-written software (Spectral Analyser, CB Johnson, Massey University, Palmerston North, NZ, 2002). Data from each individual were standardised to a percentage of baseline (pre-docking) mean, calculated over the 60 seconds immediately preceding tail docking.

Heart rates were calculated for each individual from ECG data and standardised to a percentage of baseline (pre-docking) mean, calculated over the 60 seconds immediately preceding tail docking.

For the purposes of statistical analysis, EEG and heart rate data from the final 30 seconds of baseline and for consecutive 30-second blocks (up to 180 seconds) after tail docking were compared. A single mean value for each variable was calculated for each time-period in each pig, generating a total of seven data points per pig per variable (one before and six after tail docking).

Statistical analyses

Data from each group of pigs were subjected to analysis of variance using the MIXED procedure in SAS version 9.3.1 (SAS Institute Inc., Cary NC, USA, 2012). The model incorporated sex, day of testing and treatment as fixed effects, pig as a random effect and time as a repeated measure. Statistical significance for all analyses was set at α =0.05. Where significant treatment effects were identified, post hoc tests were carried out to identify group differences, with Bonferroni adjustment for multiple comparisons.

3.4 RESULTS

Data are presented as percentages of baseline (pre tail-docking) mean, in the format mean ± standard error of the mean.

EEG and heart rate data were successfully collected from all 40 pigs. Movement responses to tail docking occurred in 12/40 (30%) pigs, with resultant EMG artefacts excluded from subsequent EEG data analyses. Of the 12 pigs that responded to tail docking, 3 belonged to the CONTROL group, 2 to the CAUT group, 4 to the MEL group and 3 to the EMLA group. Body temperature range and maximum $P_E'CO_2$ recorded during anaesthesia are summarised for each treatment in Table 3.1. Although there were some differences in maximum $P_E'CO_2$ recorded between pigs in different treatment groups, this is unlikely to have had a significant effect on cerebral blood flow over the range recorded (Olsen *et al.* 2006), and is therefore unlikely to have influenced electroencephalographic values.

There was no effect of sex or day of testing on F50, F95 or P_{TOT} (p =0.38, 0.49. 0.56, respectively for sex and p =0.81, 0.53, 0.72, respectively for day of testing).

Table 3.1 *Body temperature range (ºC) and maximum PE'CO2 (kPa) recorded during anaesthesia for pigs in each treatment group.*

Treatment	Body Temperature	Max $PE'CO_2$
Control	38.3 – 39.1	5.47
Meloxicam	38.0 – 40.3	6.33
EMLA	37.6 – 39.4	6.27
Cautery	37.8 – 40.1	6.60

There were significant effects of treatment and time on F95, but no treatment x time interaction (Table 3.2). Post hoc tests revealed that F95 was lower overall in pigs that received EMLA cream prior to tail docking than CON pigs that received no analgesia (p =0.004), but did not differ between other groups (Figure 3.1a). Across all pigs combined, F95 was elevated, relative to baseline, 30 (p <0.0001), 60 (p <0.0001) and 90 (p =0.0318) seconds after docking (Figure 3.1b).

Table 3.2 *The effects of treatment, time and their interaction on the median frequency (F50), 95% spectral edge frequency (F95) and total power (P_{TOT}) of the pig electroencephalogram (EEG) following tail docking with or without analgesic strategies.*

	Treatment		Time		Treatment*Time	
EEG variable	***F***	***p***	***F***	***p***	***F***	***p***
F50	3.87	0.0206	19.30	<0.0001	2.21	0.0040
F95	5.21	0.0060	9.24	<0.0001	1.28	0.2048
P_{TOT}	3.28	0.0367	30.95	<0.0001	2.82	0.0002

There were significant treatment by time interactions on F50 and P_{TOT} (Table 3.2). The F50 of the pig EEG increased after tail docking in the CON and MEL groups, but did not differ from baseline in the CAUT or EMLA groups (Figure 3.2a). Thirty seconds after tail docking, F50 in CON pigs was higher than that of the EMLA group (114.18 ± 3.7 *vs.* 94.36 ± 3.7; p =0.01) and remained higher 60 (124.33 ± 3.7 *vs.* 104.35 ± 3.7; p <0.01) and 90 (121.17 ± 3.7 *vs.* 98.67 ± 3.7; p <0.0001) seconds after docking. By 120 seconds after docking, F50 did not differ between groups. F50 did not differ between the CON, MEL or CAUT groups, or between the MEL, CAUT and EMLA groups, at any time point (Figure 3.2a).

The P_{TOT} of the pig EEG decreased, relative to baseline, in the CON, MEL and CAUT groups after tail docking, but did not differ from baseline in the EMLA group (Figure 3.2b). Thirty seconds after tail docking P_{TOT} in CON pigs was lower than that of EMLA pigs (82.0 ± 2.3 vs. 95.0 ± 2.3; p =0.005) and remained lower 60 seconds after docking (83.3 ± 2.3 vs. 99.4 ± 2.3; p =0.021). Sixty seconds after tail docking, P_{TOT} was also lower in CON than CAUT pigs (83.3 ± 2.3 vs. 95.7 ± 2.3; p =0.01). By 90 seconds after tail docking, P_{TOT} did not differ between treatment groups (p >0.05).

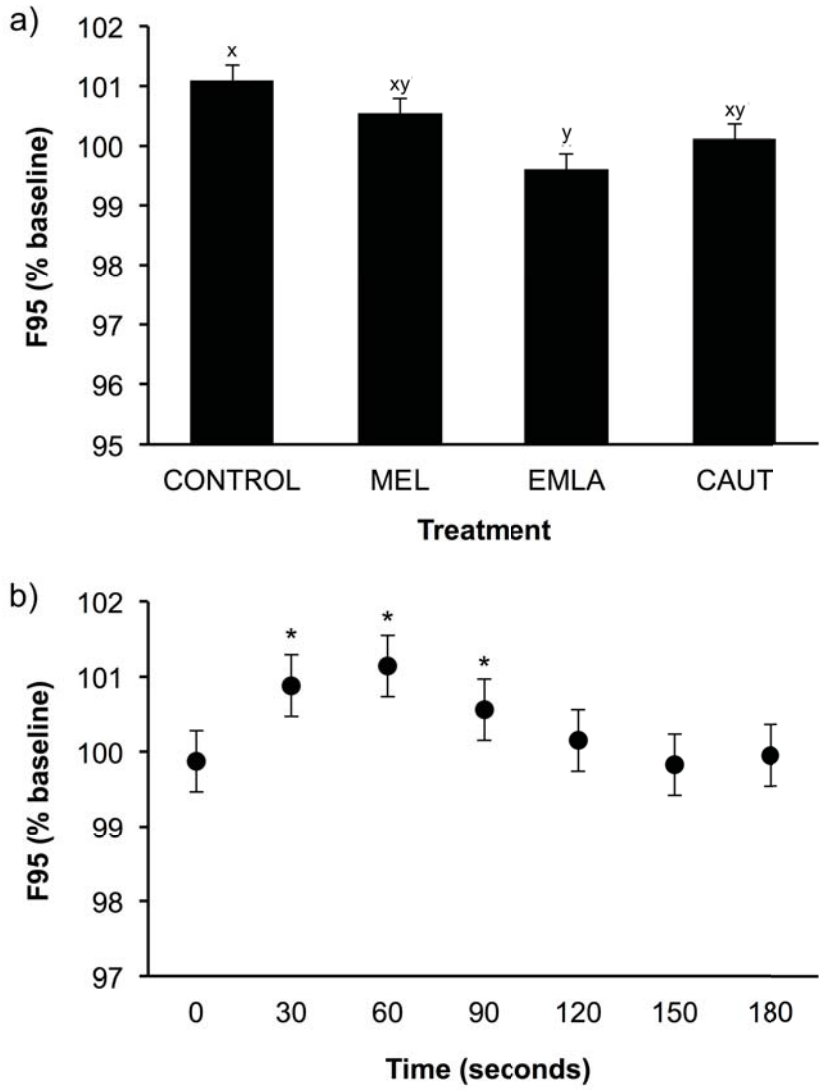

Figure 3.1 *The effects of a) treatment and b) time on the 95% spectral edge frequency (F95) of the pig EEG following tail docking. Data are presented as the mean ± SEM, standardised to a percentage of baseline (pre-docking) mean. Treatment means with different superscripts differed significantly ($p < 0.05$). Asterisks indicate post-docking time points that differed to baseline ($p < 0.05$).*

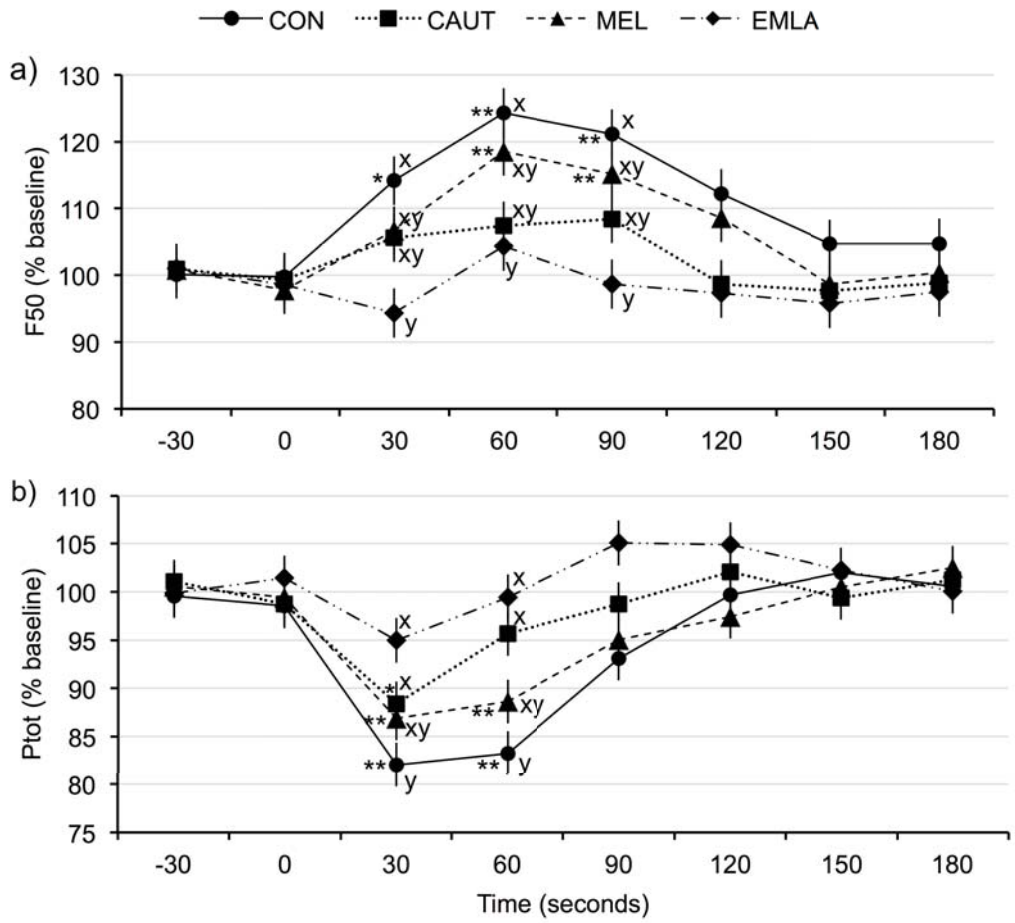

Figure 3.2 *Comparison of the changes in a) median frequency (F50) and b) total power (P_{TOT}) of the pig electroencephalogram (EEG) following tail docking (time 0) using either: clippers without prior analgesia (CON); cautery iron without prior analgesia (CAUT); clippers following prior administration of meloxicam (MEL); or clippers following prior application of topical anaesthetic (EMLA). Data are presented as the mean ± SEM of consecutive non-overlapping 30-second blocks of EEG, standardised to a percentage of baseline (pre-docking) mean. Asterisks indicate post-docking time points that differed to baseline within a treatment (* p <0.05; ** p <0.01). Different superscripts indicate means differed significantly at a given time point (p <0.05).*

There was no effect of sex or day of testing (p =0.0725 and p =0.1481, respectively) and no sex x treatment interaction (p =0.2541) on pig heart rate. Following tail docking, heart rate increased, relative to baseline, in the CON and MEL groups (p <0.05), but did not differ to baseline in the EMLA or CAUT groups (Table 3.3). Sixty seconds after tail docking, heart rate was higher in CON, MEL and EMLA pigs than CAUT pigs and higher in CON than EMLA pigs. Ninety seconds after docking, heart rate remained higher in CON and MEL pigs than CAUT pigs, but did not differ between other groups (Table 3). Heart rate did not differ between groups from 120 seconds onward.

Table 3.3 *Mean ± SEM heart rate (beats per minute) as a percentage of individual baseline at time points after tail docking (time 0) for pigs that were tail docked using: clippers without prior analgesia (CON), clippers with prior administration of meloxicam (MEL), clippers with prior application of EMLA cream (EMLA), or cautery iron without prior analgesia (CAUT).*

Time (sec)	CON	MEL	EMLA[a]	CAUT[a]
0	101.27±0.84	100.64±0.82	99.98±0.77	98.67±0.77
30	102.52±0.84	102.61±0.82	100.35±0.77	98.43±0.77
60	105.74±0.84*^{x}	103.06±0.82*^{xy}	101.29±0.77^{y}	97.35±0.77^{z}
90	104.42±0.84*^{x}	102.66±0.82^{x}	101.37±0.77^{xy}	97.67±0.77^{y}
120	103.15±0.84	102.76±0.82	101.23±0.77	98.49±0.77
150	102.67±0.84	102.35±0.82	101.14±0.77	99.52±0.77
180	102.13±0.84	102.43±0.82	100.68±0.77	99.20±0.77

[a] There were no significant differences to baseline within the EMLA or CAUT treatments (p >0.05)

* Value differed significantly to baseline within treatment (p <0.05)

^{xyz} Values in the same row with different superscripts differed significantly (p <0.05)

3.5 DISCUSSION

The objective of the current study was to evaluate the analgesic efficacy of oral meloxicam, a topical anaesthetic cream, and the use of a hot cautery iron in mitigating acute nociceptive responses to tail docking in pigs.

Noxious stimulation typically elicits desynchronisation of the mammalian EEG, characterised by a reduction in amplitude and increase in higher frequency activity (Murrel 2006), corresponding to an increase in F50 and F95, and a decrease in P_{TOT} (Johnson *et al.* 2012). Prior administration of effective analgesia has been shown to obtund or abolish such responses (Murrell *et al.* 2003; Haga & Ranheim 2005; Johnson *et al.* 2005b; Kongara *et al.* 2010).

In the present study, control pigs that received no prior analgesia demonstrated characteristic nociceptive responses to tail docking with clippers, evidenced by an increase in F50 and reduction in P_{TOT}. Similarly, pigs that received oral meloxicam prior to tail docking demonstrated an increase in F50 and decrease in P_{TOT} after docking. Although F50 in the meloxicam group was only elevated from 60–90 seconds after docking, compared with from 30–90 seconds in the control group, F50 did not differ between the two groups at any time point.

Across all treatments, F95 increased significantly after docking, suggesting that this variable does reflect nociception in pigs. F95 was also lower overall in EMLA pigs than in control pigs. Whilst there was no significant treatment x time interaction, the lower overall F95 in the EMLA group may reflect mitigation of the F95 response to docking, which is generally consistent with the F50 and P_{TOT} results. However, this variable appears to be less sensitive than F50 or P_{TOT} for discriminating the varying degrees of pain mitigation provided by the different treatments applied in this study. In support of this, other studies using the MAM have identified increases in F50 in response to noxious stimulation, even in circumstances where there were no changes in F95 (e.g. Murrell *et al*. 2007; Kongara *et al*. 2010).

Meloxicam, like other non-steroidal anti-inflammatory drugs (NSAID), exerts peripheral anti-nociceptive effects via modulation of inflammatory pathways (Engelhardt *et al.* 1995; Laird *et al.* 1997). Previous studies have demonstrated that meloxicam is effective in ameliorating inflammatory pain in pigs subsequent to castration and tail docking (Keita *et al.* 2010; Tenbergen *et al.* 2014). There is some evidence that NSAIDs exert central analgesic effects in addition to their more widely recognised peripheral actions (Cashman 1996; Vanegas 2010). A series of studies in mice demonstrated that meloxicam was effective in mitigating chemically-induced neurogenic nociception, but was ineffective in mitigating thermally-induced nociception (Santos *et al.* 1998). In the present study, prior administration of meloxicam failed to mitigate acute EEG nociceptive responses to tail docking using clippers. This is consistent with a prior study that demonstrated no effect of meloxicam in mitigating acute EEG nociceptive responses of lightly anaesthetised dogs to noxious electrical stimulation (Kaka *et al.* 2015). It may be that the central nociceptive action of meloxicam is dependent on pain modality, is not apparent within the time frame investigated in the present study, or cannot be assessed using EEG parameters.

In contrast, application of a topical anaesthetic (EMLA cream) to the base of the tail prior to tail docking completely abolished the EEG responses observed in pigs docked with clippers alone. EMLA cream contains the anaesthetic agents lignocaine and prilocaine, which penetrate the skin and block signals generated by the activation of nociceptors in the dermal and sub dermal regions, preventing them from reaching the brain (Thurmon *et al.* 1996). Application of EMLA cream to the tail for at least 86 minutes prior to tail docking appears to effectively abolish acute nociceptive responses to tail docking.

According to the manufacturer, the effective duration of anaesthesia from EMLA cream is at least 2 hours following removal of the occluding dressing. However, a study investigating the duration of topical anaesthesia in children following EMLA application reported an average effective duration of 30–60 minutes after removal of the dressing (Hallen *et al.* 1984). In the present study, tail docking was performed immediately following removal of the occluding dressing, ensuring maximum anaesthetic efficacy.

There is some possibility that the anti-nociceptive action of EMLA cream in the present study resulted from systemic absorption of the anaesthetic components. Continuous intravenous administration of lidocaine has been shown to obtund EEG changes in ponies undergoing surgical castration, providing evidence for a systemic anti-nociceptive action (Murrell *et al.* 2005). However, in a study investigating systemic absorption of EMLA cream in clinically ill cats, application of EMLA cream to a 10-cm^2 area over the jugular vein for 1 hour resulted in no detectable systemic absorption of either anaesthetic component (Wagner *et al.* 2006). In the present study, the application of approximately 2 g of EMLA over 2–3 cm^2 of tail skin for 99 minutes is therefore unlikely to have resulted in sufficient anaesthetic absorption for any systemic analgesia to occur.

Pigs that were tail docked using hot cautery iron, without analgesia, exhibited a brief reduction in EEG total power in response to tail docking, but no change in F50. The absence of an increase in F50, along with the smaller magnitude of changes in F50 and P_{TOT} compared to control pigs docked using clippers, indicates that tail docking using cautery was less acutely noxious to pigs. Both methods involve severing the tail tissues using a blade. However, in the case of cautery iron the blade is heated so that it simultaneously cauterises the tail tissues, destroying local nociceptors and reducing the animal's ability to perceive noxious stimulation (Lester *et al.* 1991; Sutherland *et al.* 2009). This likely accounts for the reduced nociceptive response to cautery observed in the current study.

Using the MAM, surgical noxious stimulation has been demonstrated to induce both increases (Gibson *et al.* 2007) and decreases (Haga & Ranheim 2005) in heart rate relative to pre-stimulus values. In the present study, heart rate increased following tail docking in CON and MEL pigs, but did not change in EMLA or CAUT pigs. This, along with the significantly greater heart rate observed in CON and MEL pigs than CAUT or EMLA

pigs at specific time points after docking, provides further evidence for the anti-nociceptive actions of cautery and EMLA cream.

Whilst the EMLA cream used in the current study was effective in mitigating acute nociceptive responses, it required that pigs be handled twice, that an occluding dressing be applied to cover the cream, and that a minimum of 60 minutes elapse between application of the cream and performance of docking. These requirements likely make the method impractical for routine use in a commercial setting. However, given the extended period of analgesia provided by EMLA cream, there would be benefit in exploring alternative methods of delivery that could eliminate the need for additional handling and application of an occluding dressing e.g. development of a spray-on rapidly absorbed formulation.

In interpreting the anti-nociceptive action of the strategies employed in the current study, it must be borne in mind that only very acute nociceptive responses were investigated i.e. those occurring in the 3 minutes immediately following tail docking. As such, any potential longer-term analgesic effects beyond this period, e.g. anti-inflammatory mediated anti-nociception, were not assessed. Further investigation, using alternative methodology, is required to determine any longer-term effects of these approaches on tail-docking induced pain.

Further, the potential long-term pain consequences of tail docking using either cautery or clippers should be compared before any recommendations regarding best practice are made.

3.6 CONCLUSION

Prior application of EMLA topical anaesthetic cream abolished EEG indicators of acute nociception in pigs undergoing tail docking using side clippers. In contrast, no effect on acute EEG responses was found following prior administration of oral meloxicam. The use of heated cautery iron ameliorated, but did not abolish, EEG indicators of acute nociception compared to tail docking with clippers. Although both EMLA and cautery iron appear to effectively mitigate acute nociceptive responses to tail docking, the practical limitations associated with the extra handling required to administer topical anaesthesia prior to docking means that use of cautery iron may be a better strategy for mitigating acute pain in pigs undergoing tail docking in a commercial setting. However, the potential long-term pain consequences of tail docking using cautery or clippers should be further investigated before any recommendations are made.

