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Title

Docking piglet tails: how much does it hurt and for how long?

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Abstract

Tail docking in pigs has the potential for evoking short- as well as long-term physiological and behavioural changes indicative of pain. Nonetheless, the existing scientific literature has thus far provided somewhat inconsistent data on the intensity and the duration of pain based on varying assessment methodologies and different post-procedural observation times. In this report we describe three response stages (immediate, short- and long-term) through the application of vocalisation, behavioural and nociceptive assessments in order to identify changes indicative of potential pain experienced by the piglets. Furthermore, we evaluated the following procedural differences: (1) cautery vs. non-cautery docking; (2) length of tail removal. Sound parameters showed a significantly greater call energy and intensity exhibited by docked vs. sham-docked piglets ($P<0.05$). Observations of general activity of the animals in a test situation failed to detect a difference among treatments ($P>0.05$) up to 48 hr post-tail docking. Similarly, no difference in mechanical nociceptive thresholds indicative of long term pain was observed at 17 weeks following neonatal tail docking ($P>0.05$). The present results highlight the potential for the use of measures of vocalisation to detect peri-procedural changes possibly associated with evoked pain. Nonetheless, activity and nociceptive measures failed to identify post-docking anomalies, suggesting that alternative methodologies need to be implemented to clarify whether tail docking is associated with short- and long-term changes attributable to pain experienced by the piglets.

Keywords: Pig, tail docking, pain, vocalization, behavior, nociception

1. Introduction

Despite the existing ban on routine tail docking in the EU (European Directive 2001/93/EC, now codified in Council Directive 2008/120/EC), it is still a common husbandry procedure performed on more than 95% of pigs across several European Member States (EFSA, 2007, Nannoni et al., 2014). Its utility as a preventive measure to reduce the risk of tail biting later in life (Larsen et al., 2016) is undermined by the ethical concern regarding any immediate pain and distress associated with the procedure and by the possible long-term consequences experienced by piglets exposed to a painful mutilation as neonates without the provision of analgesia or anaesthesia (Sutherland and Tucker, 2011).

To address the question of pain, researchers have so far focussed on the influence of different components of the procedure on behavioural and physiological measures, considered to be indicative of ongoing pain. Investigations on procedural differences have included: cautery vs. non-cautery docking (Kells et al., 2017a, Marchant-Forde et al., 2009); inclusion of anaesthesia (Sutherland et al., 2011); age at time of docking (Bovey et al., 2014, Kells et al., 2017a) and have primarily focussed on parameters of vocalisation at the time of docking, or on measures of activity immediately post-docking. More recently, docking of tails to different lengths has been suggested as having a possible effect on procedural pain responses, namely increased likelihood of squealing with increasing removal of tail tissue (Herskin et al., 2016). However the existing literature on tail docking presents contradictory results, in particular in relation to changes in general behaviours immediately following the procedure. The seeming lack of coherence may be due to the use of different sampling time points and the inclusion of additional husbandry procedures at the time of processing (e.g. castration, ear notching, teeth clipping). Behavioural recordings have been generally limited to the period immediately following docking with the most extended observations carried out until two (Sutherland et al., 2008) and five hours (Herskin et al., 2016) post-procedure.

The question of the development of long-term, possibly chronic alterations in the behaviour or physiology of the animals is yet largely unexplored. Growth rate of the pigs has been previously utilised as an outcome indicator, with contradictory reports: a reduction in weight of piglets exposed to hot cautery docking observed up to two weeks post-procedure (Marchant-Forde et al., 2009); greater weight recorded seven weeks post-docking in hot cautery and blunt trauma docking compared to sham-cut pigs (Sutherland et al., 2008); a reduction in daily gain up to ten weeks post-procedure, which included teeth clipping and tail docking (Zhou et al., 2013). Behavioural measures to investigate the development of possible chronic pain have been reported only in one study, in which all animals were recorded from the farrowing to the growing stage, and suggested no difference in social behaviours during the nursery and grower phase, but an increase in resting time in the nursery period and an overall increase in exploratory behaviours in processed vs intact pigs (Zhou et al., 2013).

