

Post-natal development of EEG responses to noxious stimulation in pigs (*Sus scrofa*) aged 1–15 days

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Abstract

This study examined electroencephalographic (EEG) indices of acute nociception in pigs (*Sus scrofa*) aged 1, 5, 7, 10, 12 and 15 days, post-natal. Ten pigs per age were anaesthetised with halothane in oxygen and maintained at a light plane of anaesthesia. EEG was recorded bilaterally using a five-electrode montage. Following a 10-min baseline period, tails were docked using side-cutter pliers and recording continued for a further 5 min. Changes in the median frequency (F50), 95% spectral edge frequency (F95) and total power (P_{TOT}) of the EEG were used to assess nociception. Tail-docking at one 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 ten days of age, with five and seven 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 post-natal age, suggesting there may be qualitative differences in pain perception between age groups. Further, the data provide some support for current recommendations that tail-docking and other painful husbandry procedures be performed within seven days of birth in order to minimise their impact on animal welfare.

Keywords: animal welfare, EEG, nociception, pain, pig, tail-docking

Introduction

Tail-docking is commonly performed on commercial pig (*Sus scrofa*) farms to reduce the incidence of tail-biting behaviour, which can have severe welfare consequences for affected animals. The procedure is typically performed within seven 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, in New Zealand, it is recommended that tail-docking of pigs be performed within 72 h of birth and minimum standards require the provision of analgesia for pigs aged seven days or over (Anonymous 2018). Similarly, both Australia and the UK recommend that tail-docking of pigs be performed within seven days of birth, with the latter stipulating that analgesia be provided for pigs > 7 days of age (Commonwealth Scientific and Industrial Research Organisation [CSIRO]

2008; Council of the European Union 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 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 (*Equus caballus*) (Murrell *et al* 2003), cattle (*Bos taurus*) (Gibson *et al* 2007), sheep (*Ovis aries*) (Johnson *et al* 2005a) and pigs (Haga & Ranheim 2005). These studies all reported transient increases in median frequency (F50) and decreases in total power (P_{TOT}) of the EEG following application of a known noxious stimulus. 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 two and 20 day old pigs to tail-docking (Kells *et al* 2017b) and identified differences in the magnitude and duration of EEG responses to tail-docking between the two ages. This led us to question how responses to noxious stimulation develop over the early post-natal period in pigs.

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.

Materials and methods

This 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 \times 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. A sample size of ten animals per age group was selected based on previous studies using the same methodology, whereby statistically significant differences were obtained using groups of ten pigs (Kells *et al* 2017a,b), ponies (Murrell *et al* 2005) and calves (Gibson *et al* 2007). Pigs had not previously undergone any potentially painful husbandry procedures (eg 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. The duration of travel from the farm to the laboratory was approximately 30 min. The maximum interval between time of collection from the farm and induction of anaesthesia was 320 min.

Experiments were conducted on 18 separate test days, with 2–4 pigs from a single litter tested per day. The mean (\pm SD) interval between collection from the farm and induction of anaesthesia for pigs tested first, second, third, or fourth on a given day was 86.8 (\pm 11.4), 142 (\pm 13.7), 203 (\pm 25.2) and 266 (\pm 27.9) min, respectively.

Anaesthesia

An established minimal anaesthesia model (MAM) was followed (Murrell & Johnson 2006). Pigs were anaesthetised with halothane (Halothane-Vet, Merial NZ Limited, Manukau City, New Zealand) vaporised in oxygen (4 L min⁻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 CO₂ tension, SpO₂ 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, UK) 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, Sarasota, FL, USA). The signals were amplified with a gain of 1,000 and a band-pass of 1.0–500 Hz and digitised at a rate of 1 kHz (Powerlab 4/20, ADInstruments Ltd, Colorado Springs, CO, USA). The digitised signals were recorded on an Apple Macintosh personal computer for off-line analysis at the conclusion of the experiment.