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MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Nicola Jean Kells

Name/Title of Principal Supervisor: Dr Ngaio Beausoleil

Name of Published Research Output and full reference:

Kells NJ, Beausoleil NJ, Sutherland MA, Morrison RM, Johnson CB. Electroencephalographic assessment of oral metacam, topical anaesthetic cream and cautery iron for mitigating acute pain in pigs (Sus scrofa) undergoing tail docking.
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In which Chapter is the Published Work: Chapter 3

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Nikki had a primary role in study design, data collection, statistical analysis, interpretation, writing of the paper and responding to reviewers' comments, with guidance from supervisors.

Nikki Kells Digitally signed by Nikki Kells Date: 2017.05.12 09:11:37 +12'00'
Candidate's Signature

12/05/2017
Date

Ngaio Beausoleil Digitally signed by Ngaio Beausoleil Date: 2017.05.15 11:42:14 +12'00'
Principal Supervisor's signature

15/05/2017
Date

CHAPTER 4 Postnatal development of electroencephalographic responses to noxious stimulation in pigs (*Sus scrofa*) between the ages of 1 and 15 days

Kells NJ, Beausoleil NJ, Sutherland MA, Johnson CB. Postnatal development of EEG responses to noxious stimulation in pigs (*Sus scrofa*) aged 1–15 days. Submitted to *Animal Welfare* 07 December 2016.

4.1 ABSTRACT

This study examined electroencephalographic (EEG) indices of nociception in pigs aged 1, 5, 7, 10, 12 and 15 days postnatal. Ten pigs per age were anaesthetised with halothane in oxygen and maintained at a light plane of anaesthesia. EEG was recorded bilaterally using a 5-electrode montage. Following a 10-minute baseline period, tails were docked using side cutter pliers and recording continued for a further 5-minutes. Changes in the median frequency (F50), 95% spectral edge frequency (F95) and total power (P_{TOT}) of the EEG of the right cerebral hemisphere were used to assess nociception. Tail docking at 1 day-of-age induced no significant changes in the EEG spectrum. A typical nociceptive response, characterised by an increase in F50 and decrease in P_{TOT}, was evident at 10 and 15 days-of-age, with 5, 7 and 12 day-old pigs exhibiting responses in either F50 or P_{TOT} only. Pooling of data into ≤7 days-of-age and >7 days-of-age revealed F50 was higher overall in the older group. Whilst P_{TOT} decreased after docking in both groups, this response was larger and more prolonged in the older group. F95 increased after docking in the older pigs only. Overall, these data provide evidence of an increase in cortical responsiveness to noxious stimulation with increasing postnatal age, suggesting there may be qualitative differences in pain perception between age groups. Further, the data provide support for current recommendations that tail docking and other painful husbandry procedures be performed within 7 days of birth to minimise their animal welfare impact.

4.2 INTRODUCTION

Tail docking is commonly performed on commercial pig farms to reduce the incidence of tail biting behaviour, which can have severe welfare consequences for affected animals. The procedure is typically performed within 7 days of birth, without the provision of analgesia. However, there is diverse evidence that the procedure is acutely painful to pigs, even when performed at a very young age (Noonan *et al.* 1994; Sutherland *et al.* 2008; Marchant-Forde *et al.* 2009).

Current animal welfare guidelines recommend that tail docking, along with other potentially painful husbandry procedures, be performed at a young age, reflecting a desire to minimise any associated pain. For example, New Zealand recommendations advocate that tail docking of pigs be performed within 72 hours of birth (Anonymous 2010). Similarly, both Australia and the UK recommend that tail docking of pigs be performed within 7 days of birth, with the latter stipulating that analgesia be provided for pigs >7 days-of-age (Council of the European Union 2008; CSIRO 2008). Despite such recommendations, there is little scientific evidence to support these age thresholds and little research has been undertaken comparing the effects of piglet age on pain responses.

The subjective nature of pain makes its assessment in animals complicated. Animals' inability to report their experiences necessitates the use of indirect indices of pain, including metabolic, endocrine and behavioural measures (Livingston & Chambers 2000). However, the interpretation of these is confounded by the fact that they are non-specific and may alter in response to non-painful stressors, such as handling and restraint. In addition, these indices represent responses to noxious stimulation, rather than pain perception (Johnson *et al.* 2005a) and some have been shown to correlate poorly with reports of pain in people (Chapman *et al.* 1985). More recently, electroencephalographic (EEG) indices of nociception have been used to infer pain and test analgesic efficacy in a range of mammals. The EEG provides a summation of electrical activity arising from the cerebral cortex. In man, changes in the frequency spectrum of the EEG mirrored changes in cortical activity relating to the cognitive perception of pain (Bromm 1984).

Changes in the EEG frequency spectra under light anaesthesia have been used to assess nociception in a range of adult mammals, including horses (Murrell *et al.* 2003), cattle (Gibson *et al.* 2007), sheep (Johnson *et al.* 2005a) and pigs (Haga & Ranheim 2005). Furthermore, prior administration of effective analgesia has been shown to obtund spectral EEG responses to noxious stimuli (Haga & Ranheim 2005; Johnson *et al.* 2005b; Murrell *et al.* 2005; Kongara *et al.* 2014). In sheep, the magnitude of changes in the EEG frequency spectrum correlated well with behavioural responses to noxious stimuli (Ong *et al.* 1997), whilst in man the magnitude of changes correlated with reports of pain intensity in response to graded noxious stimuli (Chen *et al.* 1989).

In a previous study, we investigated the EEG responses of 2- and 20-day-old pigs to tail docking (Kells *et al.* 2013) and identified differences in the magnitude and duration of responses to tail between the two ages. This led us to question how pig's responses to noxious stimulation develop over the early post-natal period.

The aim of the present study was to compare EEG responses to tail docking under light anaesthesia in pigs aged 1, 5, 7, 10, 12 and 15 days-of-age.

4.3 MATERIALS AND METHODS

The study was conducted with approval from the Massey University Animal Ethics Committee (MUAEC, protocol # 14/26). All procedures were undertaken in accordance with the MUAEC code of ethical conduct for the use of live animals for research, testing and teaching.

Sixty commercial white line (Large white x Landrace) entire male pigs aged 1, 5, 7, 10, 12 or 15 days (*n* =10 per age) were obtained from a commercial pig farm on the day of testing and housed in a temperature-controlled (30°C) indoor facility on deep straw litter with *ad libitum* access to water. Pigs had not previously undergone any potentially painful husbandry procedures (e.g. castration, tooth trimming, ear tagging, iron injection) and had intact tails. Within each age group pigs were sourced from three separate litters, with each litter being tested at only a single age.

Experiments were conducted on 18 separate test days, with 2–4 pigs from a single litter tested per day.

Anaesthesia

An established minimal anaesthesia model (MAM) was followed (Murrell & Johnson 2006). Pigs were anaesthetised with halothane (Halothane-Vet; Merial NZ Limited, Manukau City, NZ) vaporised in oxygen (4 L minute^{-1}) delivered via facemask. Halothane concentration was maintained at 3–4% during induction and instrumentation and at 0.95–1.05% during the data acquisition period. End tidal halothane and CO_2 tension, SpO_2 and heart rate were monitored throughout using an anaesthetic agent monitor (Hewlett Packard M1025B, Hewlett Packard, Hamburg, Germany). Rectal temperature was monitored using a digital thermometer (Q 1437; Dick Smith Electronics, New Zealand) and maintained at 38–40°C with the aid of a circulating warm-water heating blanket (T pump; Gaymar Industries Inc., NY, USA).

Electrophysiology

Subcutaneous 27-gauge stainless steel needle electrodes (Viasys Healthcare, Surrey, England) were positioned to record EEG from the left and right cerebral cortices, with inverting electrodes placed parallel to the midline over the left and right frontal bone zygomatic processes, non-inverting electrodes over the left and right mastoid processes and a ground electrode placed caudal to the occipital process (see Murrell & Johnson 2006).

EEG signals were fed via breakout boxes to separate amplifiers (Iso-Dam isolated biological amplifier, World Precision Instruments Inc.). The signals were amplified with a gain of 1000 and a band-pass of 1.0–500Hz and digitised at a rate of 1 kHz (Powerlab 4/20, ADInstruments Ltd, Colorado Springs, Co). The digitised signals were recorded on an Apple Macintosh personal computer for off-line analysis after the experiment.

Experimental procedure

Once end tidal halothane tension was stable at 0.95–1.05% 10 minutes of baseline EEG was recorded. Tail docking was then performed by severing the tail approximately 2 cm from the base using a pair of clean, disinfected side cutter pliers, taking care to sever between adjacent vertebrae. EEG recording was continued for 5 minutes after docking. As the pigs in this study were not yet weaned and could not be returned to the farm of origin due to biosecurity restrictions, the experimental protocol dictated that they be euthanased at the conclusion of data collection. This was carried out via I/P injection of sodium pentobarbital (250 mg/kg; Pentobarb 500, Provet NZ Pty Ltd, Auckland, NZ) administered whilst pigs were still under general anaesthesia.

Data analysis

EEG data from the right cerebral cortex only were analysed. Although EEG was recorded bilaterally, previous studies using the MAM have demonstrated equivalency in spectral EEG between hemispheres (McIlhone 2011; Murrell *et al*. 2007, 2010), suggesting data from either hemisphere alone is suitable for analysis. Data from the left cortex were collected for use in the event that right cortex data were unsuitable for analysis, for example due to electrode displacement or the presence of extensive artefact confined to a single channel. Raw EEG recordings were inspected manually and any artefacts, such as over-scale, under-scale, nystagmus or other muscular activity, were excluded from subsequent analysis.

The total power (P_{TOT}), median frequency (F50) and 95% spectral edge frequency (F95) were calculated for consecutive 1-second epochs, using purpose-written software (Spectral Analyser, CB Johnson, Massey University, Palmerston North NZ, 2002). Fast Fourier transformation was applied to each epoch, generating sequential power spectra with 1 Hz frequency bins.

Statistical analyses

All statistical analyses were performed in SAS version 9.3.1 (SAS Institute Inc., Cary NC, USA, 2012). Due to the size and complexity of the resultant dataset, a number of statistical analyses were undertaken, as follows:

Between-age comparison of baseline EEG

A comparison of baseline (pre-stimulus) F50, F95 and P_{TOT} among ages was carried out by calculating the mean F50, F95 and P_{TOT} over the final 60 seconds of the baseline recording period (prior to tail docking) for each pig. Baseline means were compared using the GLM procedure in SAS. The model included age, litter(age) and test order as fixed effects.

Analysis of all ages combined

Analysis of baseline EEG demonstrated a significant age effect on EEG variables. To account for differences in baseline EEG power between pigs of different ages, data were standardised to a percentage of pre-stimulus baseline for statistical analyses as follows: Values for F50, F95 and P_{TOT} generated over consecutive 1-second epochs were transformed to a percentage of baseline mean by dividing each variable by the mean F50, F95 or P_{TOT} calculated over the final 60 seconds of the baseline recording period and multiplying the product by 100. EEG data for consecutive 15-second blocks (up to 120 seconds) after tail docking were then

compared to that from the final 15 seconds of baseline. A single mean value for each EEG variable was calculated for each time-period in each pig, generating a total of nine data points per pig per variable (one before and eight after tail docking; (Figure 4.1).

Analysis of variance was performed using the MIXED procedure in SAS to compare post-docking means for each variable to baseline mean within-age and to compare means between ages at each time-period after docking. The model included age, litter(age) and order of testing as fixed effects, pig as a random effect, and time as a repeated measure. Statistical significance was set at p <0.05. Where significant main or interactive effects were identified, post hoc tests were carried out to identify group differences with Bonferroni correction for multiple comparisons.

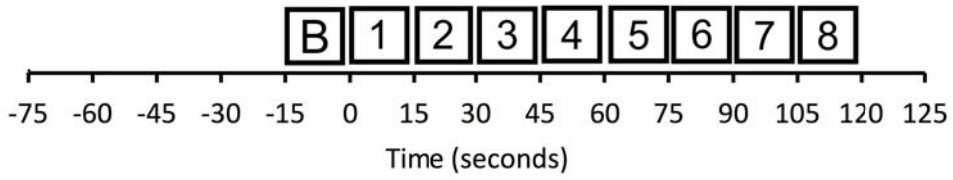

Figure 4.1 *Schematic diagram illustrating the consecutive non-overlapping time periods used for statistical analyses of transformed data. B = baseline. Mean F50, F95 and* P_{TOT} *were calculated for each period in each individual.*

Analysis of each age separately

Data from each age were also analysed separately to identify any changes in EEG variables indicative of nociception following tail docking that may have been obscured in the combined analysis due to the large number of comparisons. For each variable in each pig, means for sequential post-docking time points were compared to baseline mean using the MIXED procedure in SAS. The model included litter and order of testing as fixed effects, pig as a random effect and time as a repeated measure. Where significant main effects were found (p <0.05), Dunnett's post hoc tests were performed to identify differences from baseline.

Analysis of age-blocked data

Given various industry recommendations that tail docking be performed within the first week of life, data were combined into two age blocks for comparison: ≤7 days (data from 1-, 5- and 7-day-old pigs) and >7 days (data from 10-, 12- and 15-day-old pigs). Analysis of variance was performed using the MIXED procedure in SAS to compare post-docking EEG variables to baseline within-age and to compare values between ages at each time-period after docking. The model included age block, litter(age block) and order of testing as fixed effects, pig as a random effect, and time as a repeated measure.

4.4 RESULTS

EEG data were successfully collected from all 60 pigs. Twenty-one pigs (35%) exhibited brief movement responses to tail docking, in the form of leg or tail stump twitches, hind limb extension, or in some instances a brief 'running' motion of the hind limbs. Of these, 17 resulted in discrete movement artefacts in the EEG recording, which were excluded from subsequent analyses. Movement responses to tail docking were

observed in seven 5 day-old, one 7-day-old, six 10-day-old, three 12 day-old and four 15-day-old pigs. No movement responses to tail docking were observed in 1-day-old pigs.

Between-age comparison of baseline EEG

Pig age significantly influenced baseline F50 (F =4.18, p <0.01) and F95 (F =2.82; p =0.03), but not P_{TOT} (F =1.72; p =0.15). Baseline F50 was lower in 1-day-old than 10-, 12- or 15-day-old pigs, but did not differ between other age groups (Figure 4.2a). Baseline F95 was lower in 1-day-old pigs than 7-day-old pigs, but did not differ between other age groups (Figure 4.2b). Test order (1^{st}, 2^{nd}, 3^{rd} or 4^{th}) had a significant effect on baseline F50 (F =3.66; p =0.02), with F50 higher in pigs that were tested first on a given day compared with those tested second (p =0.02).

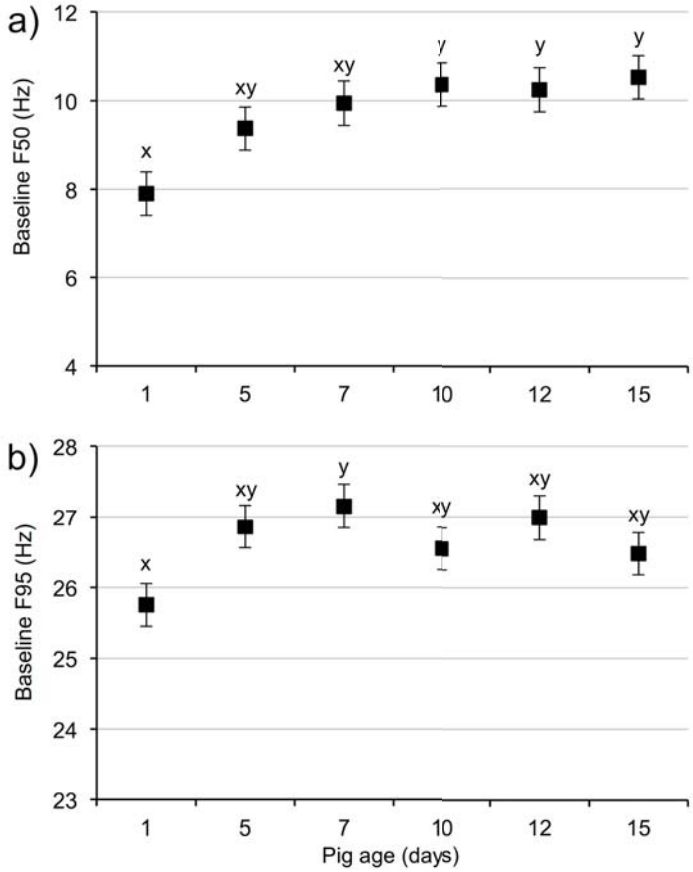

Figure 4.2 *Comparison of baseline a) F50 and b) F95 of the EEG of pigs aged 1-, 5-, 7-, 10-, 12 and 15-days-of-age. Data are presented as mean ± SEM. Superscript letters denote means that differ significantly (Bonferroni adjusted p <0.05).*

Analysis of all ages combined

Piglet age at the time of tail docking had a significant effect on all three EEG summary variables, with significant age x time interaction effects found for F95 and P_{TOT} (Table 4.1).

Table 4.1 *Effects of age, time, test order and litter on the change in EEG summary variables following tail docking in pigs aged 1–15 days. Results are based on analyses of transformed (% baseline) data.*

	Age		**Time**		**Order**		**Litter(Age)**		**Age x Time**	
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
F50	3.43	0.012	8.51	<0.001	1.69	0.185	1.66	0.116	1.26	0.136
F95	5.10	0.001	3.77	<0.001	10.94	<0.001	2.63	0.011	1.67	0.008
P_{TOT}	2.78	0.031	20.19	<0.001	0.86	0.471	3.36	0.002	1.47	0.040

Median frequency (F50)

Overall, mean F50 was lower in 1-day-old pigs than 5-day-old pigs (102.31 ± 2.00 versus 110.93 ± 2.00%; p =0.05), but did not differ among the other age groups. There was a significant effect of time on F50, with F50 being elevated, relative to baseline, from 30–105 seconds after docking (p <0.02). Mean F50 did not significantly differ between ages at any individual time point (no interaction between age and time).

Spectral edge frequency (F95)

F95 was elevated relative to baseline in 10 day-old pigs from 15–75 seconds after docking (p <0.01). Comparison at individual time points revealed that mean F95 was higher in 10-day-old pigs than 1, 5, 7 or 12-day-old pigs 30 and 45 seconds after tail docking (Figure 4.3a). Test order significantly influenced F95. Piglets tested first on a given test day exhibited lower F95 (99.68 ± 0.15%) than those tested second (100.72 ± 0.15%; p <0.001) or third (100.76 ± 0.16%; p =0.001), but did not differ to those tested fourth (100.26 ± 0.25%; p =0.30). Despite the overall effect of litter within age, post-hoc tests revealed no significant differences in F95 between the three litters tested at any age.

Total power (P_{TOT})

A reduction in P_{TOT} after docking was observed in 5, 10, 12 and 15 day-old pigs. P_{TOT} was lower than baseline 15 seconds after docking in 5, 12 and 15 day old and from 15–45 seconds after docking in 10 day-old pigs (p <0.01). Comparison at individual time points revealed that P_{TOT} was lower in 10 and 12-day-old pigs than 1-day-old pigs 45 seconds after docking (Figure 4.3b). P_{TOT} did not differ between age groups over the period 60–120 seconds after docking. Despite the overall effect of litter within age, post-hoc tests revealed no significant differences in P_{TOT} between the three litters tested at any age.

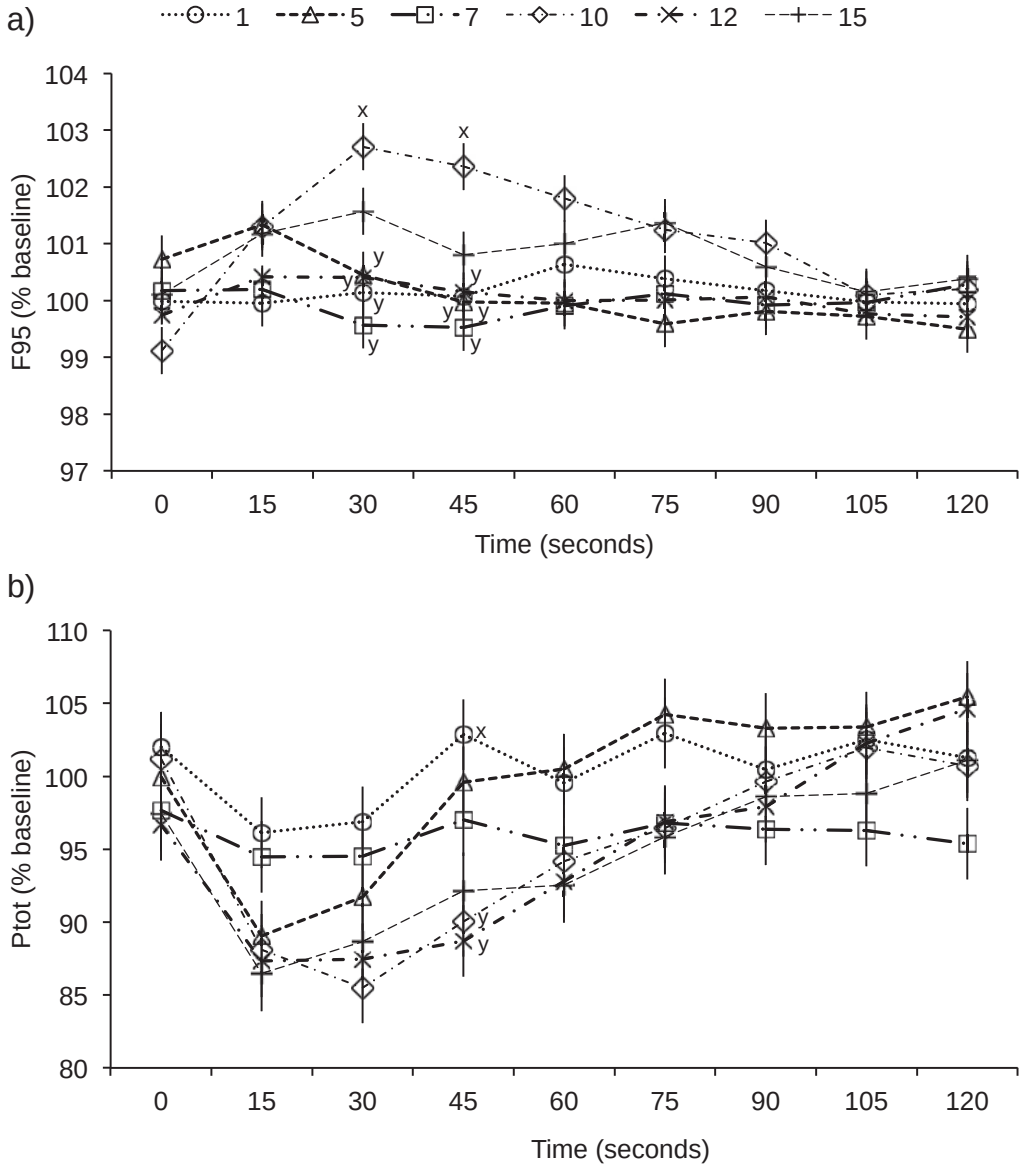

Figure 4.3 *Comparison of the changes in mean (± SEM) a) F95 and b) P_{TOT} of the EEG following tail docking (time 0) in pigs aged 1, 5, 7 10, 12 and 15 days-of-age. Data are presented as percentages of baseline mean. Means at the same time points with different letters differed significantly (Bonferroni adjusted p <0.05).*

Analysis of each age separately

Neither test order nor litter significantly influenced the EEG of pigs of any age. Time significantly influenced F50 at 1, 7, 10 and 15 days-of-age, and P_{TOT} at all ages except 7 days (Table 4.2). Only 10-day-old pigs exhibited a significant change in F95 over time after docking.