While growth rate and general behaviours are proxy measures of pain induced by tail docking, they are non-specific and linked to more than just one condition. Describing the local sensitivity of the tail region may enable detection of anomalies in sensory functionality that may implicate the presence of pain. Specific long-term physiological effects of tail docking have been suggested by recent reports on the formation of neuromas in the tail tip of pigs several weeks following the procedure (Herskin et al., 2015, Sandercock et al., 2016, Kells et al., 2017b). However, to date, no investigation has attempted to provide data on the potential effects of ongoing physiological changes using quantifiable nociceptive responses.

This study aimed at investigating the potential value of three distinct behavioural methodologies as indicators of pain at three different stages during and after tail docking: (1) the immediate reaction of the piglets to the docking, as expressed through vocalisations; (2) the acute and intermediate behavioural changes following tail-docking, as expressed through measures of spontaneous activity; (3) the long-term effects of tail docking through the assessment of changes in tail pain sensitivity.

2. Materials and Methods

2.1 Animals and housing

All animal procedures were carried out under UK Home Office License (PPL 70/7919) and approved by the Animal Welfare and Ethical Review Board of Newcastle University. All female pigs, *Sus scrofa domestica* (Landrace/Large White X synthetic sire line), belonged to the resident herd at Cockle Park Farm, Newcastle University. Three separate groups of animals were selected, with the following numbers of animals used for the three studies: 30 piglets (study 1), 72 piglets (study 2) and 24 pigs (study 3). For all studies, animals with no visible signs of injury, sickness, poor body condition, or abnormal behaviour were selected from different litters (6 piglets per litter). All animals were selected at 3 days of age and were previously subject to teeth clipping within 24 h post-farrowing.

All piglets were housed in farrowing pens measuring 1.8 m × 2.7 m, that consisted of a concrete and a partly slatted floor. Piglets had access to a creep area, which was heated by a 175 W infrared heat lamp (Interheat, Gyeonggi-do, South Korea) and had wood shavings as bedding material. Throughout the experiment, the room temperature ranged between 18 and 23°C with an 8/16 h light/dark cycle. Pigs used in study 3 were tail/sham docked and subsequently ear-tagged for identification within the first week of life (refer to study design for details). After weaning they were housed in treatment groups under standard commercial conditions for each production stage, with ad libitum access to feed and water. Animals with tail damage were not selected and the appearance of any signs of tail and body injuries at any time previous to the test determined their exclusion. During the nociception test period, they were housed in groups of 8 animals in part-slatted pens (3 m x 3 m) with a daily provision of environmental enrichment (e.g. chewable hanging toys) and fresh straw in a controlled environment room, where the temperature ranged between 18 and 20°C in the home pen. Fresh straw was provided daily.

2.2 Tail docking procedure

Tail docking was carried out according to normal commercial practice within 4-6 days of age and without the provision of anaesthesia or analgesia. At the time of docking, each piglet was picked up and restrained by a trained operator through fixation of the fore and hind legs. For study 2 and 3, a second observer recorded the total length at the lateral aspect of the tail (i.e. distance from the first proximal caudal vertebra to the tip) and drew a mark corresponding to $1/3^{\text{rd}}$ or $2/3^{\text{rds}}$ of the total length (refer to study design for details). A gas-heated docking iron (East Riding Farm Services, Driffield, UK) was applied to the tail mark and the distal proportion of the tail was removed. For study 1, tail docking was performed at $1/3^{\text{rd}}$ of the intact tail length and included docking without cautery as an additional treatment, which was carried out with 19 cm Liston Bone Cutting forceps (World Precision Instruments, Hitchin, UK). Tail docking was performed adjacent to the farrowing crate where the sow and the rest of the litter were housed.

2.3 Study 1: Vocalisation at docking

2.3.1 Experimental design

In order to evaluate the impact of different methods of tail docking on the vocalisation of piglets, the study comprised three treatment groups: intact (n=10), hot-iron docking (n=10) and non-cautery docking (n=10). A total of 5 litters were allocated to the study. Within each litter 6 female piglets were selected and individually assigned at random to one of the 3 treatments groups (i.e. all viable piglets were colour-sprayed at selection and each colour was later assigned to a treatment by a collaborator blind to the design) in order to obtain 2 piglets/treatment/litter. Sample sizes were determined by a pre-existing design developed for the study of molecular profiling of long-term changes in piglets exposed to tail docking.