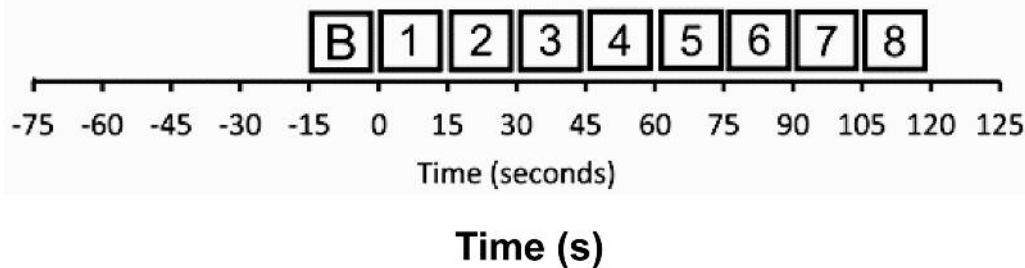
Experimental procedure

Once end tidal halothane tension was stable at 0.95–1.05%, 10 min 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 min 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⁻¹; Pentobarb 500, Provet NZ Pty Ltd, Auckland, New Zealand) administered whilst pigs were still under general anaesthesia.

Data analysis

EEG data from only the right cerebral cortex were analysed. Although EEG was recorded bilaterally, previous studies using the MAM have demonstrated equivalency in spectral EEG between hemispheres (Murrell *et al* 2007, 2010; McIlhorne 2011), suggesting data from either hemisphere

Figure 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.

alone are suitable for analysis. Data from the left cortex were collected for use in the event that right cortex data were unsuitable for analysis due, for example, 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-s epochs, using purpose-written software (Spectral Analyser, CB Johnson, Massey University, Palmerston North, New Zealand). Fast Fourier transformation was applied to each epoch, generating sequential power spectra with 1 Hz frequency bins.

Statistical analysis

All statistical analyses were performed in SAS version 9.3.1 (SAS Institute Inc, Cary NC, USA). Plots of standardised residuals versus predicted values were evaluated to test the assumption of normally distributed within-group errors, centred at 0 with constant variance. The residuals for heart rate, F50, F95 and P_{TOT} were found to approximate normal distribution and, thus, were considered suitable for parametric analysis.

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 s 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 within age, and test order as fixed effects.

Analysis of all ages combined

Analysis of baseline EEG demonstrated a significant age effect on EEG variables. In order to account for differences in baseline EEG 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-s 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 s of the baseline recording period and multiplying the product

by 100. EEG data for consecutive 15-s blocks (up to 120 s) after tail-docking were then compared to those from the final 15 s 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 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 within 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.