Table 4.2 *Effect of time on F50, F95 and P_{TOT} of the EEG following tail docking in pigs aged 1, 5, 7, 10, 12 and 15 days.*

	F50		F95		P_{TOT}	
Age (days)	**F**	***p***	**F**	***p***	**F**	***p***
1	2.91	0.01	0.29	0.97	2.93	0.01
5	1.57	0.15	1.45	0.19	5.2	<0.01
7	5.05	<0.01	0.95	0.48	1.03	0.43
10	4.89	<0.01	4.91	<0.01	5.71	<0.01
12	1.34	0.24	0.25	0.98	5.68	<0.01
15	2.38	0.02	0.96	0.48	5.48	<0.01

1-day-old pigs

Although there were significant overall effects of time on F50 and P_{TOT} in 1-day-old pigs, Dunnett's post-hoc tests revealed no significant differences to baseline mean at any time point after tail docking. There was no effect of time on F95.

5-day-old pigs

Mean F50 and F95 did not differ to baseline at any time after docking, whereas P_{TOT} was significantly lower than baseline 15 (p <0.01) and 30 (p =0.01) seconds after docking, returning to baseline values by 45 seconds after docking.

7-day-old pigs

Mean F50 was lower than baseline 15 seconds after docking (p <0.01), but did not differ to baseline from 30 seconds onward. Neither F95 nor P_{TOT} differed to baseline at any time after docking.

10-day-old pigs

Mean F50 was lower than baseline 15 seconds after docking (p =0.03), and showed a tendency toward being higher than baseline 45 and 60 seconds after docking (p =0.08 and 0.07, respectively). F95 was higher than baseline 15, 30, 45 (all p <0.01) and 60 (p =0.01) seconds after docking. P_{TOT} was lower than baseline 15, 30 (both p <0.01) and 45 (p =0.04) seconds after docking.

12-day-old pigs

Neither mean F50 nor F95 differed to baseline at any time after docking, whilst P_{TOT} was significantly lower than baseline 15 (p <0.01), 30 (p =0.01) and 45 (p =0.04) seconds after docking.

15-day-old pigs

Mean F50 was elevated relative to baseline 30 and 45 seconds after docking (p = 0.02 and 0.04, respectively), whilst P_{TOT} was lower than baseline 15 and 30 seconds after docking (p <0.01 and p =0.01, respectively). F95 did not differ to baseline at any time.

Analysis of age-blocked data

Results of statistical analyses are presented in Table 4.3.

Table 4.3 *Effects of age, time, and their interaction on the F50, F95 and P_{TOT} of the pig EEG following tail docking, using data blocked into ≤7 or >7 days of age.*

	Age		Time		Order		Litter(Age)		Age x Time	
	F	p	F	p	F	p	F	p	F	p
F50	7.98	0.008	7.88	<0.001	1.92	0.185	1.98	0.039	1.03	0.411
F95	8.64	0.006	3.37	0.001	9.37	<0.001	2.29	0.016	3.23	0.001
P_{TOT}	7.67	0.009	19.14	<0.001	0.71	0.471	2.90	0.003	3.12	0.002

Median frequency (F50)

There were significant age and time interaction effects on F50 (Table 4.3). F50 was lower overall in pigs aged ≤7 days than those aged >7 days (103.63 ± 0.16 versus 108.71 ± 0.14%). F50 was elevated relative to baseline from 30–105 seconds after docking (all p ≤0.01). Although there was an overall effect of litter within age, F50 did not differ between litters within each age group following correction for multiple comparisons.

Spectral edge frequency (F95)

There was a significant age x time interaction effect, along with a significant effect of test order on F95 (Table 4.3). F95 did not differ from baseline at any point after docking in pigs ≤7 days, whereas in pigs >7 days, F95 was greater than baseline from 15–75 seconds after docking (p <0.05) (Figure 4.4a). F95 was higher in pigs aged >7 days than those aged ≤7 days 30 (p <0.001) and 45 (p =0.014) seconds after docking, but did not differ between age groups over the period 60–120 seconds after docking (Figure 4.4). F95 was lower in pigs tested first on a given day than those tested second or third, (p <0.001). Although there was an overall effect of litter within age, F95 did not differ between litters within each age group following correction for multiple comparisons.

Total power (P_{TOT})

There was a significant age x time effect, and a significant effect of litter within age block on P_{TOT}. P_{TOT} was lower than baseline from 15–30 seconds after docking in pigs aged ≤ 7 days, and from 15–45 seconds after docking in pigs aged > 7 days (p <0.05). P_{TOT} was lower in pigs aged >7 days than those aged ≤7 days 30 (p =0.014) and 45 (p <0.001) seconds after docking, but did not differ between groups over the period 60–120 seconds after docking (Figure 4.4b). Of the nine litters aged >7 days, P_{TOT} was lower in pigs from litter 12 than from litter 13 (p =0.007).

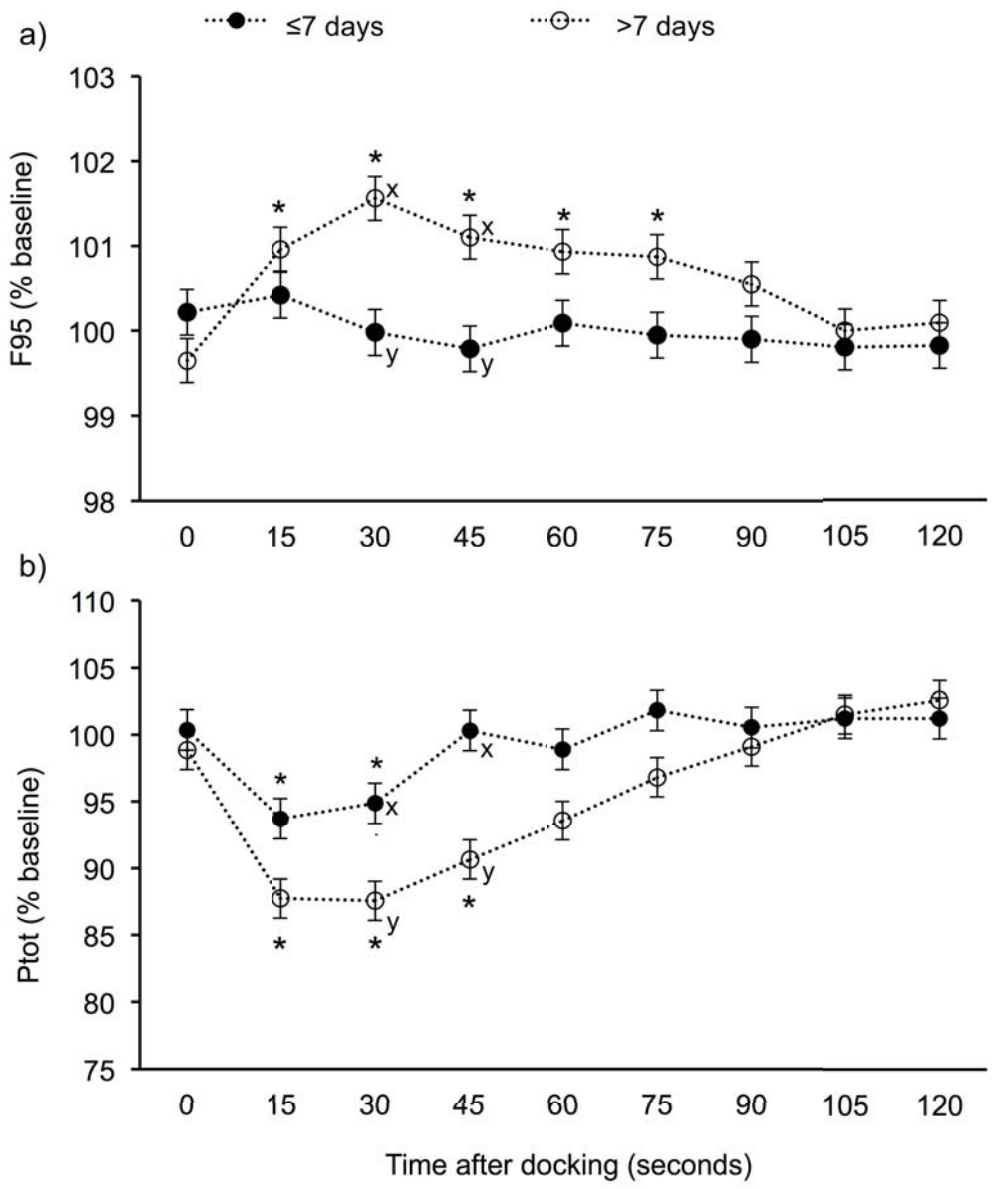

Figure 4.4 *Comparison of mean a) F95 and b) P_{TOT} of the EEG in pigs aged ≤7 days (comprised of data from 1-, 5-, and 7-day-old pigs) or >7 days (comprised of data from 10-, 12- and 15-day-old pigs) following tail docking at time 0. Asterisks indicate mean differed from baseline within age group (Dunnett's p <0.05). Superscript symbols indicate differences between age groups at common time points (Bonferroni adjusted p <0.05).*

4.5 DISCUSSION

Previously, we identified differences between the EEG responses of 2- and 20-day-old pigs to the noxious stimulus of tail docking (Kells *et al*. 2013). The aim of the present study was to examine EEG responses to tail docking in pigs aged between 1 and 15 days-of-age, to determine the manner in which cortical responses to noxious stimulation develop over this period.

The cerebral cortex remains responsive to noxious stimuli at a minimal plane of halothane anaesthesia (Murrell *et al.* 2003). Using the minimal anaesthesia model, prior studies have consistently demonstrated that noxious stimuli elicit an increase in F50 and decrease in P_{TOT} of the EEG of adult mammals (Murrell *et al*. 2003, 2005; Johnson *et al*. 2005b; Gibson *et al.* 2007; Kongara *et al.* 2010). In lambs, the degree of responsiveness of the cerebral cortex to noxious stimulation was shown to vary with postnatal age (Johnson *et al*. 2005a, 2009). The present study demonstrated that the EEG responses of pigs to the noxious stimulus of tail docking also varied with postnatal age, with an overall pattern emerging of increased responsiveness with increasing postnatal age.

At 1 day-of-age, the pig EEG showed no response to tail docking, suggesting either a marked difference in neural processing of nociceptive signals within the first day after birth, or that tail docking is not noxious to pigs at this age. A study investigating stress hormone responses to tail docking at 1 day-of-age similarly found no significant differences in plasma adrenocorticotropic hormone or cortisol concentration between docked and control pigs, leading the authors to conclude tail docking is not noxious at 1 day-of-age (Prunier *et al.* 2005). In addition, we identified differences in baseline (resting state) EEG between ages. Although P_{TOT} did not differ between ages, F50 was significantly lower at 1 day-of-age than at 10–15 days-of-age, indicating that low-frequency activity contributes a greater proportion of the total EEG power at 1 day-of-age.

Whilst at 1 day-of-age the cerebral cortex did not respond to noxious stimulation, pigs aged between 5 and 15 days exhibited at least some elements of a characteristic nociceptive response. From 10 postnatal days onward, EEG responses to tail docking were broadly consistent with those previously reported in 20-day-old pigs following castration (Haga & Ranheim 2005) and tail docking (Kells *et al.* 2013) and in other adult mammals in response to noxious stimulation (e.g. Johnson *et al.* 2005b, 2009; Kongara *et al.* 2010). Notably, at 12 days-of-age there was no significant increase in F50 after docking, although P_{TOT} decreased. This was unexpected, given that F50 increased after docking in 10 and 15 day-old pigs in the present study, and in 20 day-old pigs in a previous study (Kells *et al*. 2013). The lack of a significant increase may have been due to the high degree of individual variation among this age group.

Seven day-old pigs exhibited a reduction in F50 in response to docking, whilst 10-day-olds exhibited a brief reduction prior to a sustained increase. A reduction in F50 represents an atypical response to noxious stimulation, which has previously been reported in 2-day-old pigs following tail docking (Kells *et al*. 2013). Whilst nociception typically elicits EEG desynchronisation, characterised by a shift toward lower amplitude, higher frequency activity (Otto 2008) with corresponding increases in F50 and F95 (Johnson *et al.* 2012), paradoxical arousal, or synchronisation, characterised by a shift toward higher amplitude, lower frequency activity and corresponding decreases in F50 and F95, has also been reported. In a study of isoflurane-anaesthetised sheep undergoing orthopaedic surgery, both synchronisation and desynchronisation of the EEG were observed, with responses differing according to depth of anaesthesia and stimulation intensity (Otto & Mally 2003). In a study of EEG responses to skin incision in anaesthetised people, adult patients demonstrated desynchronisation following skin incision, whereas EEG synchronisation predominated in infants and young children, suggesting this may be an age-dependent effect (Oshima *et al.* 1981).

Age-related differences in anaesthetic requirements have previously been identified in human patients. The inspired concentration of inhalational agent required to maintain anaesthesia is up to four times higher in paediatric patients than in older adults (Gregory *et al.* 1969; Nickalls & Mapleson 2003). If such an effect were present in pigs across the relatively small age range examined in this study, it might be expected that younger pigs would have higher anaesthetic requirements than older pigs and maintaining end-tidal halothane at a constant concentration across age groups may have resulted in lighter anaesthesia in

younger pigs. If this were the case, we would have expected to see lower amplitude and higher frequency of the EEG, resulting in lower baseline P_{TOT} and higher baseline F50 and F95 in younger pigs. However, baseline EEG data do not support such an effect across the age range examined. Total EEG power did not differ between age groups and, whilst baseline F50 did vary between 1 day and 10–15 day old pigs, it was lower in the 1 day-olds, thus not indicative of a reduced state of anaesthesia.

Although there were few statistically significant differences between age groups in EEG responses to tail docking over time, there were some qualitative differences in the pattern of responses, which may be of biological significance. In terms of F50, two different response patterns emerged: Pigs aged 5, 7 and 10 days old exhibited a decrease in mean F50 relative to baseline (Time 0) immediately after docking, followed by a subsequent increase back to or above baseline mean, peaking 45–60 seconds after docking (Figure 4.5). However, pigs aged 12 and 15 days differed, in that no initial decrease but a similarly timed increase in mean F50 was observed. In terms of F95, pigs aged 10 and 15 days exhibited an increase in F95, peaking 30 seconds after docking, whereas all other ages showed little change (Figure 4.5). P_{TOT} decreased relative to baseline to varying degrees after docking in all age groups, with the duration of this appearing to be more prolonged in pigs aged 10 days and older (Figure 4.5). Thus, overall EEG responsiveness to tail docking appeared to increase with increasing age, with pigs aged 10 days and over exhibiting characteristic nociceptive response patterns.

Pooling of data into two age blocks (≤7 and >7 days-of-age) revealed significant differences in EEG responses to docking between age groups. Overall, the older group exhibited heightened responses to tail docking compared with the younger group. An increase in F95 was observed in the older group only, and although P_{TOT} decreased after docking in both groups, the magnitude and duration of the decrease was greater in pigs >7 days-of-age. Changes in the responsiveness of the cerebral cortex to noxious stimulation are thought to reflect changes in the degree to which noxious stimuli are perceived (Johnson *et al*. 2009). Therefore, the greater magnitude of EEG responses observed in the older animals suggest that tail docking may be perceived as more noxious to pigs aged >7 days compared with those aged ≤7 days. This finding is important, given current recommendations regarding the age at which husbandry procedures such as tail docking should be performed, along with requirements for provision of analgesia. These data provide support for New Zealand and Australian recommendations that tail docking be performed within the first week of life (CSIRO 2008; Anonymous 2010) and for UK policy requiring the use of anaesthesia and analgesia when performed beyond 7 days of age (Council of the European Union 2008).

It is possible that the increasing EEG responsiveness to docking with age related to differences in the magnitude of the noxious stimulus. Differences in the magnitude of noxious stimulation may occur due to variations in the size and type of tissue being transected (Johnson *et al*. 2005a). Unfortunately, tail diameter was not measured in the present study; therefore, it was not possible to determine whether tail size correlated with the magnitude of EEG responses. Whilst it is possible that tail size may have influenced the magnitude of responses, this would not explain the absence of nociceptive responses observed in very young pigs, therefore other factors must also be at play

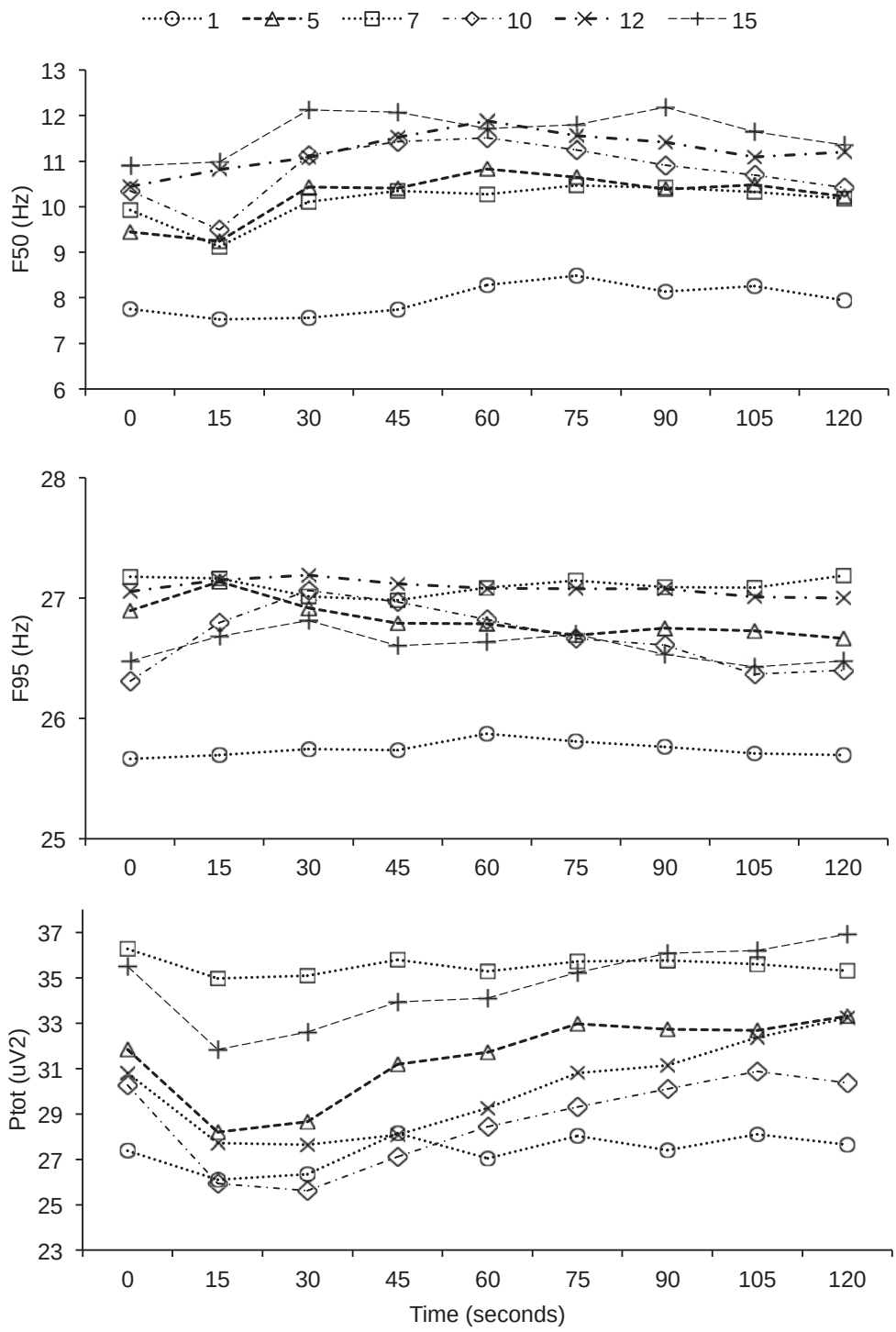

Figure 4.5 *Qualitative comparison of the changes in mean F50, F95 and P_{TOT} of the EEG following tail docking (time 0) in pigs aged 1, 5, 7 10, 12 and 15 days-of-age. For ease of distinguishing between ages, non-transformed data are presented and standard errors have been omitted.*

.