2.3.2 Experimental procedure

At the time of tail docking, the calls emitted by the piglets from the moment the tail was restrained and until its release by the operator, were recorded using a Sennheiser ME 66 Shotgun Microphone (frequency response, 40–20,000 Hz ± 2.5 dB) powered by a Sennheiser K 6 battery unit (Sennheiser

Electronic GmbH & Co. KG, Wedemark, Germany), and connected to a Zoom H6 digital recorder (48 kHz sampling frequency and 16-bit quantization; Zoom Corporation, Tokyo, Japan). All recordings were stored as uncompressed WAV files (sample frequency: 44.1 kHz, resolution: 16 bit). The microphone was held at a distance of 1 m from the head of the piglet.

Vocalisations were analysed individually by a developed algorithm in Matlab® (the Mathworks Inc., Natick, MA, USA). To remove low frequency noise (frequencies containing no useful information) produced by the ventilation system and animal grunts in the experiment unit, a 6th order Butterworth filter with pass band 1000 Hz and 10000 Hz was applied. This bandwidth was selected based on previous research suggesting that it is meaningful in the context of a painful procedure (Weary et al., 1998). In this study, the features calculated for each sound were the sound pressure level (SPL) in dB, maximum frequency in Hz and total energy of sound in Pa² s. Furthermore we estimated the peak-to-peak pressure (distance between the maximum peak and trough values for each call) from the SPL curve and calculated the average per piglet (dB). The sound analysis was performed by a treatment and time point-blind investigator.

2.4 Study 2: Post-docking behaviour

2.4.1 Experimental design

To evaluate the impact of different lengths of tail removal from the piglets at docking on general behaviours, three treatment groups were designed: 'intact' (n=24), 'short dock' - 2/3rd of original length removed (n=24), 'long dock' - 1/3rd of original length removed (n=24). Piglets were selected from a total of 12 litters (6 piglets/litter) and were assigned at random (as per study 1) within litter to one of the three treatment groups. To determine immediate and long-term changes evoked by tail docking, behavioural observations were made at the following time points: 10 min pre-, immediately (30 s) post-, 24 hr and 48 hr post-docking. The latter time points were chosen in order to obtain behavioural data in immediate response to the acute trauma, and then while in the post-acute stages of inflammation associated with the tail injury. Sample sizes were determined by a pre-

existing design developed for the study of molecular profiling of long-term changes in piglets exposed to tail docking.

2.4.2 Experimental procedure

Video recordings of piglets were obtained pre- and post-docking following the procedure described in Di Giminiani et al. (2016). Briefly, the piglets were confined in pairs (one experimental and one companion littermate) in a custom-built observation arena located adjacent to the farrowing pen. The arena consisted of an opaque PVC wall and a rubber floor and was heated by an overhead IR heat lamp. A high-definition video camera (Canon Legria HF R606, Canon, Japan) was placed at a distance of 1 m above the arena and allowed filming of the two piglets for 5 min at each sampling time point. Filming piglets in pairs was adopted as a preventive measure against isolation stress. After each filming period, the piglets were returned to their home pen. All videos were analysed using ELAN annotation software (Wittenburg et al., 2006) and behavioural scores were obtained via focal continuous sampling with recording of duration of each bout of behaviour. The ethogram included the following behaviours: 'standing' (motionless, body weight supported by four legs), 'walking' (slow movement one foot in front of the other), 'sitting' (motionless, body weight supported by hind quarters and front legs), 'lying' (motionless with shoulder or sternum in contact with the floor) and 'interaction' (any behaviour directed to the other piglet). Any behaviour potentially indicative of ongoing pain, such as posterior scooting or trembling, was also recorded. The ethogram was developed based on previous reports (Herskin et al., 2016, Di Giminiani et al., 2016). Blinding to treatment was not possible at this stage since the injury was clearly visible in the recorded videos.