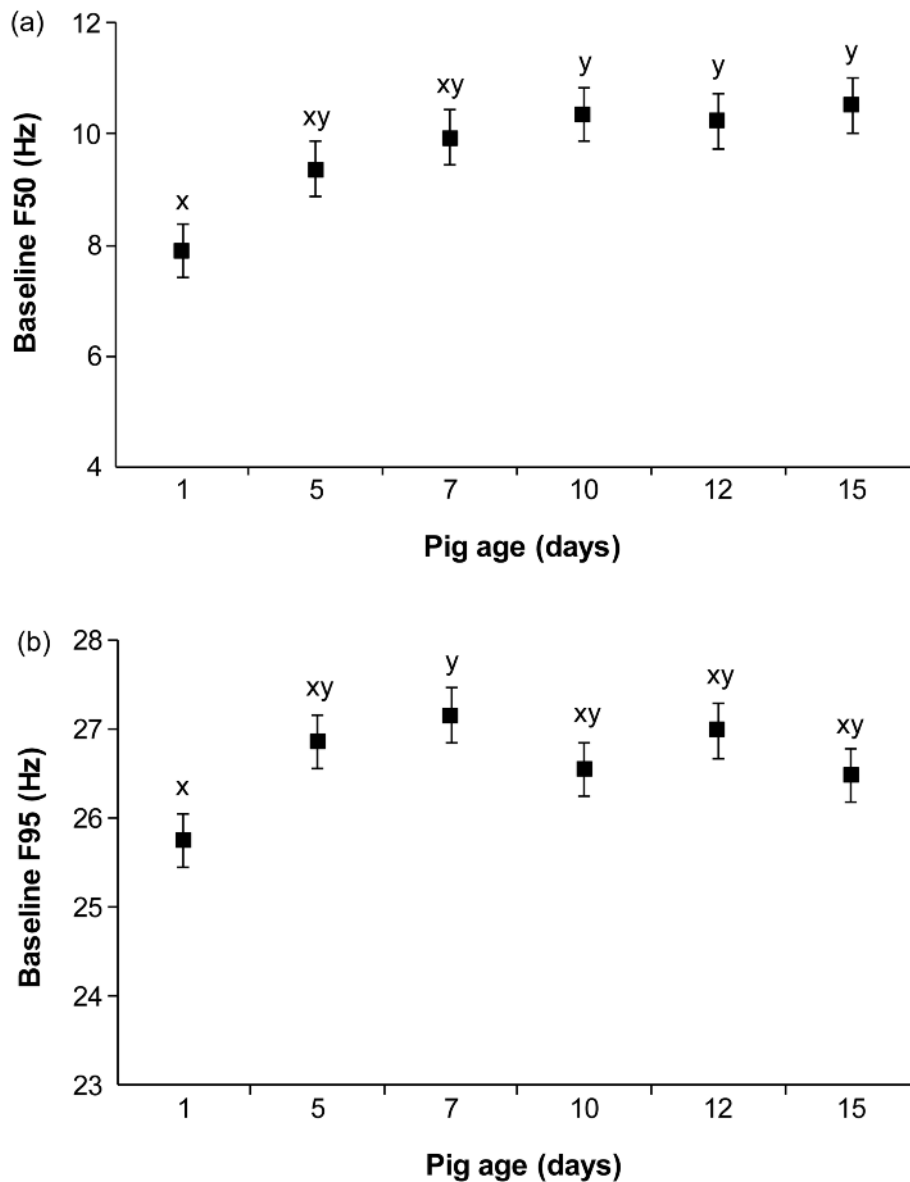
Analysis of each age separately

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. 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 within age block and order of testing as fixed effects, pig as a random effect, and time as a repeated measure.

Figure 2



Comparison of baseline (a) F50 and (b) F95 of the EEG of pigs aged one, five, seven, ten, 12 and 15 days of age. Data are presented as mean (\pm SEM). Superscripts denote significant differences between means (Bonferroni adjusted $P < 0.05$).

Table 1 Effects of age, time after tail-docking, order of testing and litter on the median frequency (F50), 95% spectral edge frequency (F95) and total power (P_{TOT}) of the EEG following tail-docking in 60 pigs aged 1–15 days. Results are based on analyses of transformed (% baseline) data.

	Age		Time		Order		Litter (Age)		Age \times Time	
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
F50	4.82	0.002	9.67	< 0.001	1.12	0.114	1.62	0.128	1.31	0.102
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

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. Periods affected by artefact ranged from 1–8 (mean = 4.6) consecutive epochs. Within each individual recording, periods containing artefact were excluded from subsequent analyses. Movement responses to tail-docking were observed in seven five day old, one seven day old, six ten day old, three 12 day old and four 15 day old pigs. No movement response to tail-docking was observed in one day old pigs.

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 one day old than ten, 12 or 15 day old pigs, but did not differ between other age groups (Figure 2). Baseline F95 was lower in one day old pigs than seven day old pigs but did not differ between other age groups (Figure 2). Test order 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 ($P = 0.02$), with no difference between those tested first and third, first and fourth, second and third, second and fourth, or third and fourth.

Combined analysis

Piglet age at the time of tail-docking had a significant effect on all three EEG summary variables, with significant age \times time interaction effects found for F95 and P_{TOT} (Table 1).

Median frequency (F50)

Overall, mean F50 was lower in one day old pigs than five or 15 day old pigs (102.31 [± 1.63] versus 110.18 [± 1.63]%) ($P = 0.006$) and 108.8 [± 1.63]% [$P = 0.037$], respectively) and lower in seven than five day olds (102.63 [± 1.76] versus 110.18 [± 1.63]%) [$P = 0.003$]). There was a significant effect of time on F50, with F50 being elevated, relative to baseline, from 15–105 s after docking ($P \leq 0.02$). Mean F50 did not differ significantly between ages at any individual time-point (no interaction between age and time).

Spectral edge frequency (F95)

F95 was elevated relative to baseline in ten day old pigs from 15–75 s after docking ($P < 0.01$). Comparison at individual time-points revealed that mean F95 was higher in ten day old pigs than one, five, seven or 12 day old pigs, 30 and 45 s after tail-docking (Figure 3[a]). Test order (1st, 2nd, 3rd or 4th) 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 from 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 each age.