The findings of this study are consistent with other animal studies that have identified age-related differences in cortical responses to noxious stimuli. A recent study of noxious-evoked EEG activity in rats aged 21 or 40 postnatal days identified age-specific changes in the frequency spectrum of the EEG recorded from the rat primary somatosensory cortex (Devonshire *et al.* 2015). Whilst post-stimulus total EEG power did not differ between 21 and 40 postnatal days of age, the authors identified an increase in theta power (4–8 Hz), which correlates with F50, in the older rats only. The authors concluded the differences were due to alterations in the cortical processing of nociceptive inputs as a result of postnatal maturation of the cerebral cortex. Although the rats used in the study were older than the pigs in the present study, the neurological immaturity of rats at birth may explain the absence of an increase in theta power at 21 postnatal days. It is well known that cortical development continues postnatally in humans, with maturation not complete until adolescence. Importantly, one third of the total cortical development over the first six years of life takes place within six weeks of birth, in response to both intrinsic and sensory-driven neuronal inputs (Shankle *et al.* 1998). In pigs, the most rapid neural development occurs between 50 days prenatal and 40 days postnatal (Dickerson & Dobbing 1967).

An earlier study investigating the effects of postnatal age on EEG responses to castration in lambs over the first six weeks of life identified an increase in cerebral responsiveness to noxious stimulation over the period 7–10 days (Johnson *et al.* 2009). In this case, the authors concluded that the lingering effects of fetal neurosuppressive mechanisms might have been responsible for the lesser responsiveness in younger lambs. In mammals, several circulating factors act to maintain the fetus in a permanent sleep-like state, and are thought to be responsible for the observed absence of fetal responses to noxious and nociceptive stimuli (Mellor *et al.* 2005). In sheep, plasma concentrations of the neurosuppressive agents pregnanolone and allopregnanolone were found to be significant up to 3 days after birth (Nguyen *et al.* 2003), leading to the suggestion that these chemicals may continue to exert some cerebral effects in the early postnatal period (Mellor & Diesch 2006). Whether significant concentrations of these chemicals are present in the plasma of pigs at birth has not been investigated.

In the present study, the lack of nociceptive response at 1 day postnatal supports the presence of neurosuppressive mechanisms acting to inhibit cerebral processing of nociceptive stimuli. The increasing cortical responsiveness observed over the period 5–15 postnatal days might be explained, in part, by the withdrawal of these neurosuppressive mechanisms. In addition, it is likely that postnatal maturation of pain processing pathways, including cortical components, contributed to the observed increase in responsiveness. Thus, the observed pattern of increasing cerebral responsiveness to nociception with increasing postnatal age may be a result of postnatal cortical development, combined with the gradual withdrawal of neurosuppressive mechanisms.

Whilst only male pigs were examined in the current study, the finding that sex did not influence pig responses to tail docking or analgesia in a previous study (Chapter 3) suggests that the results may be equally applicable to females.

In conclusion, we identified an increase in cerebral responsiveness to the noxious stimulus of tail docking with increasing postnatal age in pigs. This may be due to both the persistence of fetal neurosuppressive mechanisms in the first days of postnatal life, along with rapid cerebrocortical development after birth.

4.6 ANIMAL WELFARE IMPLICATIONS

Whilst the absence of a nociceptive response to tail docking at 1 day-of-age suggests that it may be preferential to carry out painful procedures as soon as possible after birth, information regarding the long-term consequences of noxious stimulation in the neonatal pig must be considered before any recommendations are made. In humans, noxious stimulation without analgesia in the very early postnatal period has been associated with increased reactivity to later painful stimuli (Taddio *et al.* 1997; Grunau 2013), which can persist for months (Taddio *et al.* 1997) or years (Buskila *et al.* 2003). A similar phenomenon was observed in lambs, whereby those castrated at 1 day-of-age exhibited greater behavioural responses to subsequent tail docking than did those castrated at 10 days-of-age (McCracken *et al.* 2010). In addition, lambs castrated without analgesia at 1 day old exhibited more pronounced EEG responses to tail docking 3 weeks later than those handled only at 1 day old (Impey 2015). The presence and extent of any such phenomenon in pigs should be investigated.

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STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Nicola Jean Kells

Name/Title of Principal Supervisor: Dr Ngaio Beausoleil

Name of Published Research Output and full reference:

Kells NJ, Beausoleil NJ, Sutherland MA, Johnson CB. Postnatal development of EEG responses to noxious stimulation in pigs (Sus scrofa) aged 1–15 days. Submitted to Animal Welfare, 07 December 2016

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Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Nikki had a primary role in study design, data collection, statistical analysis, interpretation and writing of the paper, with guidance from supervisors.

Nikki Kells Digitally signed by Nikki Kells Date: 2017.05.12 09:14:30 +12'00'
Candidate's Signature

12/05/2017
Date

Ngaio Beausoleil Digitally signed by Ngaio Beausoleil Date: 2017.05.15 11:44:27 +12'00'
Principal Supervisor's signature

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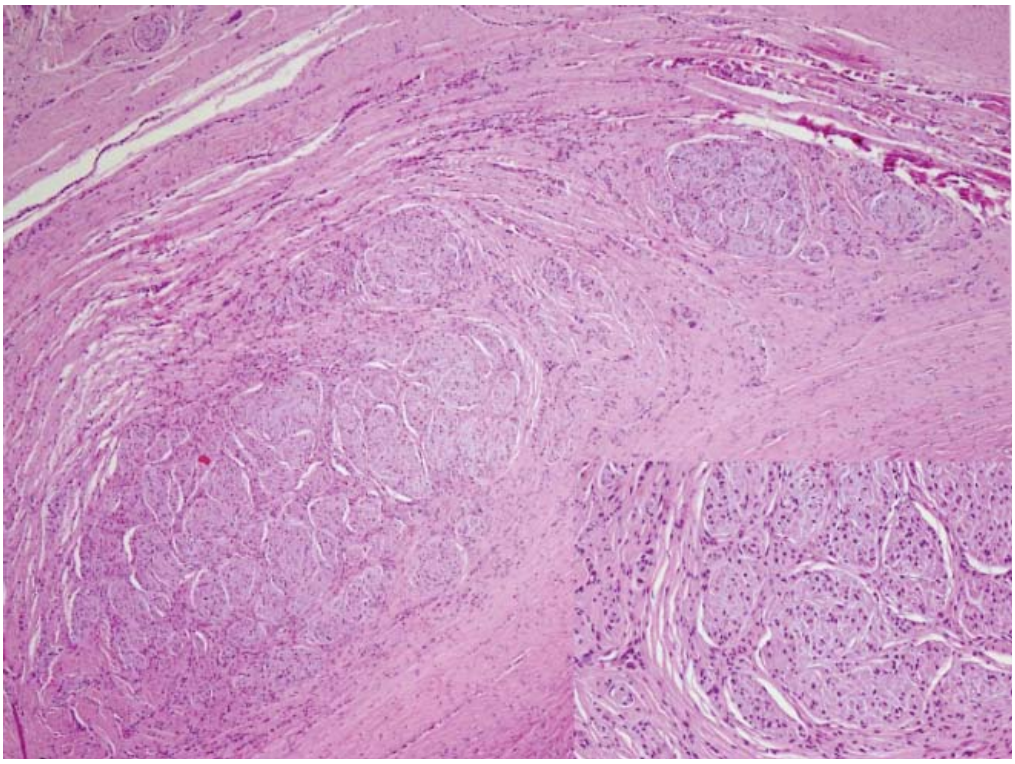
CHAPTER 5 Comparison of neural histomorphology in tail tips from pigs (*Sus scrofa*) docked using clippers or cautery iron



Kells NJ, Beausoleil NJ, Johnson CB, Morrison RS, Roe W. Comparison of neural histomorphology in tail tips from pigs docked using clippers or cautery iron. Accepted for publication in *Animal* 23 September 2016; DOI: http://dx.doi.org/10.1017/S1751731116002500

5.1 ABSTRACT

Tail docking of pigs is commonly performed to reduce the incidence of unwanted tail biting behaviour. Two docking methods are commonly used: blunt trauma cutting (i.e. using side-clippers), or cutting and concurrent cauterisation using a hot cautery iron. A potential consequence of tail amputation is the development of neuromas at the docking site. Neuromas have been linked to neuropathic pain, which can influence the longer-term welfare of affected individuals. To determine whether method of tail docking influences the extent of neuroma formation, 75 pigs were allocated to one of three treatments at birth: tail docked using clippers; tail docked using cautery iron; tail left intact. Tail docking was performed at 2 days-of-age and pigs were kept under conventional conditions until slaughter at 21 weeks-of-age. Tails were removed following slaughter and subjected to histological examination. Nerve histomorphology was scored according to the following scale: 1 = discrete well organised nerve bundles; 2 = moderate neural proliferation and disorganisation affecting more than half of the circumference of the tail; 3 = marked neural proliferation to form almost continuous disorganised bundles or non-continuous enlarged bundles compressing the surrounding connective tissue. Scores of 2 or 3 indicated neuroma formation. Scores were higher in docked pigs than undocked pigs ($P<0.001$), but did not differ between pigs docked using clippers and those docked using cautery ($P=0.23$). The results indicate that tail docking using either clippers or cautery results in neuroma formation, thus having the potential to affect long-term pig welfare.

5.2 IMPLICATIONS

Tail docking may have both short and long term welfare consequences for pigs. In addition to the short-term pain and distress caused by docking itself, long-term changes in pain processing may arise through abnormal nerve proliferation at the docking site. The present study found evidence of abnormal nerve proliferation in the tail stumps of pigs docked using both clippers and cautery iron, indicating the potential for long-term pain consequences with either method. However, as the degree of abnormal nerve proliferation did not differ between docking methods, no evidence for a longer-term welfare advantage of one method over the other was found.

5.3 INTRODUCTION

Tail biting, involving the destructive chewing of pen-mates' tails, has been a recognised problem among commercially farmed pigs for the past century (Dougherty 1976; EFSA 2007). It is an abnormal behaviour, the occurrence of which is thought to indicate reduced welfare amongst some or all pigs in an affected pen (Schrøder-Petersen and Simonsen 2001; EFSA 2007; Stafford, 2010). Tail-bitten pigs may experience severe welfare compromise because of tissue damage, haemorrhage, infection, abscesses and septicaemia, which, in severe cases, may lead to death (Schrøder-Petersen and Simonsen 2001). Whilst management factors such as reduced stocking density and the provision of rooting material have been advocated to reduce tail-biting incidence (EFSA 2007; Sonoda *et al.* 2012; Taylor *et al.* 2012), tail docking remains the most widely adopted on-farm preventive strategy (EFSA 2007; Nannoni, 2014).

Tail docking involves amputating some portion of the distal end of the tail, typically by severing with side clippers, or using a heated cautery iron to cut and cauterise in a single step (Sutherland and Tucker 2011). Whilst there is evidence that tail docking reduces the frequency of tail biting among intensively farmed pigs

(Hunter *et al.* 2001; Sutherland *et al.* 2009), the mechanisms underlying this phenomenon are not clear. Although the reduced length likely impairs the ability of pigs to grasp and manipulate the remaining tail, this probably does not sufficiently explain why tail-biting lesions are less common in 'long' docked pigs (tip only or distal third removed) than undocked pigs (Hunter *et al.* 1999; Sutherland *et al.* 2009; Scollo *et al.* 2015). It has been hypothesised that neuroanatomical changes in the tip of the docked tail result in hypersensitivity of the tail stump, stimulating protective behaviour toward this area (Simonsen *et al.* 1991), although this has yet to be confirmed.

Following nerve transection, the proximal nerve stump seals off, forming a terminal swelling. Numerous fine projections (sprouts) arise from this, and attempt to connect with peripheral target tissues. When reconnection is prevented, as is the case with amputation, terminal swellings persist and nerve sprouts turn back on themselves forming tangled masses, or neuromas (Devor and Seltzer 1999), exacerbated by excessive fibrous tissue proliferation in the region (Foltán *et al.* 2008).

In humans, neural lesions such as neuromas have been associated with neuropathic pain following limb amputation and peripheral nerve injury (Lewin-Kowalik *et al.* 2006). Neuropathic pain is evoked through alterations in the structure and function of the somatosensory nervous system such that pain may occur spontaneously and responses to both noxious and innocuous stimuli are pathologically amplified (Costigan *et al.* 2009). However, whilst neural lesions are necessary precursors for neuropathic pain, not all neuromas are associated with alterations in pain perception (Costigan *et al.* 2009). In the human clinical literature, the reported prevalence of symptomatic (painful) neuromas subsequent to digital amputations of the hand is relatively low, ranging from 2.7 to 7.8% (van der Avoort *et al.* 1983; Fisher and Boswick 1983; Sullivan 1985)

Terminal neuromas and irregular innervation of the tail stump have been reported in lambs (French and Morgan 1992) and pigs (Simonsen *et al.* 1991; Herskin *et al.* 2015) following tail docking and in chickens following partial beak amputation (Breward and Gentle 1985). In addition, both clinical and experimentally induced neuromas have been linked to neuropathic pain in animals (Reviewed by Zimmermann 2001). For example, abnormal spontaneous activity was recorded in afferent neurons in the region of neuroma formation following partial de-beaking in chickens and was accompanied by heightened thermal sensitivity and behavioural signs of persistent pain up to six weeks after amputation (Breward and Gentle 1985). Likewise, Gross and Carr (1990) identified neuromas in the tail stump of six tail-docked dogs with histories of persistent autotomy, behaviour considered indicative of chronic pain (Zimmermann 2001). Thus, the presence of neuromas after amputation has the potential to cause neuropathic pain and impact detrimentally on the longer-term welfare of animals.

Whilst neuromas have previously been identified in the healed tail tips of pigs docked using both blunt trauma (Simonsen *et al.* 1991; Done *et al.* 2003) and cautery iron (Herskin *et al.* 2014), the incidence of neuroma formation has not previously been compared between these methods. The aim of the present study was to compare neuroma development in healed tail tips from pigs docked using clippers and cautery iron, to determine whether docking method influenced neuroma formation.

5.4 MATERIALS AND METHODS

Animals and housing

All experimental procedures were approved by the Rivalea Australia Animal Ethics Committee (protocol number 13B068C). The experiment was conducted at the Rivalea Australia Research and Innovation Unit, Corowa NSW, Australia between March and September 2014. The animals used in the study were part of a larger cohort also used to investigate mortality and tail damage. The cohort consisted of forty healthy, viable sows (Large White x Landrace) that had the same farrowing date and their litters. The sows farrowed in individual farrowing crates. Three healthy, entire male piglets per litter were randomly assigned to one of three treatments, performed at 2 days-of-age: no tail docking (CONTROL); tail docking using clippers (CLIP); tail docking using cautery iron (CAUT). Allocation of pigs within a litter to each treatment was based upon computer-generated random selection, following confirmation of good health. Pigs were mixed at weaning (i.e. not kept in groups of similar tail length) and remained in a conventional (semi-slatted) housing system until market. Pig health and welfare was monitored daily. Pigs identified as having superficial wounds in a localised area, minimal bleeding and no evidence of bleeding or infection received first aid and were placed in a hospital pen prior to market. Pigs showing signs of severe infection, or with extensive tail damage, were euthanased (as per the Australian Pesticides and Veterinary Medicines Authority guidelines). The total number of piglet deaths and removals per treatment, along with causes, are shown in Table 5.1. There were no significant differences between treatments in the number of piglet deaths and piglet removals due to illness or injury (χ^2 =0.09; *P* =0.95). Pigs were slaughtered at commercial premises at 21 weeks-of-age and their tails/tail stumps harvested for subsequent analysis.

Table 5.1 *Number of pig deaths and removals due to illness or injury among pigs with intact tails (CONTROL, n=40) and those tail-docked using clippers (CLIP, n=40) or cautery iron (CAUT, n=40) from the full cohort of 120 pigs used in the wider study.*

Cause of death/removal	CONTROL	CLIP	CAUT
Overlain by sow	5	1	4
Scours	1	1	3
Unthrifty	1	5	1
Tail bite (euthanasia)	2	0	0
Other	1	2	1
Total (%)	10/40 (25%)	9/40 (23%)	9/40 (23%)

Experimental procedures

Tail docking

Piglets were quietly picked up from their home pen and were held, supported under the arm of the technician with their hind area exposed. Piglets in the CONTROL treatment were held in the same manner

for approximately 30 seconds and returned to their pen. Pigs in the CLIP treatment had their tails docked using clean, sanitised side clipper pliers (Bahco 2101G side cutter pliers, SNA Europe). Pigs in the CAUT treatment had their tails docked using a clean, sanitised gas operated cautery iron (Stericut® Tail Docker, Cotran Corporation, Portsmouth RI, USA). In both docking treatments, the tail was cut approximately 2 cm from the base, in between the first and second visible external vertebrae, and a chlorhexidine disinfectant spray was applied immediately.

Collection and preparation of tail tissue

A random sample of 20 tail tips from CLIP docked pigs were collected at the time of tail docking (2 days-of-age) and fixed in 10% neutral buffered formalin (NBF) until the conclusion of the experiment, at which time all pigs were slaughtered. Following slaughter, tails/tail stumps from 25 pigs per treatment were cut off at the root and individually fixed in 10% NBF for seven days. All samples were then removed, wrapped in formalin-soaked towels and sealed in individual plastic bags for transport to the laboratory. Upon receipt, samples were unpacked and submerged in 10% NBF for storage prior to processing within the following four weeks.

Histology and immunohistochemistry

Preparation and staining of formalin-fixed samples was undertaken at Massey University, New Zealand. Prior to sectioning, all tail samples were examined for gross evidence of trauma that may be indicative of tail biting (tail lesions, scabs or scarring), and the tail length (mm) was measured and recorded. All tails collected at slaughter were cross-sectioned 5 mm from the distal tip, whilst control (undocked) tails were also cross-sectioned at a second location, 92 mm from the tail base (Figure 1). The average length of docked tails at the time of slaughter was 92 mm, thus cross sections were prepared at this level in control tails to visualise normal tail neuroanatomy in the region equivalent to the site of amputation of docked tails.

All tail sections were decalcified and dekeratinised in potassium hydroxide and Veet® (Reckitt Benckiser, Berkshire, UK) according to standard laboratory protocol, then processed through graded alcohols and xylene before being embedded in paraffin. The formalin-fixed, paraffin-embedded tissues were sectioned at 4–5 µm and stained with haematoxylin and eosin (H&E).

Where required, additional sections were stained with Masson's trichrome and S-100 to further optimise nerve visualisation and confirm neuroma presence. Tissue sections were deparaffinised, blocked for endogenous peroxidase, and treated with primary antibodies towards S-100 (polyclonal rabbit anti-S-100; FLEX Anti-S100, Dako) as described by Nielsen *et al.* (2011).

Tail sections were examined under light microscope (Olympus BX51) at 4, 20 and 40X magnification. Two pathologists, both blinded to treatment, examined sections independently using the same equipment, and assigned scores of 1, 2, or 3 as follows: 1 = discrete well organised nerve bundles (normal morphology); 2 = moderate proliferation and disorganisation within fibrous connective tissue, affecting less than half the circumference of the tail; 3 = marked proliferation to form almost continuous disorganised bundles or non-continuous enlarged bundles compressing the surrounding, densely fibrous, connective tissue. The presence of disorderly, proliferative nerve bundles surrounded by fibrous connective tissue (i.e. scores of 2 or 3) indicated neuroma formation.

Following initial independent examination of tail sections, scores were compared between pathologists. Where discrepancies were identified, both scorers re-examined the slides in question together and arrived at a consensus as to the appropriate classification prior to statistical analysis.

Statistical analyses

All statistical analyses were performed using SAS version 9.3.1 (SAS Institute Inc., Cary NC, USA, 2012).

Inter-rater reliability of scores (based on initial independent scores) was determined using Cohen's Kappa coefficient.

Non-parametric one-way analysis of variance (Kruskal-Wallis test) was carried out to compare neuroanatomy scores between treatments. Where a significant treatment effect was found, post-hoc pairwise comparison of mean scores was performed using the Wilcoxon-Mann Whitney rank-sum test. In addition, Fisher's exact test was performed to determine whether the distribution of scores differed from expected.

5.5 RESULTS

Descriptive neuroanatomy

Tail tips collected at time of docking (2 days-of-age).

Cross sections from twenty randomly selected tail tips from pigs in the CLIP treatment were examined. Discrete, well-organised nerve bundles were observed in all tail sections, providing evidence for normal innervation at the site of amputation at the time of docking.

Tails harvested at slaughter (21 weeks-of-age).

Although 25 tails per treatment were targeted, difficulties with sample adhesion meant that histological scoring could only be performed on a total of 15 control (undocked), 19 clipper-docked and 18 cautery-docked pigs (N =52). In some cases, poor tissue adhesion meant that only part of the sample could be examined. Samples were only scored where the entirety of the cross section could be visualised.

No abnormal nerve proliferation was observed in sections from the distal portion (section *x*, Figure 5.1) of control tails (Table 5.2; Figure 5.2). Gross evidence of tail tip trauma (lesions, scabs or scarring) was observed in 5/15 control tails, with corresponding evidence of inflammation and/or scarring apparent on histological analysis. Neural histomorphology at the site approximating the point of tail docking (section *y,* Figure 5.1) was unremarkable, with discrete nerve fibres present and no signs of neural proliferation observed.