2.5 Study 3: Nociceptive threshold testing

2.5.1 Experimental design

The long-term impact of tail docking on mechanical nociceptive thresholds (MNT) was tested at 17 weeks following the procedure. This specific time point was chosen based on the reported presence

of neuromas in tail tissues collected from animals of similar age (Herskin et al., 2015). To evaluate the changes in MNTs determined by different extent of tail removal, pigs were allocated at the time of neonatal selection (refer to study 2 and 3 for randomisation) to three treatment groups: 'intact' (n=8), 'short dock' - 2/3rd of original length removed (n=8), 'long dock' - 1/3rd of original length removed (n=8). Sample sizes were determined by a pre-existing design developed for the study of molecular profiling of long-term changes in piglets exposed to tail docking.

2.5.2 Experimental procedure

Testing of MNTs required temporary confinement of the pigs inside a custom-made crate situated in a test room adjacent to the home pens. The crate, recently described by Di Giminiani et al. (2016), consisted of steel mesh side panels (1.8 m x 1 m) and prevented the animals from escaping and turning around. It comprised a front and a rear gate, to allow the animals to consistently enter at one end and leave from the other, and a drinker (5L volume), fixed to the front gate, which contained a 5% sucrose solution to occupy the pig during testing. A laptop computer was placed in proximity of the crate and allowed the experimenter to observe the screen while concurrently applying the stimuli. Tail MNTs were measured with a handheld digital Pressure Application Measurement (PAM) device (Ugo Basile, Varese, Italy), applied to the dorsal surface of the tail at a rate of 120 g force per second (gF/s) until a withdrawal response was induced (tail flick or tail clamp) or when the maximal cut-off force of 1500 gF was reached. All stimuli were applied to a site immediately adjacent to the injured section of the tail, at a maximum distance of one cm, and at equivalent anatomical landmarks across the three tail-length treatments (as described in Di Giminiani et al., 2017). Force application was monitored on a computer screen through dedicated software (DCA, Ugo Basile, Varese, Italy) and allowed the operator to provide a stable stimulation by matching the actual rate against a pre-set visualised value. A total of 3 individual mechanical stimuli were delivered per animal with a minimum of 30 s inter-stimulus interval.

The PAM device was consistently operated by one investigator (PDG) throughout the study. Blinding to treatment was not possible due to the constraints of the study (i.e. visible tail treatment).

2.6 Statistical analysis

All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). All data are presented as means $\pm$ SEM with significance at $P < 0.05$. Differences in sound parameters across methods of tail docking (study 1) were analysed with a linear mixed model (PROC MIXED) and Tukey's pair-wise comparison. The model included 'docking method' as fixed factor and 'litter' as random factor. Differences in behavioural variables (Study 2) following tail docking were analysed with a linear mixed model (PROC MIXED) with 'tail docking' (3 levels) as fixed factor and 'litter' as random factor. The model included baseline values (pre-docking) as covariate. A compound symmetry correlation structure was used to account for the clustering of repeated measurements across sessions. Differences in MNTs across treatment groups were analysed with a general linear model (GLM) with tail treatment (3 levels) included as a fixed factor and 'subject' as a random factor.

3. Results

3.1. Study 1: Vocalisation at docking

A preliminary comparison of the typical waveforms and spectrograms allowed detection of differences between intact, hot-iron docking and non-cautery docking treatments. Figure 1 shows an example of the sound wave and spectrogram for each treatment. Figure 2 shows an example of time-domain waveforms for pressure and sound pressure level for each treatment and describes the specific call parameters analysed. Tail docking treatment affected the total call energy produced by the piglets while being processed. Intact (sham-docked) piglets produced significantly lower levels of energy ($2.5 \pm 0.5 \text{ Pa}^2\text{sec}$) compared to animals exposed to cautery ($7.3 \pm 0.7 \text{ Pa}^2\text{sec}$; $P < 0.001$) and non-cautery docking ($8.8 \pm 0.8 \text{ Pa}^2\text{sec}$; $P < 0.001$) (Figure 3A). Similarly, sound pressure levels were different between the treatments, with significantly lower values recorded in intact ($86.2 \pm 2.3 \text{ dB}$) compared to cautery ($91.7 \pm 0.23 \text{ dB}$, $P = 0.008$) and non-cautery ($92.1 \pm 0.10 \text{ dB}$, $P = 0.005$) docked piglets (Figure 3B), but no difference recorded between the latter two treatments ($P = 0.969$). Average peak-to-peak pressure was significantly greater in intact ($15.2 \pm 1.1 \text{ dB}$) compared to