Total power (P_{TOT})

A reduction in P_{TOT} after docking was observed in five, ten, 12 and 15 day old pigs. P_{TOT} was lower than baseline 15 s after docking in five, 12 and 15 day old and from 15–45 s after docking in ten day old pigs ($P < 0.01$). Comparison at individual time-points revealed that P_{TOT} was lower in ten and 12 day old pigs than one day old pigs 45 s after docking (Figure 3[b]). P_{TOT} did not differ between age groups over the period 60–120 s 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 each age.

Analysis of each age separately

Neither test order nor litter significantly influenced the EEG of pigs at any age. Time significantly influenced F50 at one, seven, ten and 15 days old, and P_{TOT} at all ages except seven days (Table 2). Only ten day old pigs exhibited a significant change in F95 over time after docking.

One day old pigs

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

Five day old pigs

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

Seven day old pigs

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

Ten day old pigs

Mean F50 was lower than baseline 15 s after docking ($P = 0.03$). F95 was higher than baseline 15, 30, 45 (all $P < 0.01$) and 60 ($P = 0.01$) s after docking. P_{TOT} was lower than baseline 15, 30 (both $P < 0.01$) and 45 ($P = 0.04$) s after docking.

Twelve day old pigs

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

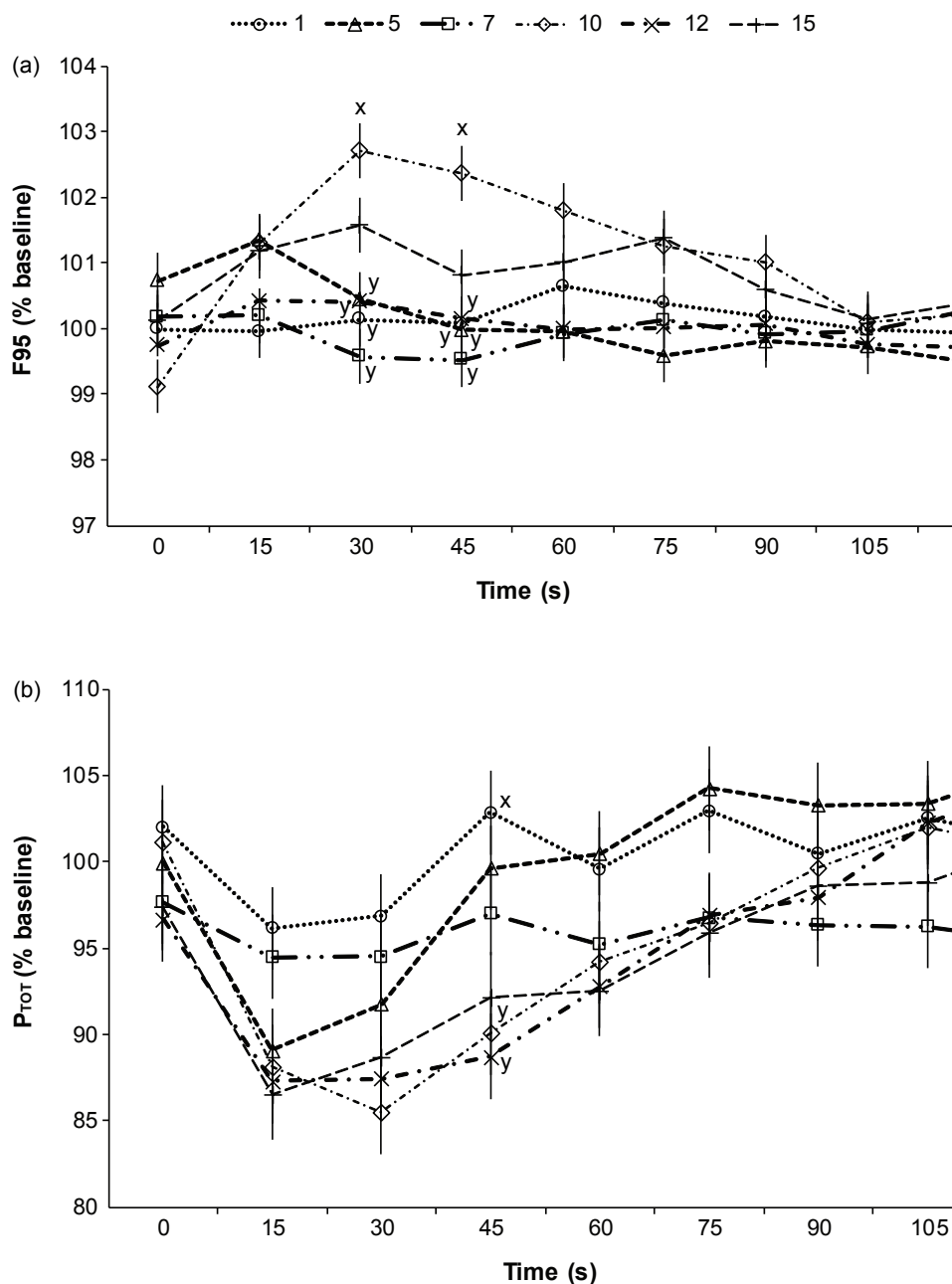
Fifteen day old pigs

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

Analysis of age-blocked data

Statistical results are presented in Table 3.

Figure 3



Comparison of the changes in mean (\pm SEM) (a) F95 and (b) P_{TOT} of the EEG following tail-docking (time 0) in pigs aged one, five, seven, ten, 12 and 15 days. Data are shown as percentages of baseline mean. Means at the same time-points with different superscripts differed significantly (Bonferroni adjusted $P < 0.05$).

Median frequency (F50)