Table 5.2 *Summary of nerve histomorphology scores from cross sections from the distal tail tip of undocked (CONTROL) clipper-docked (CLIP) and cautery-docked (CAUT) pigs. Where score of 1 = discrete well organised nerve bundles (normal morphology); 2 = moderate proliferation and disorganisation within fibrous connective tissue, affecting less than half the circumference of the tail; 3 = marked proliferation to form almost continuous disorganised bundles OR non-continuous enlarged bundles compressing the surrounding densely fibrous connective tissue. Scores of 2 or 3 indicate neuroma formation.*

Score	CONTROL	CLIP	CAUT
1	15	2	1
2	0	9	14
3	0	8	3
n	15	19	18

The average length of all docked tails (*N* =50) collected at slaughter was 92 (range 75–140) mm. Evidence of abnormal neural proliferation consistent with neuroma formation (i.e. score 2 or 3) was observed in 17/19 tails from pigs docked using clippers and 17/18 tails from pigs docked using cautery iron (Table 5.2; Figures 5.3 and 5.4).

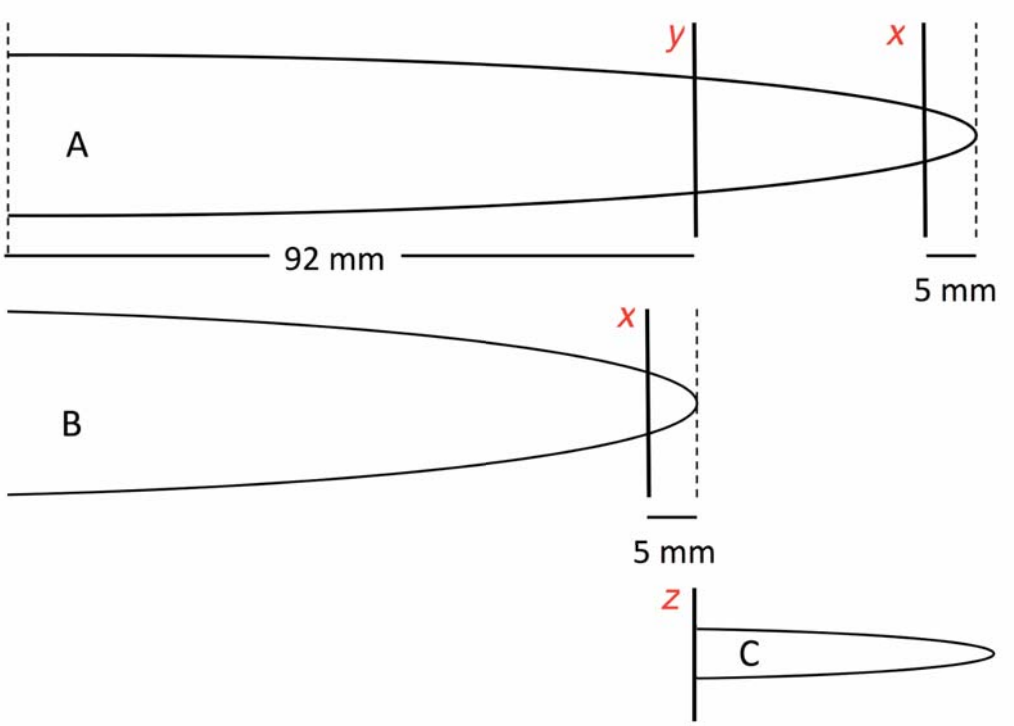


Figure 5.1 *Schematic diagram indicating sampling sites for histological analysis. Undocked tails (A) and docked tail stumps (B) collected at slaughter were cross-sectioned 5 mm from the distal tip (x). Undocked tails were additionally cross-sectioned 92 mm from the root (y), representing the mean length of all docked tails. Tail tips collected from 2-day-old pigs at the time of docking (C) were cross-sectioned at the docking site (z).*

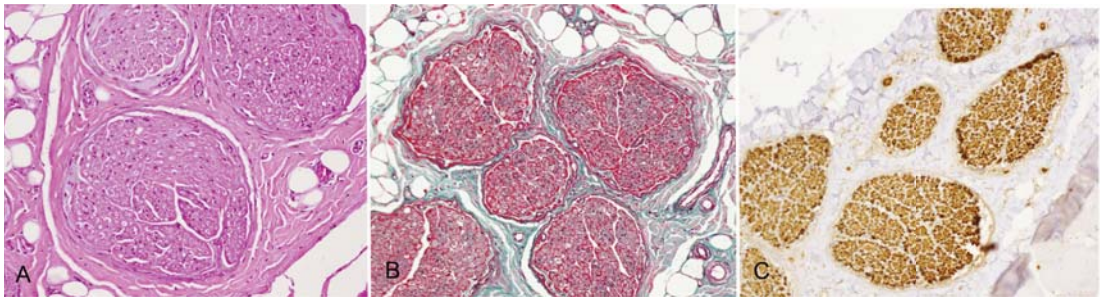


Figure 5.2 *Control (undocked) pig tail sectioned at level equivalent to docking site, showing nerve histomorphology score=1. A: H&E; B: Masson's trichrome; C: S100 (all 20X magnification).*

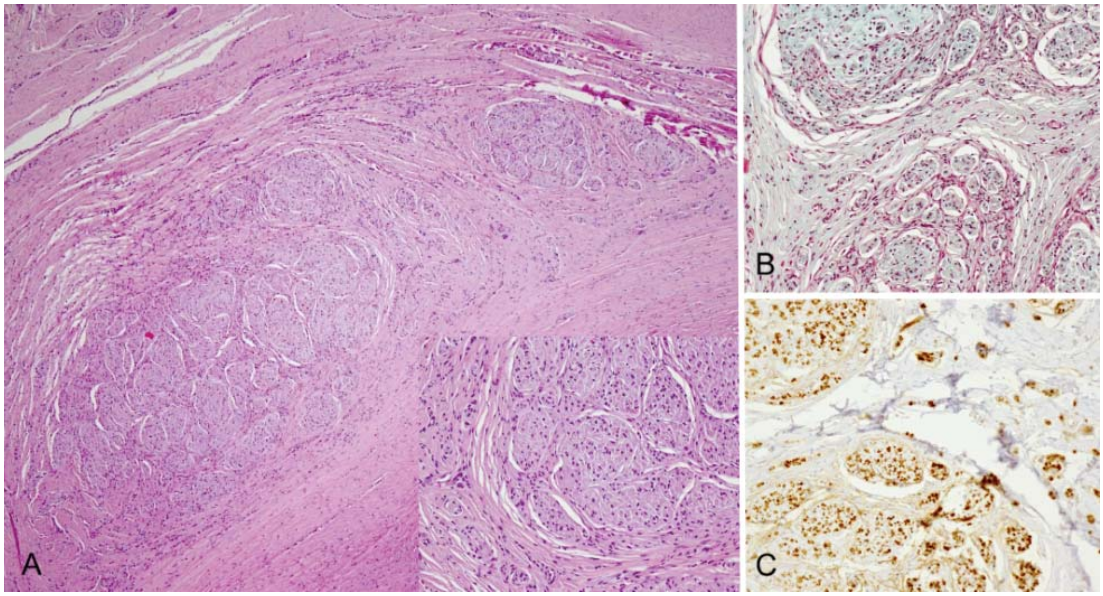


Figure 5.3 *Cross section 5 mm from the tip of a docked pig tail, showing nerve histomorphology score=2. A: moderately disorganised proliferative nerve bundles of varying sizes, surrounded by thin, frequently loosely arranged layers of fibrous connective tissue. H&E, 4X magnification. Inset: 20X magnification at same site. B: Masson's trichrome (magnification 20X). C: Small to medium nerve fibres stained brown with S100 (magnification 20X).*

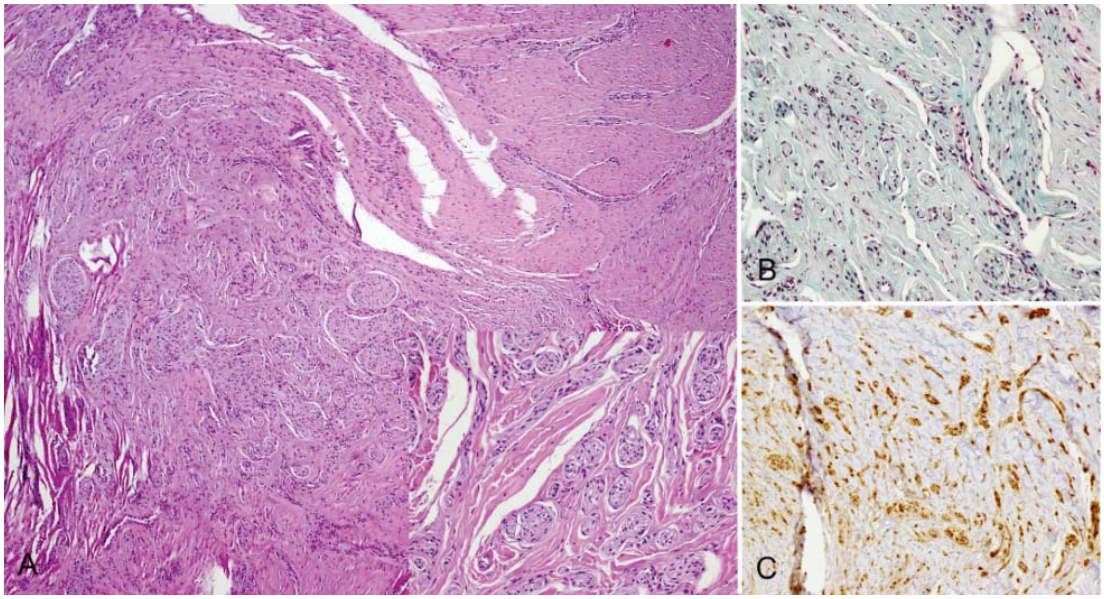


Figure 5.4 *Cross section 5 mm from the tip of a docked pig tail, showing nerve histomorphology score=3. A: Poorly organised highly proliferative nerve bundles, often within dense fibrous connective tissue. H&E (magnification 4X). Inset: 20X magnification at same site. B: Note numerous small proliferating nerves within connective tissue. Masson's trichrome (magnification 20X). C: Large numbers of small, disorganised, immunopositive nerve fibres. S100 (magnification 20X).*

Inter-rater reliability

Initial independent examination of tissue sections revealed 83% agreement in the assigned scores. Inter-rater reliability was substantial (Cohen's Kappa coefficient = 0.73; 95%CI 0.58–0.89). Following consultation and re-examination, consensus scores were reached for all samples prior to statistical comparisons being performed.

Comparison of neural histomorphology

Kruskal-Wallis analysis revealed a significant effect of treatment on neuroanatomy scores (χ^2 =31.25, *P* <0.001). Post-hoc tests revealed that mean neural histomorphology scores for both the CLIP and CAUT groups (Wilcoxon rank means = 35.0 and 31.7, respectively) were higher than those in the CONTROL group (rank mean = 9.5) (*P* <0.001). Whilst the percentage of tails scored as 3 (marked neural proliferation) was higher in pigs docked using clippers than those docked using cautery (42 *vs.* 17%, respectively), mean nerve histomorphology score did not differ between the two docking methods (*P* =0.23). Fisher's exact test likewise revealed no difference in the distribution of histomorphology scores between CAUT and CLIP docked pigs (*P* =0.16).

5.6 DISCUSSION

The aim of the present study was to compare the neural histomorphology in healed tail tips of pigs docked using clippers and cautery iron, to determine whether docking method influenced the extent of neuroma formation.

Due to the often marked and haphazard degree of neural proliferation observed in the tips of docked tails, the number and/or size of individual neuromas could not be determined, as it was not clear whether large regions of proliferation resulted from the regeneration of single or multiple severed nerves. Therefore, a unique descriptive scale was developed and used to compare neural morphology.

Evidence of neuroma formation, including the presence of disorganised excessive nerve proliferation within moderate to dense connective tissue, was observed in almost all tails docked using both clippers and cautery iron, whereas no evidence of neuromas was found in tail tips from control, undocked pigs, despite gross evidence of trauma due to tail biting in 5/15 tails. The incidence of neuroma formation in tails docked using hot cautery in the present study (94%) was higher than that reported by Herskin *et al*. (64%; 2015). This may, in part, be attributable to the fact that in the latter study pig tails were halved midsagitally prior to cross sectioning one half, whereas the present study examined cross sections of the entire tail.

Given the reported associations between neuroma formation and the occurrence of neuropathic pain (Zimmermann 2001; Lewin-Kowalik *et al.* 2006), the presence of neuromas in almost all the tail stumps of pigs docked using clippers and cautery iron suggests that tail docking by either method has the potential to induce longer-term alterations in pain processing and attendant welfare compromise in pigs. This does however need to be confirmed before any robust conclusions about pig welfare can be drawn. This might be achieved, for example, by assessing thermal and mechanical nociceptive thresholds in the tail tip over the lifetime in commercial pigs with intact tails and those docked using clippers or cautery.

The present study indicates that there was no difference in the incidence of tail-tip neuroma formation between clipper- and cautery-docked pigs, suggesting that the longer-term pain consequences of tail docking using clippers and cautery may be similar. In their study of the anatomical and pathological consequences of tail docking using scissors or a turkey cautery de-beaker, Done *et al.* (2003) reported evidence of tail tip neuroma formation in 100% of docked pig tails at 4–4.5 months, with undocked control pigs showing no evidence of neuromas.

Previous studies have indicated that hot cautery may induce less acute pain than blunt trauma docking methods. Both Sutherland *et al.* (2008) and Morrison *et al.* (2013) reported higher plasma cortisol concentrations in pigs docked using blunt trauma compared to those docked using cautery in the period immediately following docking. Further, pigs' tail-docked using cautery exhibited less electroencephalographic evidence of nociception than those docked using clippers (Kells *et al.* 2013). A comparison between control (undocked), clip- and cautery-docked pigs revealed no difference in tail-biting lesion scores between clipper and cautery docked pigs over the seven weeks following docking (Sutherland *et al.* 2009), suggesting the two methods do not differ in their ability to prevent tail biting. Therefore, if it can be confirmed that the longer-term welfare consequences do not differ between the two docking methods, the smaller acute pain responses previously identified may be cause for recommending the use of cautery over blunt trauma for tail docking pigs.

It must also be noted that the relatively small sample size examined (*n* =18 or 19 per group) may have reduced the ability of this study to discriminate between docking methods. In addition, the inclusion of male pigs only meant that any effects of sex could not be determined.

5.7 CONCLUSION

In conclusion, tail docking of pigs using either side clippers or cautery iron resulted in the formation of neuromas in the healed tail tip. The degree of abnormal nerve regeneration in the tail tip did not differ between docking methods, providing no evidence for a longer-term welfare advantage of one method over the other, at least in terms of the potential for alterations in pain processing.

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MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Nicola Jean Kells
Name/Title of Principal Supervisor: Dr Ngaio Beausoleil

Name of Published Research Output and full reference:

Kells NJ, Beausoleil NJ, Johnson CB, Morrison RS, Roe W. Comparison of neural histomorphology in tail tips from pigs docked using clippers or cautery iron. Accepted for publication in Animal 23 September 2016.

In which Chapter is the Published Work: Chapter 5

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Nikki Kells designed the study, determined the sampling regime, and collaborated with Dr Roe on sample staining and scoring, which was subsequently undertaken by expert histopathologists. Nikki analyzed and interpreted the resulting data, wrote the first draft of the paper and revised the paper after editorial inputs from co-authors and referees.

Digitally signed by Nikki J Kells
Nikki J Kells Date: 2016.11.17 11:57:51 +13'00'

Candidate's Signature — Date: 17/11/2016

Ngaio Beausoleil Digitally signed by Ngaio Beausoleil Date: 2016.11.29 23:53:24 +13'00'

Principal Supervisor's signature — Date: 29/11/2016

CHAPTER 6 General Discussion

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Despite associations being found between a number of environmental and management factors and the risk of tail biting behaviour, tail docking remains the most widely adopted strategy for its prevention among commercially farmed pigs (Nannoni *et al*. 2014). Thus far, the 2003 introduction of legislation in the European Union banning the routine use of tail docking as a preventive measure (EU Directive 2001/93/EC amending Directive 91/630/EEC, now codified in Council Directive 2008/120/EC) has had little impact on the rate of tail docking in this region (EFSA 2007; Harley *et al*. 2012). It has been proposed that this is due to failure of the legislation to specify in sufficient detail the steps that producers are required to take before turning to tail docking as a last resort (D'Eath *et al*. 2016). It is likely that similar reasons lie behind the continued performance of tail docking in other regions in which its routine use is discouraged.

In time, given increasing public awareness of animal welfare and increasing consumer demand for high welfare across all aspects of animal production, it is possible that tail docking as a routine measure will be phased out of commercial pig production. However, tail docking is likely to continue to be employed as a means of reducing tail biting behaviour in instances where alternatives (such as changes in housing, environment or provision of rooting substrates) are ineffective, or where serious tail-biting outbreaks occur. As such, it is important to identify the best practice for performing docking in terms of minimising any short- or long-term animal welfare compromise.

In this body of work, I undertook to extend the existing knowledge regarding the potential for acute and longer-term pain as a consequence of tail docking, with respect to docking method, age at the time of docking, and provision of pain-mitigation strategies.

6.1 MAJOR FINDINGS AND FUTURE RESEARCH

The following summarises the major findings of each individual study, highlighting any limitations and proposing future research extensions.

Use of side cutter pliers versus hot cautery iron

Docking using either side cutter pliers or hot cautery iron induced transient changes in the EEG consistent with nociception in 20 day-old pigs, indicating that docking by either method likely results in perceived pain when performed on conscious pigs of this age. This finding is consistent with previous behavioural and physiological studies, which identified increases in vocalisation (Noonan *et al.* 1994; Marchant-Forde *et al.* 2009; Torrey *et al.* 2009), escape attempts and pain-related behaviour (Noonan *et al.* 1994; Sutherland *et al.* 2008; Marchant-Forde *et al.* 2009; Torrey *et al.* 2009), and plasma cortisol concentrations (Sutherland *et al.* 2008; Morrison *et al.* 2013) in pigs tail docked using either method.

However, I found evidence of a quantitatively smaller EEG response to docking using cautery iron than side cutter pliers in 20-day-old pigs (Chapters 2 and 3), suggesting that conscious pigs may experience less acute pain when docked using this method. Again, this is consistent with other studies which identified smaller increases in plasma cortisol in 2 or 6 day-old pigs docked using cautery compared to those docked using clippers at 30 or 60 min after processing (Sutherland *et al.* 2008; Morrison *et al.* 2013). In contrast, Marchant-Forde *et al.* (2009) found no differences in plasma cortisol or ß-endorphin concentrations between clipper and cautery docked pigs aged 2, 3 and 8 days at 45 min, 4 h, 48 h, 1 week or 2 weeks after processing. This discrepancy may have been due to differences in the frequency of sampling between studies meaning transient changes may not have been detected.

Tail docking using cautery iron appears to be less acutely painful to pigs than tail docking using clippers; however further study is required to determine whether there are differences in long-term welfare outcomes between the two methods.

Acute pain mitigation strategies for pigs undergoing tail docking

A comparison of the efficacy of oral meloxicam and a topical anaesthetic (EMLA) cream in mitigating acute nociception associated with docking using clippers showed that EMLA cream completely abolished acute EEG responses, whilst meloxicam had little effect on EEG responses to docking.

The use of topical anaesthetic (EMLA) cream was the most effective analgesic strategy evaluated. However, this, along with oral administration of other analgesic drugs, poses the disadvantage of requiring pigs to undergo additional handling to administer analgesia prior to processing. It is evident that handling and restraint alone is stressful to piglets (Prunier *et al.* 2005), therefore additional handling is undesirable unless there is substantial benefit to be gained. Any requirement for additional handling will also be more labour intensive, thus increasing production costs over and above the cost of purchasing analgesic drugs. Such limitations in the use of topical analgesia could be overcome by the development and use of a readily absorbed spray-on formulation. Transdermal drug delivery via drug-infused adhesive patches has been successfully used to administer local analgesia and other drugs to cats and dogs (e.g. Hofmeister and Egger 2004; Hill *et al.* 2011). There would be merit in investigating whether similar transdermal formulations of local anaesthetic could be successfully applied in a spray-on form to the tail of pigs prior to docking.

An alternative method of systemic analgesia delivery that has been investigated in pigs is trans-mammary delivery, through analgesic dosing of the lactating sow. Bates *et al*. (2014) investigated trans-mammary meloxicam dosing of piglets over a three-day period, with piglets undergoing castration and tail docking on dosage day 2. The authors demonstrated successful transfer of meloxicam from sow to piglets, as evidenced by a decrease in cortisol and prostaglandin E2 levels and maintenance of cranial skin temperature subsequent to docking, relative to placebo treated piglets. The authors took blood samples 1, 6 and 12 hours after processing on day 1 and at 12 hourly intervals until 72 hours after processing. The observed effects of meloxicam likely related to its anti-inflammatory actions. No attempt was made to assess the efficacy of meloxicam in mitigating acute pain responses during or immediately following processing. There could be value in investigating the efficacy of alternative analgesics, administered via this route, in mitigating acute primary nociceptive and inflammatory-mediated responses to tail docking in piglets.

Docking by cautery iron was also included as an experimental treatment in the pain mitigation study, based on the reduced EEG responses observed in cautery-docked pigs relative to clipper-docked pigs in experiment 1 (Chapter 2). Whilst cautery is not an analgesic strategy per se, comparing this with commercially viable analgesic strategies provides further information on the potential value of this as an anti-nociceptive strategy for routine use in commercial pig production.