cautery (10.1 ± 0.8 dB, $P < 0.001$) and non-cautery (7.0 ± 0.6 dB, $P < 0.001$) docked piglets (Figure 3C). For this parameter, cautery compared to non-cautery treatment also differed significantly ($P = 0.007$), with lowest levels of peak-to-peak pressure recorded in non-cautery docked piglets. No difference was observed in mean ($P = 0.961$) or maximum call frequencies between the treatments ($P = 0.607$) (Table 1). In addition, the temporal characteristics of the individual call were not affected by tail docking treatment: call rate ($P = 0.665$) and average ($P = 0.258$), maximum ($P = 0.291$) and minimum ($P = 0.200$) call duration (Table 1). All procedures took on average 14 ± 2 s, from the time the piglet was restrained by the operator to its release, without a difference due to tail docking methods.

3.2 Study 2: Post-docking behaviour

No differences in any of the behavioural measures of activity were observed between piglets exposed to sham or tail docking (of either length) at any of the sampling time points (standing: $P = 0.378$; walking, $P = 0.309$; sitting, $P = 1.000$; lying, $P = 0.729$; interaction, $P = 1.000$) (Figure 4). Regardless of tail docking treatment, an effect of time was present immediately post-docking relative to pre-docking values, with a significant increase in duration of the following behavioural variables: standing ($P = 0.001$), walking ($P < 0.05$), lying ($P = 0.045$). This difference was not observed through the remaining sampling time points ($P > 0.05$). In contrast, sitting ($P = 0.081$) and interaction ($P = 0.073$) did not differ significantly over time. No instances of specific pain-related behaviours were observed in any treatment. The litter effect was not significant in any analysis ($P > 0.05$).

3.3 Study 3: Nociceptive threshold testing

MNTs recorded at 17 weeks of age did not differ significantly ($P = 1.000$) among treatments, with pigs with intact tails responding at an average threshold of 666.2 ± 93.4 gF compared to 680.7 ± 120.5 gF and 712.4 ± 35.3 gF for short- and long-docked respectively (Figure 5).

278 Discussion

279 Different experimental approaches have been previously employed to determine peri- and post-
280 procedural changes indicative of pain in piglets exposed to tail docking. Here we describe three
281 relevant sequential stages of response to the procedure: immediate (vocalisation), short-
282 (behaviour) and long-term (nociception). Vocalisation data recorded at the time of processing
283 suggested differences in features of the calls emitted by piglets exposed to docking vs sham-docking.
284 Tail docking did not appear to induce behavioural changes in a test situation up to 48 h following the
285 procedure and long-term differences in local pain sensitivity were absent at 17 weeks post-docking.

286 The present results on the features of calls produced by piglets exposed to tail docking do not
287 confirm previous observations. However, they provide novel information of potential value to the
288 evaluation of procedural vocalisations as a measure of pain, in particular the association between
289 the total energy and the intensity of the calls emitted by piglets exposed to docking. It has been
290 previously reported that tail docking induced an increase in the rate of high-pitched calls (i.e.
291 squeals/s) and mean frequencies in processed vs. non-processed piglets (Marchant-Forde et al.,
292 2009). A different study confirmed that the procedure was associated with a greater mean
293 frequency of vocalisations without however having any effect on the total call rates (Torrey et al.,
294 2009). On the contrary, a more recent report by Zhou et al. (2013) suggested that tail docking did
295 not induce differences in call frequencies, while resulting in higher call rates. This ambiguity is also
296 observed in the context of castration, with reports of similar frequencies but different call rates
297 (Weary et al., 1998, Taylor and Weary, 2000) as opposed to different frequencies but similar call
298 rates between sham and castrated animals (Puppe et al., 2005, Kluivers-Poodt et al., 2012).