There were significant effects of age and time on piglet F50 (Table 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 s after docking (all $P \leq 0.01$). 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 \times time effect, and a significant effect of test order on F95 (Table 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 s after docking ($P < 0.05$) (Figure 4). F95 was higher in pigs aged > 7 days than those aged ≤ 7 days 30 ($P < 0.001$) and 45 ($P = 0.014$) s after docking but did not differ between age groups over the period 60–120 s after

Table 2 Effect of time after tail-docking on the median frequency (F50), 95% spectral edge frequency (F95) and total power (P_{TOT}) of the EEG following tail-docking in ten pigs aged one, five, seven, ten, 12 and 15 days ($n = 60$ pigs in total).

Age (days)	F50		F95		P_{TOT}	
	F-value	P-value	F-value	P-value	F-value	P-value
1	2.91	0.01	0.29	0.97	2.93	0.01
5	1.57	0.15	1.45	0.19	5.20	< 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

Table 3 Effects of age, time after docking, and their interaction on the median frequency (F50), 95% spectral edge frequency (F95) and total power (P_{TOT}) of the pig EEG following tail-docking, using data grouped into ≤ 7 ($n = 30$) or > 7 ($n = 30$) days of age.

	Age		Time		Order		Litter (Age)		Age \times Time	
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
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

docking (Figure 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 \times time effect, and a significant effect of litter within age block on P_{TOT} . P_{TOT} was lower than baseline from 15–30 s after docking in pigs aged ≤ 7 days, and from 15–45 s 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 ($P < 0.001$) s after docking, but did not differ between groups over the period 60–120 s after docking (Figure 4). Of the nine litters aged > 7 days, P_{TOT} was lower in pigs from litter 12 than from litter 13 ($P = 0.007$).