Consistent with the observations in experiment 1, cautery-docked pigs in this study exhibited smaller EEG responses than control pigs docked using clippers. Pigs docked using cautery demonstrated no change in F50 following docking and a less prolonged reduction in P_{TOT} than clipper docked pigs. This is an important finding, as the use of cautery iron may be more appealing to pork producers than the use of analgesic drugs to mitigate acute pain, due to the smaller costs involved i.e. purchase of a cautery iron plus the ongoing cost of electricity or gas to power this, versus the cost of purchase and administration of analgesic drugs to each individual pig. Additionally, there are no concerns regarding drug residues and no requirement for additional handling with cautery alone.

The influence of piglet age on acute EEG nociceptive responses to tail docking

In Chapter 2, I identified a difference in the EEG responses of 2- and 20-day-old pigs to the noxious stimulus of tail docking using side clippers. Pigs docked at 2 days-of-age demonstrated little evidence of nociception compared with pigs docked at 20 days-of-age and exhibited lower EEG power overall. These findings suggest that there are differences in neural maturity and/or processing of nociceptive signals between the two ages. This prompted a more in-depth examination of the development of EEG nociceptive responses to tail docking in pigs between birth and 20 days of age, at which time pigs exhibited a characteristic adult nociceptive response.

The finding that 1-day-old pigs exhibited no EEG evidence of acute nociception following tail docking was consistent with a previous study of stress hormone responses, which reported no increases in plasma ACTH or cortisol in 1 day-old pigs following tail docking or teeth trimming (Prunier *et al.* 2005). The authors proposed that the lack of response to tail docking or teeth clipping at this age may have been due to either: immaturity of the HPA axis; marked stimulation of the HPA axis at birth preventing a response to supplementary stimulation; stress responses elicited by prior handling and blood sampling masking any subsequent responses to tail docking, or due to tail docking/teeth clipping being insufficiently noxious to elicit a physiological response. The suggestion that HPA axis immaturity might account for the lack of stress response at 1 day of age is unlikely, given the critical role of the fetal HPA axis in parturition in pigs necessitating its functionality prior to birth (Lay Jr 2000). In terms of tail docking being an insufficiently noxious stimulus, evidence from studies involving pigs aged 2 days and older has shown that tail docking elicits physiological and/or behavioural responses consistent with pain (Sutherland *et al.* 2008; Marchant-Forde *et al.* 2009; Torrey *et al.* 2009). This leaves the possible explanation of handling-induced distress masking stress responses to docking at 1 day-of-age. This seems feasible, given that all aspects of the physical environment are novel in the period following birth, therefore possibly eliciting stress responses at this age that would not occur at a later age.

Whilst there is behavioural evidence of pain and/or distress in response to tail docking in pigs aged 2 days and older, little data is available for 1-day-old pigs. In their study of piglet responses to tail docking at 1 or 3 days-of-age, Torrey *et al*. (2009) found that those docked at 3 days exhibited more tail jamming (a pain-specific behaviour) in the 10 minutes after treatment than control or sham docked pigs; however, behavioural responses of pigs docked at I day of age did not differ to undocked controls. Noonan *et al*. (1994) reported vocalisation, tail wagging and tail jamming in response to tail docking in newborn pigs; however, the pigs involved in that study were aged 1–3 days, therefore the observed responses could have been attributable to the older pigs. Additionally, it is possible that behavioural responses to painful stimuli in the very early postnatal period may be brainstem-driven reflexes as opposed to part of a coordinated response to tissue damage, given that such behavioural responses to noxious simulation also occur in utero in the absence of pain perception (Mellor and Lentle 2015).

An alternative hypothesis for the absence of nociceptive responses at 1 day-of-age is the continued action of fetal pain-suppressive mechanisms in the period following birth. Whilst the fetal nociceptive system is mature in late gestation, a number of circulating chemicals function to maintain the fetus in a quiescent state, such that perception of nociceptive stimuli is inhibited (Mellor *et al.* 2005). Research in sheep has demonstrated the gradual withdrawal of suppressive mechanisms after birth (Nguyen *et al.* 2003), suggesting that analgesic actions may continue to be exerted in the period immediately after birth (Mellor and Diesch 2006). If this is the case in pigs, it might account for the lack of both EEG and stress hormone responses observed at 1 day-of-age. Future research should investigate whether neurosuppressive

chemicals, such as pregnanolone and allopregnanolone, are present at detectable levels in pigs at birth and, if so, for how long these persist.

Whist there were few statistically significant differences in EEG responses to docking between pigs aged 5, 7, 10, 12 or 15 days-of-age, a qualitative pattern of increasing responsiveness to docking with increasing post-natal age emerged. This was reinforced by the analysis of age-blocked data, which demonstrated significantly smaller nociceptive responses in pigs aged ≤7 days than in those aged >7 days. Based on this finding, tail docking pigs within the first week of birth may be advantageous in terms of reducing the acute pain associated with the procedure.

There are some considerations, however, in electing to perform painful procedures on animals at a very young age. As well as eliciting acute responses, nociception can also be followed by a period of hyperalgesia, or increased pain sensitivity, characterised by a decrease in stimulus threshold and enhanced response to suprathreshold stimulation (Raja *et al.* 2000). Hyperalgesia has been demonstrated in human infants subsequent to surgery or tissue damage without analgesia (Fitzgerald *et al.* 1989; Taddio *et al.* 1997), and has been shown to persist for months (Taddio *et al.* 1997) or years (Buskila *et al.* 2003). Research suggests that such hyperalgesia is a result of central sensitisation, as evidenced by hyperalgesia to noxious inputs in body locations distal to the original stimulus (Taddio *et al.* 1997). A previous study involving lambs demonstrated heightened behavioural responses to tail docking at 1 month of age among lambs castrated at <24 hours of age compared with those castrated at 10 days of age (McCracken *et al.* 2010). Further, lambs castrated without analgesia at 1 day old exhibited more pronounced EEG responses to tail docking 3 weeks later than those subjected to handling only at 1 day old (Impey 2015).

Given the EEG and cortisol evidence suggesting a lack of nociceptive response to tail docking at 1 day-of-age, it is important to investigate the potential, at various ages, for docking to result in longer-term pain sensitivity. Such effects should be investigated across the range of ages at which tail docking is currently performed, to determine whether there are advantages or disadvantages of docking at a particular age, or within a particular age range. Should evidence of hyperalgesia be demonstrated as a result of docking at a very young age, the welfare implications would need to be carefully weighed against the welfare implications of docking at a later age if this is likely to induce more acute pain. This is particularly important, given that tail docking is usually performed at the same time as other painful procedures such as teeth trimming, ear notching or tagging and castration (in countries other than New Zealand).

Histological changes in tail tips after docking using clippers or cautery iron

A comparison of the neural histomorphology of healed tail tips from slaughter weight pigs that had been docked at 2 days-of-age using either side cutter pliers or cautery revealed no differences between methods in either neuroma incidence, or the degree of neural proliferation in healed tissues. The incidence of neuromas was high in tails docked using either method (89% and 94% for side clippers and cautery, respectively). The lack of a significant difference in neural histomorphology suggests there is no longer-term welfare advantage of one docking method over the other, at least in terms of the potential for alterations in pain processing due to neuroma formation at the docking site. Moreover, clinical research has shown that not all neuromas are painful (Costigan *et al.* 2009). Therefore, further work is required to determine whether hyperalgesia or other forms of neuropathic pain occur subsequent to tail docking in pigs and, if so, whether this relates to observable changes in neural histomorphology. It is conceivable that hyperalgesia of the tail tip, or more distal tissues in the case of spinal or central sensitisation, might occur in the absence of neuromas or other observable changes in neural morphology, and that there may be differences in the extent of this between docking methods. Future studies could explore this by undertaking nociceptive threshold testing, at the tail tip and other regions of the body, before docking (by cautery or clippers) and at

intervals after docking until the time of slaughter and comparing these to undocked controls. In parallel, post-mortem histological analysis of tail tissues could be performed at the same intervals. This would allow determination of whether alterations in nociceptive thresholds occur over time subsequent to docking using clippers or cautery, and whether such changes relate to observable changes in neural histomorphology in the tail tip.

It should be noted that the number of tails successfully examined in the present study was low (15, 19 and 18/25 control, clipper and cautery docked, respectively), as only those samples in which the entire cross section could be visualised were scored. Poor sample adhesion to slides, resulting in partial cross sections, occurred in 40% of control and 26% of docked tails, meaning these were not included in the statistical comparison. The reason for poor sample adhesion was uncertain, given that the preparation method was based on that previously reported for pig tail-sections. The only difference was that I elected to prepare transverse sections of the intact tail, therefore enabling examination of the entire cross sectional area, whereas the prior studies sectioned tails in the mid-longitudinal plane before preparing mediolateral cross-sections. This may have resulted in improved sample adhesion compared with using entire cross-sections, due to properties associated with the cutaneous tissue around the circumference. If this were the case, this issue could be overcome by preparing mediolateral cross-sections from identical regions of the longitudinally halved tail, thus still allowing visualisation of the entire cross sectional area. This approach may improve successful slide preparation in future studies.

Comparison of EEG data from the left and right cerebral cortices

A comparison of EEG data recorded from the left and right cerebral cortices in a single study (Appendix A) revealed that these were largely equivalent, which is consistent with previous reports from other species (McIlhone 2011; Murrell *et al*. 2007; Murrell *et al*. 2010). However, there were some differences in P_{TOT} responses between hemispheres that were suggestive of greater right hemisphere sensitivity in nociceptive processing. As such, this should be further investigated with a larger data set to determine whether it is advantageous to analyse right hemisphere recordings. In the meantime, whilst analysis of data from either hemisphere allows detection of EEG changes in response to nociception, there may be merit in preferentially analysing right hemisphere data where this is available. These findings provide justification for the initial decision to preferentially analyse right hemisphere data throughout the studies presented in this thesis.

Acute nociception following intraperitoneal sodium pentobarbitone injection

Intraperitoneal injection of sodium pentobarbital euthanasia solution induced a characteristic EEG nociceptive response in 10–15 day old pigs in the 60-second period following administration. Based upon this, it is likely that conscious pigs experience pain in response to I/P pentobarbital injection prior to loss of awareness. This finding is consistent with behavioural and physiological studies in rodents, in which researchers also concluded that I/P administered pentobarbital induces pain (Ambrose *et al.* 2000; Svendsen *et al.* 2007).

A limitation of this study was the failure to assess the accuracy of dose placement. Common landmarks were used to determine placement site and depth and dose rate was calculated based on individual live weight. However, the extreme variations in time to onset of an isoelectric EEG trace (indicative of pentobarbital-induced cerebral depression) between individuals (min 144, max 640 seconds) suggests differences in the rate of drug uptake. It is likely this was a result of differences in the site of dose placement affecting the rate of drug entry into general circulation (Claasen 1994). Previous studies have demonstrated incorrect dose placement (i.e. delivery to sites other than the peritoneal cavity) rates between 10 and 50% for I/P

administered drugs in rodents and cats (Steward *et al.* 1968; Miner *et al.* 1969; Arioli and Rossi 1970; Claasen 1994). It would have been useful to have include a dye in the pentobarbital solution to permit post-mortem determination of exact delivery sites to confirm this.

Future studies should investigate whether modifications to I/P pentobarbital solution can effectively mitigate acute nociceptive responses in pigs. The addition of a fast-acting local anaesthetic (lidocaine) has been shown to mitigate (but not abolish) nociceptive responses to I/P injection in rats (Ambrose *et al.* 2000; Svendsen *et al.* 2007); therefore, this may also be effective for pigs.

6.2 METHODOLOGICAL CONSIDERATIONS

In interpreting the EEG analyses presented in this thesis, it must be borne in mind that the use of EEG for pain assessment does have limitations. Whilst it provides a real-time record of changes in cerebrocortical activity following noxious stimulation, such EEG changes are specific to primary nociceptive stimulation – i.e. these only occur for as long as the duration of the nociceptive stimulus, fading once the peripheral noxious stimulus is removed. Whilst this is advantageous for assessing the magnitude and duration of primary nociceptive inputs, EEG analysis provides no information on subsequent inputs relating to inflammatory pain, thus cannot be used to study the full-time course of nociceptive responses.

In addition, the use of general anaesthesia precludes the simultaneous collection and analysis of additional pain indicators. For example, behavioral indicators of pain cannot be assessed in anaesthetised animals and stress hormone studies may be compromised by the manipulations required to anaesthetise the animals, i.e. the handling and anaesthetic induction procedures can influence plasma stress hormone levels (Taylor 1989), thus confounding the effects of docking. However, the use of other variables, such as behaviour and stress-hormone production, for evaluating pain in pigs undergoing tail docking has been reported elsewhere and is referred to throughout this document in the interpretation of EEG nociceptive responses.

It should also be noted that FFT is not the only tool available for analysing EEG. In the human clinical setting, EEG is traditionally analysed in terms of frequency band analysis, whereby processed EEG is classified according to the relative activity in the delta (0–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), and beta (>12 Hz) frequency bands. In humans, these frequency bands have associated functionality, e.g. oscillations in alpha and theta activity are linked to cognitive memory and performance (Klimesch 1999). Although some animal studies have adopted band analysis for studying EEG (Otto *et al.* 1996; Ong *et al.* 1997), use of these bands is arbitrary, as no associated functionality has been defined, therefore false functionality might be inferred (Murrell and Johnson 2006).

A bispectral index (BIS) monitor has been developed for use in the clinical setting as a means of real-time monitoring of anaesthetic adequacy in man. This processes the raw EEG into a single numerical output, indicative of sedation depth. The process underlying BIS score calculation is derived from analysis of numerous human bifrontal EEG recordings. In man, BIS has been shown to correlate more strongly with sedation than nociceptive indices (Vernon *et al.* 1995; Leslie *et al.* 1996), meaning it is of limited use in nociceptive studies. Although BIS has been investigated for monitoring anaesthetic depth and nociception in animals (e.g. Haga *et al.* 1999; Haga and Dolvik 2002; Carrasco-Jiménez *et al.* 2004; Lamont *et al.* 2004), its use is questionable, given its derivation from human data.

An alternative to FFT for EEG frequency analysis is wavelet transform (WT). Initially developed for seismic signal analysis, WTs have been applied to a range of complex signal analyses, including turbulence, magnetic resonance imaging and speech discrimination (Schiff *et al.* 1994). Both FFT and WT are window-based

techniques that analyse data in small portions or 'windows', yielding both time and frequency information. However, they utilise different window formations, thus achieving different resolutions. FFT uses a single fixed window, meaning that frequency resolution may be compromised if the window is too narrow, whereas a wide window reduces time resolution. By contrast, WT utilises a window with variable-sized regions, thus achieving better overall resolution (Akin 2002). In the medical field, WT is used for EEG spike and seizure detection, and more recently, has been applied to the study of thermal nociception (Mouraux and Plaghki 2004). To date, there are no reports of WT being used for EEG analysis in animals.

The use of FFT for spectral analysis of the EEG in the current series of studies was based on this being the predominant method of EEG analysis reported in the literature in animal studies of nociception and analgesic efficacy. Using the same methodology facilitated data interpretation in the context of previous research.

6.3 IMPLICATIONS FOR ANIMAL WELFARE AND TAIL DOCKING PRACTICE

The finding that tail docking using cautery elicits less acute nociception, combined with evidence from prior studies in pigs showing that cautery elicits smaller stress hormone responses, along with the finding that tail tip neuroma incidence does not differ between cautery and clipper docked pigs, provides a good evidence base for advocating the use of cautery in place of clipping for tail docking of pigs in the absence of analgesia. The use of a method that is associated with less acute pain would improve the welfare of pigs undergoing tail docking.

The data presented in this thesis, in conjunction with the wider body of research, clearly indicate that tail docking is painful to pigs aged 2 days and older and that provision of analgesia is beneficial in mitigating acute pain responses, thus improving the welfare of pigs. However, as yet no commercially viable analgesic strategy that effectively mitigates both acute primary nociceptive and inflammatory pain responses has been identified. In addition, provision of analgesia may be hindered in some countries due to regulations surrounding the use of analgesic drugs in food producing animals, as well as the cost and availability of such drugs. In the absence of analgesia, the use of cautery iron in place of clippers has the potential to improve the welfare of large numbers of pigs worldwide annually.

In terms of age, performance of tail docking at a younger age appears to be associated with less acute nociception. Although tail docking at 1 day-of-age induced no significant nociceptive response, the mechanisms underlying this absence, along with the potential for long term heightened pain sensitivity, need to be determined before docking at this age is recommended. However, the evidence provided by this research indicates that docking within seven days of birth is preferable to docking at a later age, in terms of acute nociception.

6.4 CONCLUSIONS

The following conclusions are drawn from the results of the research presented in this thesis:

- Pigs tail docked at 2 days-of-age demonstrated little evidence of nociception compared with pigs docked at 20 days-of-age, and exhibited lower EEG power overall, suggesting there are differences in neural maturity and/or processing of nociceptive signals between the two age groups. Similarly, pigs tail docked at 1 day-of-age exhibited no EEG evidence of nociception; therefore, tail docking within 2 days of birth may be less acutely painful to pigs compared with docking at 20 days-of-age.
- Acute EEG responses to noxious stimulation varied significantly with postnatal age in pigs over the first 15 days after birth, with responses increasing in magnitude with increasing postnatal age.
- Tail docking within the first week of life is likely less acutely painful to pigs than tail docking at a later age.
- Tail docking using cautery iron elicits a smaller nociceptive response than tail docking using clippers; therefore, docking with cautery may be less acutely painful to pigs than docking with clippers.
- Tail docking of pigs using either side clippers or cautery iron resulted in the formation of neuromas in the healed tail tip, therefore both methods have the potential to induce long-term changes in the processing and perception of afferent neural signals arising from the tail tip region.
- Neither the proportion of tails with neuromas, nor the degree of abnormal nerve proliferation in the tail tip differed between pigs docked using clippers and those docked using cautery iron.
- Prior application of EMLA topical anaesthetic cream abolished EEG indicators of acute nociception in pigs tail-docked using clippers.
- Prior administration of topical anaesthesia, or the use of cautery iron in place of clippers, has the potential to reduce acute pain in pigs undergoing routine tail docking, thus improving welfare.
- Intraperitoneal injection of sodium pentobarbital for euthanasia elicited an immediate nociceptive response in anaesthetised pigs, suggesting that conscious pigs are likely to experience pain prior to loss of awareness when this method is used.
- Where bilateral EEG recording is undertaken in pigs using the MAM, data from the right cerebral cortex may provide greater resolution of nociceptive responses than data from the left, therefore should be preferentially analysed.

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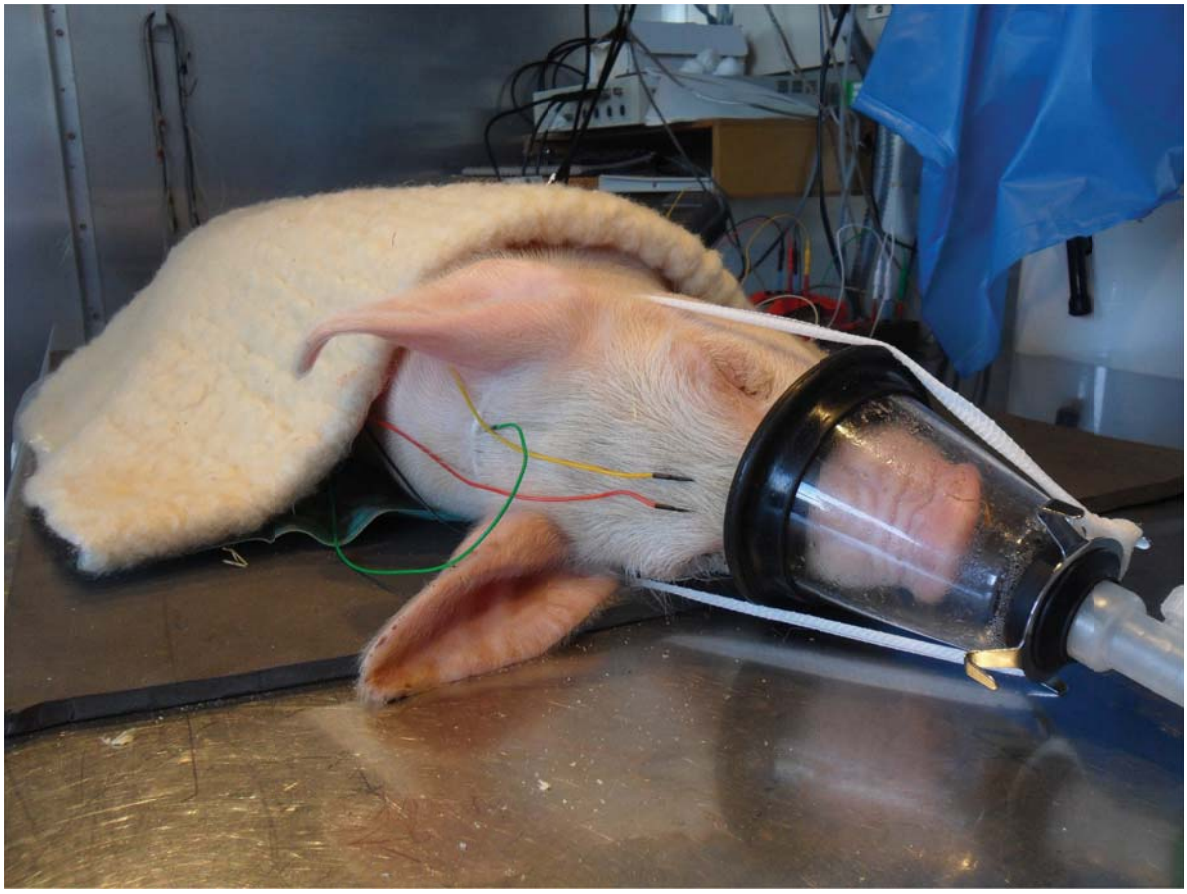
APPENDIX A Comparison of EEG data recorded from the left and right cerebral cortices in Chapter 3

(Electroencephalographic assessment of pain mitigating strategies for pigs undergoing tail docking; Chapter 3)



A.1 ABSTRACT

The minimal anaesthesia model (MAM) utilises electroencephalography (EEG) to monitor changes in cerebrocortical electrical activity related to the processing of nociceptive signals. Using this model, EEG may be recorded and analysed from one (unilateral) or both (bilateral) cerebral hemispheres. Previous studies using the MAM to study nociception in chickens and rats found negligible hemispheric differences in bilateral EEG recordings, with researchers concluding there was no evidence for lateralisation of nociceptive responses in these species. In contrast, brain-imaging studies in humans have provided evidence of a right hemispheric bias in nociceptive processing. To determine whether hemispheric lateralisation of nociceptive processing occurs in pigs, statistical comparison of bilateral EEG data recorded from 40 pigs subjected to tail docking with or without prior analgesia was carried out. The summary variables median frequency (F50), 95% spectral edge frequency (F95) and total power (P_{TOT}) were derived from EEG recorded from each hemisphere in each pig. Statistical analyses revealed no effect of hemisphere on F50, whilst overall F95 was higher in EEG recorded from the left hemisphere than the right (across all treatments and time points). In control pigs that received no prior analgesia, P_{TOT} was lower in EEG recorded from the right hemisphere compared with the left. A reduction in P_{TOT} is associated with nociception; therefore, the observed effect on P_{TOT} may indicate a greater right-side sensitivity to nociceptive inputs in pigs. However, given the absence of a hemisphere effect on F50, which typically increases in response to noxious stimulation, further investigation is required to confirm the presence or absence of lateralisation in nociceptive processing among pigs.