299 Focussing on the energy and the intensity of the vocalisations may be a viable approach to overcome
300 the aforementioned limitations. In the present study, they appear to be effective in detecting a
301 difference in the quality of the calls produced by sham and docked animals, with greater values
302 recorded for the latter. Higher call intensities and call energy have been previously reported in

piglets exposed to castration compared to sham-castrated animals (Marx et al., 2003, Kluivers-Poodt et al., 2012). In the context of tail docking, only one study has thus far focussed on the call intensity and failed to discover any differences between processed and unprocessed animals (Zhou et al., 2013). However, vocalisations were recorded during the combined exposure of piglets to tail docking and teeth clipping. Future research should address the efficacy of this parameter in the context of tail docking, with the potential of achieving a level of sensitivity sufficient to identify procedural differences (e.g. different docking methods). Average values of call energy and peak-to-peak pressure levels may imply a greater impact of non-cautery docking on call outputs.

Behavioural and physiological consequences of different methods of docking have been addressed by a limited number of studies, which recorded no difference in plasma cortisol levels and in acute phase proteins (Prunier et al., 2005, Sutherland et al., 2009), a transient increase in cortisol concentration observed only 90 minutes following non-cautery docking (Sutherland et al., 2008), no difference in histomorphological changes (i.e. formation of traumatic neuromas) in cautery compared to non-cautery tail-docked animals (Kells et al., 2017b), and a difference, although non-significant, in characteristics of vocalisation (Marchant-Forde et al., 2009). In particular, greater call frequencies and call rates have been recorded from piglets docked with cautery compared to a non-cautery method (Marchant-Forde et al., 2009). These differences have been primarily attributed to the longer duration of cautery docking. In the current study both docking procedures lasted the same amount of time hence other procedural factors (e.g. prolonged handling and restraint of the animals) did not influence our results.

If tail docking by cautery is performed promptly and effectively, an immediate desensitisation of the skin nociceptors around the site of injury may be expected due to the intense heat of the hot iron, thus reducing subsequent pain signalling (Junger et al., 2002). The results from this study confirm that cautery docking evokes a painful response of lower magnitude than the non-cautery method. The heated blade facilitates the destruction of soft tissues, with an almost simultaneous severing of

caudal nerve trunks. The absence of heat in the non-cautery method may imply a non-synchronous truncation of the nerves, possibly leading to greater perceived pain.

The post-procedural results from this study appear to add to the uncertainty of the existing literature. Tail docking, regardless of length of tail removal, did not induce significant changes in duration of walking, standing, lying, sitting or interactive behaviours, in line with previous reports (Torrey et al., 2009, Zhou et al., 2013). Furthermore, no other behavioural variables possibly indicative of acute pain (e.g. posterior scooting, trembling) were identified. It is important to bear in mind that the present and the previous investigations comprised different experimental conditions: here, the animals were observed while removed from the sow and the rest of the litter, whereas other reports included more husbandry procedures at the time of processing. Caution is needed in interpreting the results due to the specific behavioural recording setup and consequent changes evoked by the exposure to a novel environment.

The observation arena was intended to reduce confounding effects on the behavioural observations, such as activity directed towards the sow or other littermates. Nevertheless, filming the piglets in a condition of temporary separation from the sow may have introduced an element of stress sufficient to conceal the exhibition of any pain-related changes. Stress-induced analgesia (i.e. a mammalian suppression response occurring during or following exposure to a stressful or fearful stimulus (Butler and Finn, 2009)), may have modulated the perception of pain. Social isolation has been previously linked in laboratory rodents to increased release of opioids and consequent decreased sensitivity at the opiate receptor level (Puglisialleggra and Oliverio, 1983). Similarly, single exposure to temporary isolation has been demonstrated to be sufficient to stimulate behavioural arousal, neuroendocrine stress hormones, and stress-related gene expression in the porcine brain (Kanitz et al., 2009). In the present study, stress was evident in the first exposure to separation from the sow and the rest of the litter (pre-docking), with frequent escape attempts and longer duration of walking behaviour (i.e. exploratory activity). Nevertheless, the reduction in walking and the increase in lying behaviour

observed in all treatments in the following exposures to the observation arena could signify habituation to the experimental setup and a consequent reduction in stress. Furthermore, the presence of a familiar conspecific may have resulted in the reduction of stress-induced HPA activity, possibly alleviating the stress response and acting as a potential buffer through social support (Tuchscherer et al., 2014, Kanitz et al., 2016).