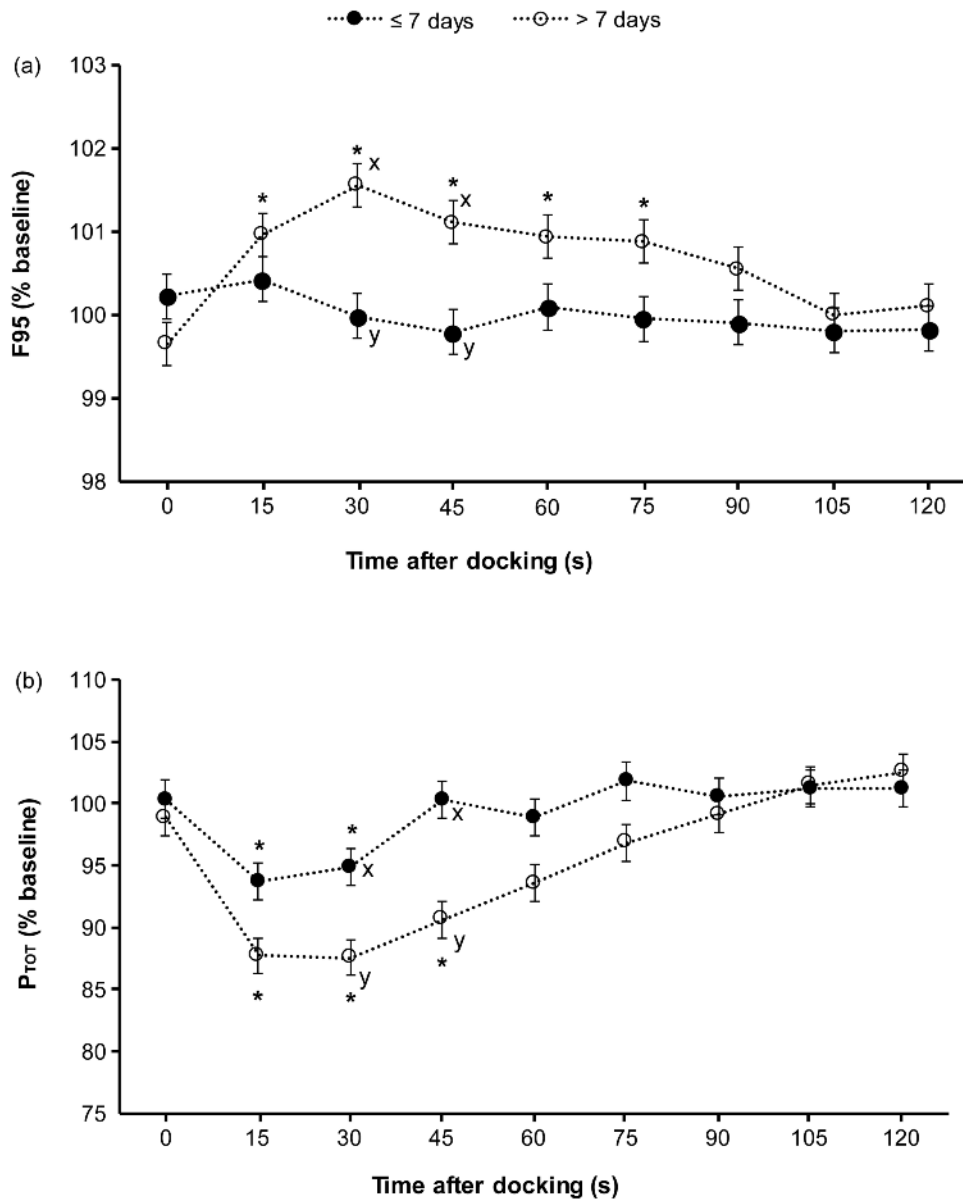
Discussion

Previously, we identified differences between the EEG responses of two and 20 day old pigs to the noxious stimulus of tail-docking (Kells *et al* 2017a). The aim of the present study was to examine EEG responses to tail-docking in pigs aged between one and 15 days of age, to determine the manner in which cortical responses to acute 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; Johnson *et al* 2005b; Murrell *et al* 2005; 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 post-natal 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 post-natal age, with an overall pattern emerging of increased responsiveness with increasing post-natal age.

At one 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 one day of age similarly found no significant differences in plasma ACTH or cortisol between docked and control pigs, leading the authors to conclude tail-docking is not noxious at one day old (Prunier *et al* 2005). In addition, we identified differences in baseline (resting state) EEG between ages. Although P_{TOT} did not differ

Figure 4



Comparison of mean (a) F95 and (b) P_{TOT} of the EEG in pigs aged ≤ 7 days (comprised of data from one, five and seven day old pigs) or > 7 days (comprised of data from ten, 12 and 15 day old pigs) following tail-docking at time 0. Asterisks indicate mean differed from baseline within age group (Dunnett's $P < 0.05$). Superscripts indicate differences between age groups at common time-points (Bonferroni adjusted $P < 0.05$).

between ages, F50 was significantly lower at one 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 one day of age.