A.2 INTRODUCTION

Throughout this series of experiments EEG was recorded simultaneously from both the left and right cerebral cortices. However, the data analysed and presented throughout are those recorded from the right cerebral cortex only. Previous studies using the same methodology (MAM) have shown that EEG data recorded from the left and right cerebral cortices are largely equivalent. In a study of EEG responses of domestic chickens to increasing concentrations of different inhalational anaesthetic agents, it was reported that EEG recorded from the left and right cortices were equivalent, both in terms of spectral analysis and burst suppression ratios (McIlhone 2011). In a study of rat spectral EEG responses to a noxious surgical stimulus, Murrell *et al.* (2010) reported negligible differences between data recorded from the left and right cortices following surgical noxious stimulation, with no consistent patterns or trends indicative of lateral bias found.

In contrast, neuroimaging studies in human subjects have provided evidence for right hemispheric lateralisation in pain processing. Utilising positron emission tomography (PET) or functional magnetic resonance imaging (fMRI) techniques, researchers have provided support for a right hemispheric bias in neuronal activity in specific cortical regions following application of nociceptive stimuli (Coghill *et al*. 2110; Brooks *et al*. 2002; Bingel *et al*. 2003; Youell *et al*. 2004). Using fMRI, Symonds *et al*. (2006) identified five discrete cortical regions in which activation in response to an acute noxious electrical stimulus occurred solely in the right hemisphere, or was significantly more intense and occurred over a greater area in the right hemisphere than the left, independent of lateralisation of stimulus application. These regions were the anterior cingulate, middle frontal gyrus, medial and superior frontal gyri, along with regions in the inferior frontal gyrus and inferior parietal lobule.

The lack of evidence for hemispheric lateralisation of cortical activity in response to noxious stimuli in animal studies using the MAM may indicate the absence of any such lateralisation in the mammalian species examined. Alternatively, it may be that the methodology employed was not sensitive to hemispheric differences. Whilst PET and fMRI provide detailed spatial resolution, the MAM provides a representation of global activity arising from each hemisphere. Therefore, although overall cortical activity arising from each hemisphere may be similar, the specific origin of neuronal activity within each hemisphere might differ. As such, the observed similarities between cortices seen with the MAM may be due to a lack of sensitivity to regional differences in signal processing between hemispheres, rather than the absence of any such differences.

The decision to analyse data from the right cortex in the present studies was based upon the evidence of a right hemisphere bias in pain processing derived from human imaging studies. However, data were recorded from both cerebral cortices in all studies, with data from the left cortex essentially being held as back-up in the event of data from the right cortex being unsuitable for analysis, for example due to recording failure, electrode displacement, or the presence of extensive artefact confined to a single channel.

For completeness, it is important to evaluate the general equivalence of EEG data recorded from the left and right cortices of pigs, to determine whether there is any detectable lateral bias in nociceptive processing in this species using the MAM. As such, a statistical comparison of spectral EEG data derived from the left and right cerebral cortices in Study 2 (Chapter 3) was undertaken. Data from this study were selected, as they were obtained from pigs of a single age. In Study 1 (Chapter 2), examination of EEG responses to tail docking in 2 and 20 day-old pigs revealed differences in the cortical processing of noxious stimuli as a function of age. To reduce variability in the data set and improve statistical power, it was decided to examine data from pigs of a single age.

A.3 METHODOLOGY

The following text describes the methods used for data collection, processing and statistical analyses. Full details, including animals, experimental treatments and anaesthesia, are provided in Chapter 3.

EEG recording

Subcutaneous 27-gauge stainless steel needle electrodes (Viasys Healthcare, Surrey, England) were positioned to record EEG and electrocardiograph (ECG) activity. A five-electrode montage was used to record EEG from the left and right cerebral hemispheres, with inverting electrodes placed parallel to the midline over the left and right frontal bone zygomatic processes, non-inverting electrodes over the left and right mastoid processes and a ground electrode placed caudal to the occipital process (see Murrell & Johnson 2006).

EEG signals from each hemisphere were fed via breakout boxes to separate amplifiers (Iso-Dam isolated biological amplifier, World Precision Instruments Inc.) where the signals were amplified with a gain of 1000 and a band-pass of 1.0–500Hz and digitised at a rate of 1 kHz (Powerlab 4/20, ADInstruments Ltd, Colorado Springs, Co). The digitised signals were recorded on an Apple Macintosh personal computer for off-line analysis at the conclusion of the experiment.

Data analysis

Raw EEG recordings from both hemispheres were inspected manually and any artefacts, such as over-scale, under-scale, nystagmus or other muscular activity, excluded from subsequent analysis. The total power (P_{TOT}), median frequency (F50) and 95% spectral edge frequency (F95) were calculated for consecutive 1-second epochs, using purpose-written software (Spectral Analyser, CB Johnson, Massey University, Palmerston North, NZ, 2002). Data from each hemisphere for each individual were standardised to a percentage of baseline (pre tail-docking) mean.

EEG data from the final 30 seconds of baseline and for consecutive 30-second blocks (up to 180 seconds) after tail docking were compared between the two hemispheres. A single mean value for each EEG variable was calculated for each time-period in each hemisphere for each pig, generating a total of seven data points per pig per variable (one before and six after tail docking) for each hemisphere.

Statistical analyses

Summary variables from Fourier-transformed EEG data were subjected to analysis of variance using the MIXED procedure in SAS version 9.3.1 (SAS Institute Inc., Cary NC, USA, 2012). The model incorporated treatment (control, EMLA, meloxicam or cautery) and hemisphere (left or right) as fixed effects, pig as a random effect and time as a repeated measure. Statistical significance was set at $\alpha = 0.05$. Where significant main or interaction effects were identified, post hoc tests were carried out to identify group differences, with Bonferroni adjustment for multiple comparisons.

A.4 RESULTS

The results of the statistical analyses are presented in Table A.1 below. The effects of treatment and/or time on F50, F95 and P_{TOT} are presented in Chapter 3 and are not discussed here.

Median frequency (F50)

There were no effects of hemisphere, or hemisphere interaction effects, on the F50 of the pig EEG (Table A.1).

95% spectral edge frequency (F95)

Overall, F95 was higher in EEG recorded from the left cerebral cortex than the right cerebral cortex (100.58 *vs*. 100.26 (SE 0.16) %, respectively).

There was a tendency toward a significant hemisphere x treatment interaction effect on F95. A comparison of mean F95 between hemispheres within each treatment showed that F95 was higher in EEG recorded from the left hemisphere than that from the right (100.76 *vs*. 100.15 ± 0.38%, $p = 0.003$) in pigs that received meloxicam prior to docking. There were no differences in F95 between treatments within either hemisphere.

Table A.1 *Results of statistical comparison of EEG spectral data recorded from the left and right cerebral cortices of 21-day-old pigs undergoing tail docking with or without prior analgesia.*

	F50		**F95**		**P_{TOT}**	
	F	*p*	*F*	*p*	*F*	*p*
Hemisphere	2.59	0.116	11.38	0.002	1.60	0.214
Treatment	4.02	0.030	1.88	0.180	0.31	0.815
Time	32.04	<0.001	12.99	<0.001	50.63	<0.001
Hemisphere x treatment	0.90	0.449	2.63	0.065	3.67	0.021
Hemisphere x time	1.25	0.283	1.03	0.408	0.65	0.692
Hemisphere x treatment x time	0.17	1.0	0.37	0.992	0.43	0.980

Total power (P_{TOT})

There was a significant hemisphere x treatment interaction on the P_{TOT} of the pig EEG. In the control treatment (pigs docked using clippers without prior analgesia) P_{TOT} was significantly lower in EEG recorded from the right cerebral cortex compared with that recorded from the left (94.78 *vs*. 97.79 (SE 2.2)%, respectively; p =0.022). There were no differences in the P_{TOT} of the EEG recorded from the left and right cortices of pigs in the cautery (p =1.0), EMLA (p =0.3884) or meloxicam (p =1.0) treatments. There were no differences in P_{TOT} between treatments within either hemisphere.

There were no hemisphere x time, or hemisphere x treatment x time interaction effects on any EEG spectral variable.

A.5 DISCUSSION

The purpose of this analysis was to determine whether there was any lateralisation of spectral EEG responses to noxious stimulation in lightly anaesthetised pigs undergoing tail docking with or without prior analgesia. The results demonstrated minor, non-systematic differences between hemispheres, in terms of overall F95 (all treatments and times combined) and in P_{TOT} of pigs in the control treatment only.

The typical mammalian EEG response to nociception is an increase in F50 and concurrent decrease in P_{TOT} (Murrell & Johnson 2006). In the present study, such a response was observed in control pigs docked using clippers without analgesia and in those docked using clippers after prior administration of meloxicam (see Chapter 3). Had there been hemispheric differences in nociceptive signal processing, it would be expected that EEG responses to tail docking would differ between hemispheres in both control and meloxicam-treated pigs. However, this was not the case, as evidenced by the absence of a hemisphere x treatment interaction effect on F50, or a hemisphere x treatment x time interaction effect on either F50 or P_{TOT}.

The lower P_{TOT} recorded from the right hemisphere observed in control pigs may be interpreted as an indication of greater right side sensitivity to nociception, given that a reduction in P_{TOT} is associated with nociception. The lack of hemispheric differences in P_{TOT} among pigs that received meloxicam or EMLA prior to docking, or those docked using cautery, may be explained by the antinociceptive properties associated with these treatments. However, there were significant reductions in P_{TOT} following tail docking in the meloxicam and cautery treatments (see Chapter 3), therefore we would have expected to see a hemispheric

effect on P_{TOT} in these groups if there were greater right side sensitivity to nociception. This merits further investigation.

Whilst some studies utilising the MAM have identified elevated F95 concurrent with elevated F50 in response to noxious stimulation (e.g. Johnson et al. 2005; Gibson *et al*. 2007), others have found no change in F95, even in situations where F50 increased (e.g. Murrell *et al*. 2007; Kongara *et al*. 2010). F50 is considered a more sensitive index of nociception than F95 (Murrell *et al*. 2003, 2007; Johnson *et al*. 2005). Therefore, it is likely that the hemispheric differences in total F95 in the current study were related to aspects of cortical activity unrelated to nociception. Given that the tendency toward a treatment x hemisphere interaction effect was confined to pigs that received oral meloxicam, it is possible that the observed lateralisation related to hemispheric differences in the cerebral effects of meloxicam. This is further supported by the absence of any significant interaction effects of hemisphere with treatment and/or time.

A.6 CONCLUSIONS

The results of these analyses largely support those of previous studies using the MAM, which demonstrated no (McIlhone 2011) or negligible (Murrell *et al*. 2007; Murrell *et al*. 2010) effects of hemisphere on spectral EEG responses to noxious stimuli. However, the potential for greater right-side sensitivity in terms of changes in P_{TOT} following noxious stimulation warrants further investigation. Considering this, there may be merit in preferentially recording and analysing EEG data from the right hemisphere in future nociceptive studies in pigs using the MAM.

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APPENDIX B Electroencephalographic responses of anaesthetised pigs to intraperitoneal injection of sodium pentobarbital

Kells NJ, Beausoleil NJ, Sutherland MA, Johnson CB. *Electroencephalographic responses of anaesthetised pigs to intraperitoneal injection of sodium pentobarbital*. Submitted to *Animal Welfare* 4 April 2017.

B.1 ABSTRACT

Laboratory animals are commonly euthanased via intraperitoneal (IP) injection of sodium pentobarbital. However, there is concern that animals may experience pain prior to loss of consciousness with this delivery route. The present study investigated electroencephalographic (EEG) nociceptive responses of anaesthetised pigs to IP sodium pentobarbital injection using an established minimal anaesthesia model. Thirty commercial white line entire male pigs were minimally anaesthetised with halothane in oxygen. Following 10 minutes of baseline EEG data collection, pigs had their tails docked using side cutters and, after a further 5 minutes of data collection, were euthanased via IP injection of sodium pentobarbital (250 mg/kg). The summary variables median frequency (F50), 95% spectral edge frequency (F95) and total power (P_{TOT}) were derived from the resultant EEG data. For each variable in each individual, means were calculated for the following 60-second periods: immediately prior to tail docking (baseline 1), immediately prior to pentobarbital injection (at least 4 minutes after docking; baseline 2), and for two consecutive 60-second periods immediately following pentobarbital injection (P1 and P2). Statistical analyses revealed no differences between the two baseline periods, indicating that transient EEG changes induced by tail docking had resolved prior to pentobarbital injection. IP pentobarbital injection induced a significant increase in F50 and decrease in P_{TOT} of the EEG during P1. All EEG measures returned to baseline during P2. This response is characteristic of acute nociception, indicating that conscious pigs likely perceive IP sodium pentobarbital as painful in the period prior to loss of consciousness.

B.2 INTRODUCTION

Whilst it is generally accepted that the use of live animals is a requirement for some types of biological research, there is increasing awareness of the importance of maximising the welfare of research animals, particularly with regard to minimising any pain or distress incurred during the performance of research (Fenwick *et al.* 2009). Intraperitoneal (IP) injection of sodium pentobarbital is frequently employed for anaesthesia and euthanasia of laboratory rodents (Svendsen *et al.* 2007). Whilst intravenous delivery is preferred as it achieves more rapid distribution of the agent and has lower potential to cause tissue irritation (Wolfensohn and Lloyd 2003; Svendsen *et al.* 2007), IP delivery is most common in small animals such as rodents due to its relative ease of administration. However, there is concern that pentobarbital preparations may cause pain or irritation to the parietal and visceral peritoneum and associated tissues when delivered IP, particularly at concentrations required for euthanasia (Ambrose *et al.* 2000; Wolfensohn and Lloyd 2003). In addition, there is substantial evidence that the accuracy of placement of IP injections is poor, with reported misplacement rates ranging from 10–24% in rats and mice (Steward *et al.* 1968; Miner *et al.* 1969; Arioli and Rossi 1970; Claasen 1994) to greater than 50% in cats (Grier and Schaffer 1990). Dose misplacement may affect subsequent absorption and metabolism, thus speed of action and efficacy, as well as the extent of associated irritation or pain (Svendsen *et al.* 2007).

Previous studies have investigated pain and/or distress associated with IP pentobarbital injection in rodents through measurements of behaviour, plasma stress hormone concentration and expression of c-fos (a marker of neuronal activity) in spinal nociceptive neurons following peripheral stimulation (Svendsen *et al.* 2007). Ambrose *et al*. (2000) reported writhing in rats in response to IP sodium pentobarbital injection. Abdominal writhing is a recognised sign of pain in rats that can be experimentally induced through injecting a known irritant, such as acetic acid, into the peritoneal cavity (Siegmund *et al.* 1957). Further, plasma cortisol was elevated in rats that were decapitated following IP sodium pentobarbital anaesthesia, relative to control rats decapitated without prior anaesthesia (Vahl *et al.* 2005; Wu *et al.* 2015).

Elevations in plasma stress hormone concentrations are frequently used as indices of distress induced by painful or noxious procedures. However, such measures are not specific to pain, instead providing a measure of the overall noxiousness of an experience, including both physical and emotional components (Mellor *et al.* 2000). Indeed, elevations in plasma stress hormone concentrations have been shown to occur in rats administered IP physiological saline solution prior to decapitation, relative to those that did not receive IP saline (Baek *et al.* 2015; Wu *et al.* 2015), suggesting that the process of IP injection itself is stressful, independent of the compound being delivered.

Whilst changes in behaviour and stress hormone concentration can occur in response to non-painful stressors, assessment of spinal dorsal horn nociceptor activation provides a more specific and quantitative measure of nociception. IP pentobarbital injection reportedly caused an increase in spinal nociceptive neurone c-fos expression in rats, which was attenuated by concurrent lidocaine administration (Svendsen *et al.* 2007). Together, these data provide evidence of pain and distress in rodents following IP pentobarbital injection.

The use of electroencephalographic (EEG) parameters is gaining popularity for the quantitative assessment of nociception in mammals. Using an established minimal anaesthesia model (MAM) (Murrell and Johnson 2006), changes in the EEG frequency spectrum associated with nociception may be studied in anaesthetised animals, thus providing an ethical model for pain assessment which also minimises the influence of extraneous variables such as novelty and handling. The EEG provides a summation of electrical activity arising from neurons in the cerebral cortex. In man, changes in the frequency spectrum of the EEG have been found to mirror changes in cortical activity relating to the cognitive perception of pain (Bromm 1984). Using the MAM, EEG changes in response to the application of a known noxious stimulus have been demonstrated in a range of mammals, including horses (Murrell *et al.* 2003), sheep (Johnson *et al.* 2005b), cattle (Gibson *et al.* 2007), deer (Johnson *et al.* 2005a), pigs (Haga and Ranheim 2005) and rats (Murrell *et al.* 2010). In sheep, the magnitude of changes in the EEG frequency spectrum correlated well with behavioural responses to noxious stimuli in conscious animals (Ong *et al.* 1997). Likewise, in man the magnitude of changes correlated well with reports of pain intensity in response to graded noxious stimuli (Chen *et al.* 1989), highlighting the quantitative value of EEG as an index of nociception. Furthermore, prior administration of effective analgesia has been shown to obtund spectral EEG responses to noxious stimuli (Haga and Ranheim 2005; Johnson *et al.* 2005a; Murrell *et al.* 2005; Gibson *et al.* 2007; Kongara *et al.* 2010; Kongara *et al.* 2014), further demonstrating the specificity of this model.

It is recommended that euthanasia via injected overdose of barbiturates should be administered IV; however, in situations where IV access may be distressful, dangerous or impractical due to small animal size, IP administration is deemed acceptable (Leary *et al.* 2013). Thus, whilst mature research pigs would typically be euthanased via IV-administered pentobarbital, neonatal pigs may be euthanased via the IP route. No previous studies investigating the noxiousness of IP pentobarbital euthanasia in pigs were identified. Given the increasing use of pigs and mini pigs as general surgical models, as well as in toxicology and translational research (Swindle *et al*. 2012), it is important to determine the welfare implications of using IP pentobarbital for euthanasia of pigs.

The aim of the present study was to evaluate the nociceptive responses of piglets to IP pentobarbital injection, using the MAM.

B.3 ANIMALS

This study used 30 commercial white line (Large white x Landrace) entire male pigs (*Sus scrofa*) aged 10, 12 or 15 days (n=10 per age). Body weight ranged from 3.17–6.96 kg, with mean (± SEM) body weights of 3.6 ± 0.07, 4.1 ± 0.09 and 5.6 ± 0.09 kg recorded for 10, 12 and 15 day old pigs, respectively. All animals were sourced from a single commercial premises and formed part of a larger cohort of 60 pigs used to study the development nociceptive responses to tail docking with increasing postnatal age. As the pigs were not yet weaned and could not be returned to the farm of origin due to biosecurity restrictions, the experimental protocol dictated that they be euthanased via IP pentobarbital injection following data collection, whilst still under anaesthesia. This presented the opportunity to investigate nociceptive responses to IP pentobarbital injection in pigs.

B.4 MATERIALS AND METHODS

This study was conducted with approval from the Massey University Animal Ethics Committee (MUAEC, approval number 14/26). All procedures were undertaken in accordance with the MUAEC code of ethical conduct for the use of live animals for research, testing and teaching.

Pigs were obtained from a single commercial pig farm on the day of testing and housed in a temperature-controlled (30°C), ventilated indoor facility on deep straw litter with *ad libitum* access to water until the time of testing. All experiments were conducted within 6 hours (min 1.0, max 5.3) from the time of collection, in order to minimise any distress associated with separation from the sow. The pigs had not previously undergone any painful husbandry procedures (i.e. castration, tooth trimming, ear tagging, iron injection) and had intact tails on arrival. Within each age group, pigs were sourced from three separate litters, with each litter being tested at a single age.

Experiments were conducted over 10 separate test days, with 2–4 pigs tested per day.