Having considered the specific features of the study and the possible confounding effects, we cannot exclude that the behavioural variables recorded in this study may not have been appropriate to detect changes induced by pain arising from tail docking. Differences in duration of active or resting behaviours have not thus far provided unequivocal evidence of changes primarily induced by the discomfort or pain associated with tail docking. Researchers have focussed on more complex interactions, such as behaviours directed towards the sow (e.g. nursing), social interaction with other piglets (e.g. lying in contact, aggression) or changes in relation to environmental factors (i.e. time spent in heated creep area) (Sutherland et al., 2008; Herskin et al., 2015). In the current study these behavioural variables were precluded by the experimental setup, except for interaction with the conspecific, which failed to detect differences among treatments.

To the best of our knowledge, this is the first investigation focussing on behavioural consequences of tail docking for a period exceeding 2-5 hours post-procedure. Only one study has reported changes in the amount of time spent by piglets in different locations of the pen, particularly in the creep area, observed up to 5 hours following tail docking (Herskin et al., 2015). Once again, the behavioural variables reported here failed to detect any difference up to 48 hr post-procedure. More investigation is needed to integrate those behavioural variables that have so far proved useful in the evaluation of pain related to tail docking and to extend their recording to several hours or days following the injury. In addition to the question of the longitudinal characteristic of possible pain indicators following tail docking, this study attempted to assess differences in animal responses induced by two lengths of tail removal. The rationale behind this investigation was based on

378 previous indication that the extent of tail removal influenced differences in behavioural responses of
379 the piglets (Herskin et al., 2015). However, the current results did not demonstrate any difference
380 between 'short' and 'long dock' and more sensitive outcome measures are required to further assess
381 this possibility.

382 Long-term effects of tail docking in pigs have not been sufficiently addressed in previous research to
383 assess whether an amputation performed early in the life of the animals can evoke a potentially
384 chronic painful condition. The results of this study, namely the absence of differences in MNTs
385 between docked and intact pigs, seem to confirm previous observations on pain sensitivity assessed
386 in the tail root of 8 week-old pigs (Sandercock et al., 2011), despite the different proximity of the
387 test site to the area of insult (stump vs tail root). Although the absence of peripheral hypersensitivity
388 by itself is not sufficient to exclude changes in pain processing typical of central sensitisation
389 (Latremoliere and Woolf, 2009, Woolf, 2011), it may appear to contradict claims that docking shortly
390 after birth causes chronic pain and subsequent long-term welfare detriment to the animals. Recent
391 reports on the sustained neuroanatomical changes occurring in injured tails, explicitly the formation
392 of neuromas up to 22 weeks following tail docking (Herskin et al., 2015, Sandercock et al., 2016), and
393 on the emergence of sustained sensitisation in non-neonatal pigs exposed to surgical tail amputation
394 (Di Giminiani et al., 2017) prompted investigation of the association with MNTs. Amputation injuries
395 in humans result in neuromas and can consequently induce changes in somatosensory nerve
396 function, manifested through increased mechanical sensitivity (Wall and Gutnick, 1974, Nikolajsen et
397 al., 1996, Hsu and Cohen, 2013). The lack of hypersensitivity described here does not seem to
398 support this hypothesis in the specific context of porcine neonatal tail docking. However, the small
399 sample size warrants caution in interpreting our results.

400 The effects of peripheral nerve injury on somatosensory processing and pain are known to depend
401 upon the age at the time of the insult in both humans and animals (Fitzgerald and McKelvey, 2016).
402 A major consequence of nerve damage in adult humans and laboratory animals is the onset of pain

hypersensitivity, typical of a neuropathic pain condition (Gilron et al., 2015). Remarkably, the same symptoms are not observed when nerve damage occurs in rat pups (Howard et al., 2005) or they appear only later in life (McKelvey et al., 2015). In humans, natural developmental changes in the neuro-immune system have been indicated as responsible for the late emergence of hypersensitivity (Fitzgerald and McKelvey, 2016). In light of these considerations, the absence of tail hypersensitivity in 17 week-old docked pigs observed in this study may therefore suggest that the injury has no long-term effects on local sensitivity or that the sampling age did not fall within the age range that would have allowed detection of a delayed onset of hypersensitivity. The current knowledge on long-term consequences of early post-natal injuries in both animals and humans is still limited (Walker et al., 2016) therefore more research is needed, in particular targeting the longitudinal profiling of sensory activity at regular time intervals.