Whilst, at one day of age, the cerebral cortex did not respond to noxious stimulation, pigs aged between five and 15 days exhibited at least some elements of a characteristic nociceptive response. From ten post-natal 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* 2017a) and in other adult mammals in response to

noxious stimulation (eg Johnson *et al* 2005b, 2009; Kongara *et al* 2010); ie an increase in F50 and decrease in P_{TOT} . Notably, at 12 days old, there was no significant increase in F50 after docking, although P_{TOT} decreased. This was unexpected, given that F50 increased after docking in ten and 15 day old pigs in the present study, and in 20 day old pigs in previous studies (Kells *et al* 2017a,b). 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 ten 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 two day old pigs following tail-docking (Kells *et al* 2017b). 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 EEG amplitude and higher EEG 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 one and 10–15 day old pigs, it was lower in the one day olds, thus not indicative of a reduced state of anaesthesia. Nevertheless, there would be value in determining age-specific halothane MAC for pigs in future studies.

Based on previous data collected from two and 20 day old pigs undergoing tail-docking (Kells *et al* 2017b), we anticipated seeing significant changes in two or more EEG variables in pigs docked at all interim ages. This was not the case. In particular, the failure to see any changes in F50 at either five or 12 days of age was unexpected. The observed decrease in F50 at seven days old was consistent with the previously observed decrease in F50 in pigs docked at two days old (Kells *et al* 2017b). The biphasic response seen at ten days (initial decrease, followed by an increase) suggests a transition toward the characteristic increase in F50 observed in 15 and 20 day old pigs and other adult mammals. Similarly, given the reductions in P_{TOT} seen after docking at five, ten, 12 and 15 days of age, the absence of such a response in seven day old pigs was surprising. Again, there is no obvious explanation for this — no changes in

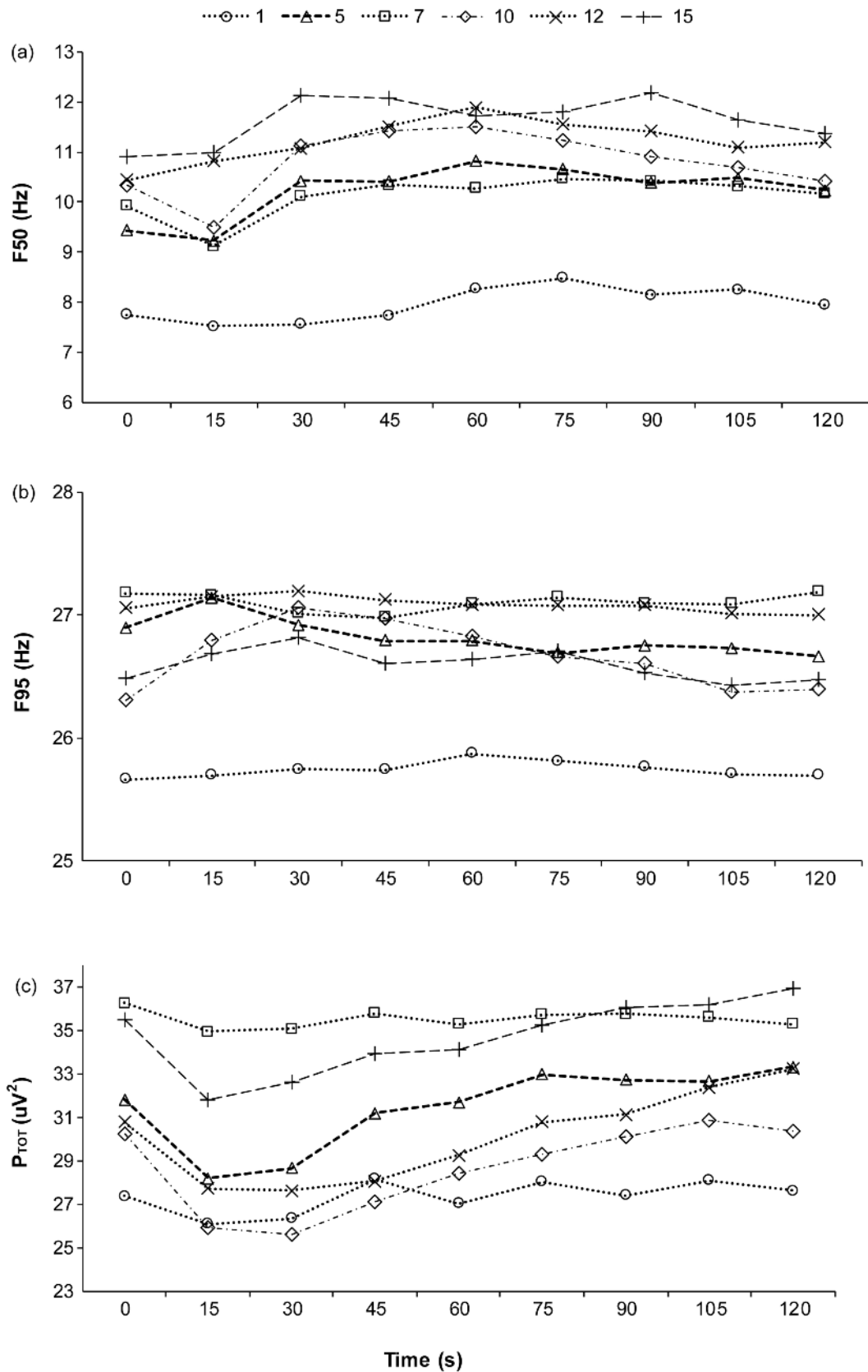
diet, management or other on-farm practices that might have influenced pig responses were implemented at any time within the age range examined.