Anaesthesia

Pigs were anaesthetised with halothane (Halothane-Vet; Merial NZ Limited, Manukau City, NZ) vaporised in oxygen (4 L minute^{-1}) delivered via facemask. Halothane delivery was maintained at 3–4% during induction and instrumentation and between 0.95 and 1.05% end-tidal concentration during the data acquisition period. End tidal halothane and CO_2 tension, SpO_2, respiration rate and heart rate were monitored throughout using an anaesthetic agent monitor (Hewlett Packard M1025B, Hewlett Packard, Hamburg, Germany). Rectal temperature was monitored using a digital thermometer (Q 1437; Dick Smith Electronics, New Zealand) and maintained at 38–40°C with the aid of a circulating warm-water heating blanket (T pump; Gaymar Industries Inc., NY, USA).

Electrophysiology

Subcutaneous 27-gauge stainless steel needle electrodes (Viasys Healthcare, Surrey, England) were positioned to record EEG from the left and right cerebral cortices, with inverting electrodes placed parallel to the midline over the left and right frontal bone zygomatic processes, non-inverting electrodes over the left and right mastoid processes and a ground electrode placed caudal to the occipital process.

EEG signals were fed via breakout boxes to separate amplifiers (Iso-Dam isolated biological amplifier, World Precision Instruments Inc.). The signals were amplified with a gain of 1000 and a band-pass of 1.0–500Hz

and digitised at a rate of 1 kHz (Powerlab 4/20, ADInstruments Ltd, Colorado Springs, Co). The digitised signals were recorded on an Apple Macintosh personal computer for off-line analysis at the conclusion of the experiment.

Experimental procedure

Once end tidal halothane tension was stable at 1.0 ± 0.05% 10 minutes of baseline EEG data was recorded. Pigs were then tail docked using a pair of clean, disinfected side cutter pliers. After a minimum interval of 5 minutes (range 5 min 0 sec – 9 min 59 sec), to allow for resolution of acute EEG responses to tail docking, pigs were euthanased via IP injection of sodium pentobarbital (Pentobarb 500, Provet NZ Pty Ltd, Auckland, NZ) at a dose rate of 250 mg/kg. Anaesthesia was maintained and EEG recording continued until death (indicated by isoelectric EEG in conjunction with respiratory and cardiac arrest).

Data analysis

Raw EEG recordings were inspected manually and any artefacts, such over-scale, under-scale, nystagmus or other muscle activity, were excluded from subsequent analysis. The total power (P_{TOT}), median frequency (F50) and 95% spectral edge frequency (F95) were calculated for consecutive 1-second epochs, using purpose-written software (Spectral Analyser, CB Johnson, Massey University, Palmerston North, NZ, 2002). Fast Fourier transformation was applied to each epoch, generating sequential power spectra with 1 Hz frequency bins. Data from each individual were standardised to a percentage of pre-docking baseline, calculated over the 5-minute period preceding tail docking. For the purposes of statistical analyses, mean F50, F95 and P_{TOT} were calculated for four non-overlapping 60-second periods for each individual pig: immediately preceding tail docking (Baseline 1; B1), immediately preceding pentobarbital injection (Baseline 2; B2), from 1 to 60 seconds after pentobarbital injection (P1) and from 61 to 120 seconds after pentobarbital injection (P2). A single mean value for each EEG variable was calculated for each time period in each pig, generating a total of four data points per pig per variable.

In addition, the interval (in seconds) from delivery of pentobarbital to the onset of isoelectric EEG was determined for each individual, following visual inspection of the raw EEG traces.

Statistical analyses

All statistical analyses were performed using SAS version 9.3.1 (SAS Institute Inc., Cary NC, USA, 2012). Between-period comparisons of EEG data were performed using proc GLM. The model incorporated age as a fixed effect, pig as a random effect and period as a repeated measure. Statistical significance was set at α =0.05. Where a significant period effect was identified, post hoc tests were carried out to identify differences, with Bonferroni adjustment for multiple comparisons.

Analysis of variance was undertaken to compare the time to onset of isoelectric EEG between age groups. Statistical significance was set at α =0.05, with Tukey's HSD test performed to identify group differences.

B.5 RESULTS

EEG data were successfully collected from 29/30 pigs, with data from one 15-day-old pig excluded due to equipment failure during data collection. Body temperatures recorded during anaesthesia ranged from 37.0 to 39.4°C. $PE'CO_2$ did not exceed 57.5 mmHg in any animal at any time during anaesthesia. Although there were some differences in maximum $PE'CO_2$ recorded between pigs, this is unlikely to have had a significant

effect on cerebral blood flow over the range recorded (Olsen *et al.* 2006), and is therefore unlikely to have influenced electroencephalographic values.

Results are presented as mean ± SEM unless otherwise specified. Age did not significantly influence any EEG summary variable. There was a significant effect of period on F50 and F95, and a significant age x period interaction on P_{TOT} (Table B.1).

Table B.1 *Effect of piglet age (10, 12 or 15 days) and recording period (pre tail-dock baseline, pre pentobarbital injection and post pentobarbital injection), and their interaction on the summary variables F50, F95 and P_{TOT} of the EEG.*

	F50		**F95**		**P_{TOT}**	
	F	***P***	**F**	***P***	**F**	***P***
Age	1.82	0.182	0.34	0.716	2.31	0.119
Period	28.50	<0.001	2.45	0.050	16.77	<0.001
Age*period	1.67	0.114	0.72	0.671	2.70	0.010

Mean F50 was significantly greater in P1 compared with B1 (P <0.001) or B2 (P <0.0001; Figure B.1). Mean F50 in P2 did not differ from either baseline period or from P1 (P =1.0; Figure B.1).

Although there was an overall effect of period on F95, there were no significant differences between periods following adjustment for multiple comparisons (Figure B.1).

Mean P_{TOT} differed between periods in an age-dependent manner (Figure B.1). Whilst P_{TOT} did not differ between age groups within periods (adjusted P =1.0 for all comparisons), within-age differences between periods were observed in 10 and 12-day-old pigs. In 10-day-old pigs P_{TOT} was lower in P1 than P2 (P <0.001), but did not differ from either baseline period. In 12-day-olds P_{TOT} was lower in P1 than B2 (P =0.006) and lower in P1 than P2 (P <0.001). In 15 day-old pigs there was a tendency for P_{TOT} to be lower in P1 than P2 (P =0.08).

A summary of the changes in spectral EEG variables over time is provided in Figure B.2. The increase in F50 and decrease in P_{TOT} following pentobarbital injection (vertical dashed line) can be clearly seen, along with the subsequent resolution of these responses.

The mean interval between administration of pentobarbital and the appearance of an isoelectric EEG trace for all pigs was 360 ± 32.4 (min 144, max 640) seconds. Age significantly influenced the time to onset of isoelectric EEG (F =8.15 p =0.002). The mean interval to onset of isoelectric EEG was greater for 10 day-old pigs (490.9 ± 54.0 seconds) than 12 day-old pigs (234.5 ± 34.1 seconds). However, the time to onset of isoelectric EEG in 15 day-old pigs (354.9 ± 46.8 seconds) did not differ to that of either 10 or 12 day-old pigs.

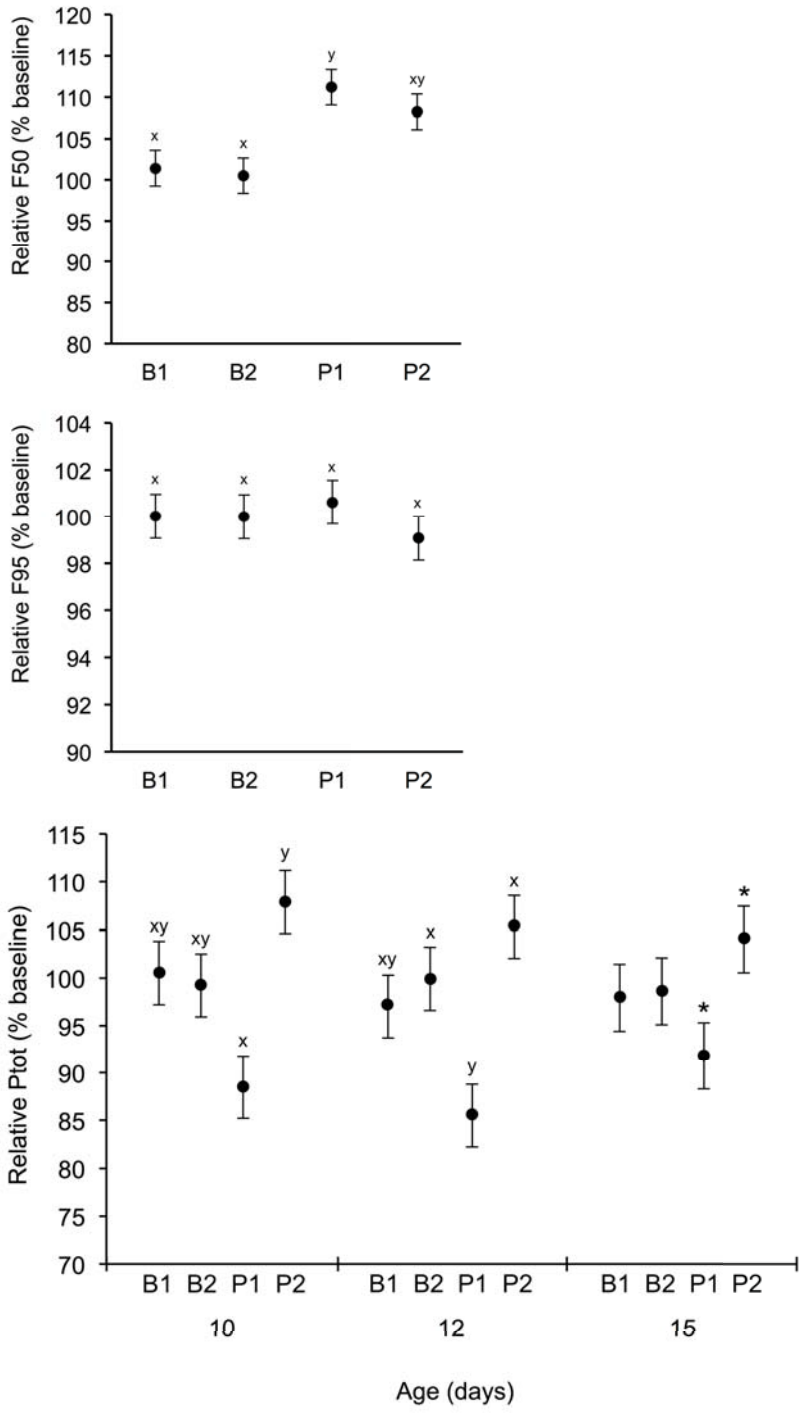

Figure B.1 *Comparison of mean (±SEM) F50, F95 and P_{TOT} of the pig EEG (n =29) over the 60-second period prior to tail docking (B1), the 60-second period prior to pentobarbital injection (B2), the 60-second period immediately after pentobarbital injection (P1) and the 60-second period from 61–120 seconds after pentobarbital injection (P2). xy Means with different superscripts were significantly different (Bonferroni adjusted P <0.05). * Means with asterisks tended to differ (adjusted P =0.08).*

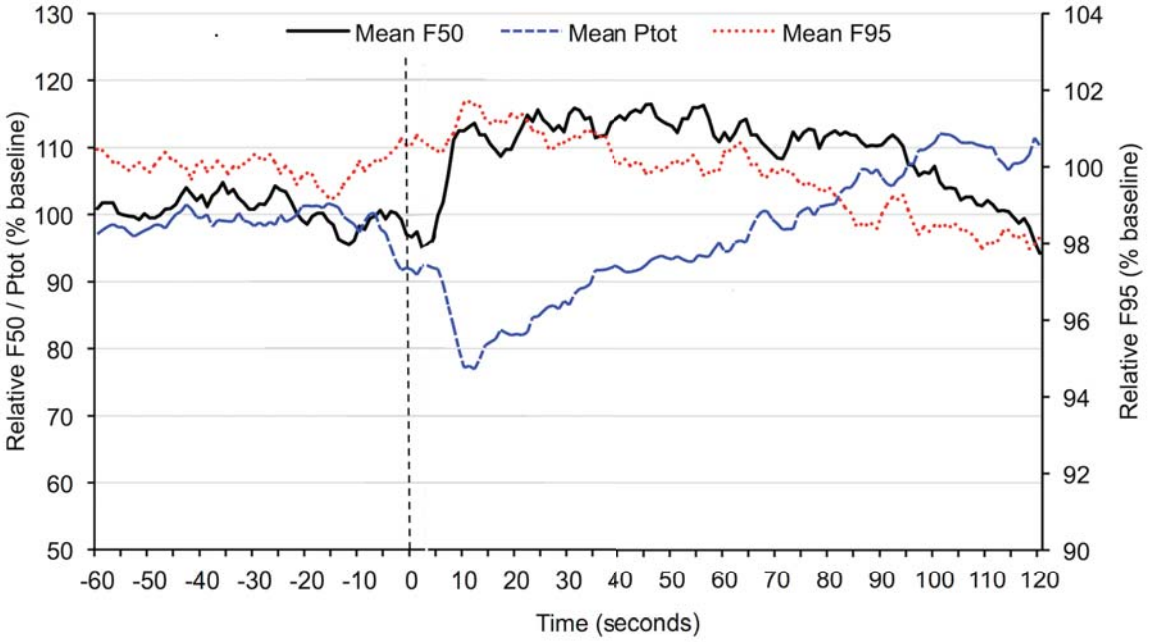

Figure B.2 *The changes in mean F50 F95 and P_{TOT} of the pig EEG (all ages combined), relative to pre-treatment baseline mean, following intraperitoneal injection of sodium pentobarbital (250 mg/kg; vertical dashed line). Note secondary axis for F95 data.*

B.6 DISCUSSION

The aim of this study was to use the MAM to determine whether administration of IP pentobarbital euthanasia induces a nociceptive response in pigs. In mammals, cortical processing of nociceptive signals is characterised by an increase in F50 and concurrent decrease in P_{TOT} of the EEG (Murrell and Johnson 2006). In the present study, across all ages combined, such a characteristic nociceptive response was observed in the period immediately following IP pentobarbital injection.

The data analysed in this study were collected from pigs that had been subjected to the noxious stimulus of tail docking shortly prior to being subjected to euthanasia via IP pentobarbital injection. In order to determine whether EEG responses to tail docking had resolved prior to the administration of pentobarbital, baseline data collected over the 60-seconds prior to pentobarbital injection were compared with baseline data collected over the 60-seconds preceding tail docking. The two baseline periods were found to be equivalent for all variables, indicating complete resolution of nociceptive responses to docking prior to pentobarbital injection. However, it is possible that tail docking may have sensitised pigs to subsequent noxious stimulation, thereby affecting the magnitude of responses to IP pentobarbital injection.

Pentobarbital is a short-acting oxybarbiturate, previously used for veterinary clinical anaesthesia, but nowadays primarily used as a euthanasia solution (Riviere and Papich 2009). It induces progressive central nervous system (CNS) depression, beginning with the cerebral cortex. Deep anaesthesia progresses to apnoea as the brainstem respiratory centres are depressed, with eventual death due to respiratory and/or cardiac arrest at doses sufficient for euthanasia (Leary *et al.* 2013). Cortical depression is reflected by a

progressive reduction in total EEG power until complete cessation of cortical electrical activity occurs, indicated by an isoelectric EEG trace.

In previous studies utilising the MAM concurrent reductions in both F50 and $P_{TOT,}$ representing a reduction in high frequency activity, have been reported with CNS depression in response to loss of cortical perfusion (Gibson *et al.* 2009) or increasing depth of anaesthesia (Johnson *et al.* 1994; Johnson *et al.* 1999). In the present study, there was a transient increase in F50 and decrease in P_{TOT} of the EEG in the initial 60-second period after pentobarbital injection, with a return to baseline state in the subsequent 60-second period. The observed increase in F50, along with the subsequent return to baseline; coupled with the extended interval to the appearance of isoelectric EEG (mean 6 minutes), strongly supports this being a nociceptive response to injection rather than CNS depression caused by the pharmacological effects of pentobarbital.

Whilst pooling of data from all pigs revealed a significant decrease in P_{TOT} immediately following pentobarbital injection, the response was found to be age-dependent. In 10-day-old pigs P_{TOT} was lower in P1 than P2 only, while in 12-day-olds P_{TOT} was lower in P1 than B2 or P2. In 15-day-old pigs, the tendency for P_{TOT} to be lower in P1 than P2 indicates a less pronounced P_{TOT} response in this age group. Given that other studies using the MAM have demonstrated patterns of increasing cortical responsiveness to noxious stimuli with increasing postnatal age (Johnson *et al.* 2005b; Johnson *et al.* 2015), the reduced response in 15-day-old pigs was unexpected.

The absence of a significant change in F95 following noxious stimulation has previously been reported in studies utilising the MAM (Murrell *et al.* 2007; Kongara *et al.* 2010), whilst others have reported an increase in F95 concurrent with increased F50 (Johnson *et al.* 2005a; Gibson *et al.* 2007). Such discrepancies likely indicate that F95 is not a sensitive indicator of nociception using this model. This is consistent with reports that F95 responds preferentially to changes in anaesthetic agent dose (Johnson *et al.* 1994; Johnson and Taylor 1998; Antunes *et al.* 2003), thereby reflecting general CNS suppression, whereas it has been proposed that F50 responds preferentially to noxious stimulation (Murrell *et al.* 2003; Johnson *et al.* 2005b). In the present study, the 2 minutes following pentobarbital injection was likely too short to allow time for sufficient quantities of the drug to enter general circulation, accounting for the lack of central depressive effects on the EEG over the 2-minute period analysed.

There was considerable variation between pigs in the time to onset of isoelectric EEG. Such variability suggests that the site of drug placement may have been inconsistent between animals. Whilst an effort was made to standardise the placement site based on anatomical landmarks, differences in the size and weight of pigs used in the study may have led to variation in the delivery site. This could then have influenced the rate of subsequent drug absorption and metabolism, affecting the speed of action (Claasen 1994). However, this could not be confirmed, as neither post mortem examination nor pharmacokinetic analyses were undertaken as part of this study.

Although pig age was found to affect the time to onset of isoelectric EEG, there was no consistent pattern of increasing or decreasing duration with increasing age. The finding that isoelectric EEG occurred sooner in 12 day-old pigs than in 10 day-old pigs, but did not differ between 12 and 15, or 10 and 15 day-olds, suggests this may have been due to random differences in dose placement rather than a true age effect. The pentobarbital dose given was calculated on pig weight, thus accounting for differences in body weight that may have influenced drug absorption.

The interval between administration of pentobarbital and the appearance of an isoelectric EEG trace is indicative of the period of potential awareness following drug administration in unanaesthetised animals. Given that isoelectric EEG is considered incompatible with consciousness (Newhook and Blackmore 1982),

awareness of noxious inputs associated with IP pentobarbital cannot occur following the onset of irreversible cortical isoelectricity. In the present study the mean interval between pentobarbital administration and the onset of isoelectric EEG was six minutes, therefore unanaesthetised pigs might experience unpleasant effects for up to several minutes following IP pentobarbital administration.

The finding that IP injection of sodium pentobarbital as doses commonly used for euthanasia induces nociception in anaesthetised pigs is consistent with previous studies in rats that demonstrated IP pentobarbital administered at concentrations sufficient for either anaesthesia or euthanasia was noxious. Svendson and co-workers showed that a dose volume of 0.4 mL/kg pentobarbital IP (from a 100 mg/mL stock solution) used for short-term anaesthesia induced a significant rise in the activity of spinal nociceptive neurones, compared with the same volume of physiological saline (Svendsen *et al.* 2007). Similarly, Ambrose *et al.* demonstrated increased activity and writhing following administration of 1.5 mL/kg sodium pentobarbital IP (from a 100 mg/mL solution) used for euthanasia (Ambrose *et al.* 2000). In both studies adverse responses were attenuated, but not abolished, by the addition of 10-mg/mL lidocaine, a fast acting local anaesthetic, to the pentobarbital formulation.

In the present study, a dose volume of 0.25 mL/kg, from a 500 mg/mL stock solution, was administered to pigs. A stronger stock solution was used in order to reduce the total volume required to achieve a lethal dose. More concentrated solutions are likely more irritant due to their higher pH; however the reduced dose volume may be more practical to administer, particularly to larger animals. In the interest of improving animal welfare, the benefits of using a more dilute solution, or the addition of local anaesthetic, should be investigated in future studies.

B.7 CONCLUSION

The transient increase in F50 and decrease in P_{TOT} observed following IP pentobarbital injection is consistent with an acute nociceptive response. It is therefore likely that conscious piglets would perceive IP administered pentobarbital as painful in the period prior to loss of consciousness. Future investigation into ways of modifying the technique in order to enhance pig welfare is warranted.

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STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Nicola Jean Kells

Name/Title of Principal Supervisor: Dr Ngaio Beausoleil

Name of Published Research Output and full reference:

Kells NJ, Beausoleil NJ, Sutherland MA, Johnson CB. Electroencephalographic responses of anaesthetised pigs to intraperitoneal injection of sodium pentobarbital. Submitted to Animal Welfare, 4 April 2016

In which Chapter is the Published Work: Appendix B

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Nikki had a primary role in study design, data collection, statistical analysis, interpretation and writing of the paper, with guidance from supervisors.

Nikki Kells Digitally signed by Nikki Kells Date: 2017.05.12 09:16:14 +12'00'
Candidate's Signature

12/05/2017
Date

Ngaio Beausoleil Digitally signed by Ngaio Beausoleil Date: 2017.05.15 11:38:36 +12'00'
Principal Supervisor's signature

15/05/2017
Date