5. Conclusions

The results presented in this report suggest that tail-docking evoked an immediate response expressed through vocalisations of greater energy and intensity produced by processed vs. non-processed piglets. Behavioural measures of activity in a standardised test failed to detect any differences between intact and docked piglets for a period of up to 48 hrs, possibly indicating that the procedure is not associated with short-term pain or that behavioural approaches may not be sufficiently sensitive techniques in this specific context. It cannot, however, be excluded that the sampling regime described here may have prevented identifying behavioural differences occurring outside of the limited observation periods. Alternative experimental approaches are desirable to identify measures more effective in detecting pain-related alterations. For example, the analysis of molecular mediators of pain signalling may be useful to detect changes in peripheral somatosensory nerve function normally observed in the presence of painful injuries. Finally, the absence of long-term differences in peripheral tail sensitivity may suggest that the injury and the associated physiological anomalies are resolved before the animals reach 17 weeks of age. Future investigations are needed to describe the temporal profile of the injury, in order to identify the initial development

and the duration of any sustained changes in peripheral sensitivity and to determine whether long-term pain is associated with neonatal tail docking in pigs.

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Conflict of interest

No conflict of interest to report.

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Figure 1. Sound wave (top) and spectrogram (bottom) of an intact (A), hot-iron docking (B) and non-cautery docking (C) of piglet.

Figure 2. Example of time-domain waveforms for pressure (top) and Sound Pressure Level (bottom) recorded in one intact (A), one hot-iron cautery docked (B) and one non-cautery docked piglet (C). The numbers in A refer to the specific call parameters analysed: (1) peak-to-peak change in sound pressure level (dB); (2) duration of an individual call (s).

Figure 3. (A) Total call energy (B) Sound Pressure Level and (C) peak-to-peak change ($\pm$ SEM) in pressure of calls emitted by the piglets while being tail-docked. Piglets belonged to the 'intact' (n=10), 'cautery' (n=10) and 'non-cautery' (n=10) treatment group. Different letters denote a statistically significant difference ($P < 0.05$).

Figure 4. Mean duration of behaviours ($\pm$ SEM) performed by piglets in the observation arena out of a total of 5-min observations pre-, immediately post-docking and at 24 and 48 hr post-docking. A: Standing; B: Walking, C: Sitting; D: Lying; E: Interaction with accompanying piglet. Piglets belonged to the 'intact' (n=24), 'short dock' (n=24) and 'long dock' (n=24) treatment group.

Figure 5. Mean mechanical nociceptive threshold ($\pm$ SEM) recorded proximal to the site of tail docking at 17 weeks post-procedure. Pigs belonged to the 'intact' (n=8), 'short dock' (n=8) and 'long dock' (n=8) treatment group.

603 **Table 1.** Characteristics of vocal responses of piglets in study 1 ($\pm$ SEM).

Treatment	Average call duration (s)	Minimum call duration (s)	Maximum call duration (s)	Average call rate (calls/s)	Maximum frequency (Hz)	Mean frequency (Hz)
<i>Intact</i>	0.5281 $\pm$ 0.11	0.348 $\pm$ 0.11	0.7148 $\pm$ 0.12	1.12 $\pm$ 0.51	3739 $\pm$ 1325	2899 $\pm$ 431
<i>Cautery</i>	0.6795 $\pm$ 0.08	0.412 $\pm$ 0.05	0.9551 $\pm$ 0.13	0.99 $\pm$ 0.09	3341 $\pm$ 555	2996 $\pm$ 248
<i>Non-cautery</i>	0.7084 $\pm$ 0.04	0.522 $\pm$ 0.04	0.9099 $\pm$ 0.06	1.06 $\pm$ 0.15	3558 $\pm$ 520	3028 $\pm$ 304
<i>p-value</i>	0.258	0.200	0.291	0.665	0.855	0.961

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Figure 1A
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