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 five, seven and ten days exhibited a decrease in mean F50 relative to baseline (Time 0) immediately after docking, followed by a subsequent increase above baseline mean, peaking 45–60 s after docking (Figure 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 ten and 15 days exhibited an increase in F95, peaking 30 s after docking, whereas all other ages showed little change (Figure 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 ten days and older (Figure 5). Thus, overall EEG responsiveness to tail-docking appeared to increase with increasing age, with pigs aged ten 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. 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). This is supported by data from sheep, in which the magnitude of changes in the EEG frequency spectrum correlated well with behavioural responses to noxious stimuli (Ong *et al* 1997) and in man, where the magnitude of EEG changes correlated with reports of pain intensity in response to graded noxious stimuli (Chen *et al* 1989).

Therefore, the greater magnitude of EEG responses observed in the older group suggests that the qualitative perception of tail-docking is different between the two age groups and that tail-docking without analgesia 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 some support for New Zealand and Australian recommendations that if tail-docking is to be performed without analgesia, this should occur within the first week of life (CSIRO 2008; Anonymous 2018) and for UK and New Zealand policy requiring the use of anaesthesia and analgesia in pigs aged seven days and older (Council of the European Union 2008; Anonymous 2018).

Figure 5



Qualitative comparison of the changes in mean (a) F50, (b) F95 and (c) P_{TOT} of the EEG following tail-docking (time 0) in pigs aged one, five, seven, ten, 12 and 15 days. For ease of distinguishing between ages, non-transformed data are presented, and standard errors omitted.

In the present study, order of testing had a significant effect on baseline F50. Test order reflects the elapsed time between piglet collection from the farm and induction of anaesthesia, therefore also reflecting the length of time since the last feed from the sow. As a result, piglet nutritional status might have varied across test order. However, the effect of test order on F50 was non-linear, ie did not show a consistent increase or decrease with increasing test order. As such, it is likely that the observed effect was random, rather than a consequence of time off feed. This is further supported by the absence of order effects on baseline F95 or P_{TOT} .

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 post-natal 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 post-natal 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 post-natal 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 post-natal days. It is well known that cortical development continues post-natally 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 pre-natal and 40 days post-natal (Dickerson & Dobbing 1967).

An earlier study investigating the effects of post-natal age on EEG responses to castration in lambs which, like pigs, are born neurologically mature, identified an increase in cerebral responsiveness to noxious stimulation over the first 7–10 days of life (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, a number of 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 pregnanalone and allopregnanalone were found to be significant up to three days after birth (Nguyen *et al* 2003), leading to the suggestion that these chemicals may continue to exert some cerebral effects in the early post-natal 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 one day post-natal supports the presence of neurosuppressive mechanisms acting to inhibit cerebral processing of nociceptive stimuli. The increasing cortical responsiveness observed over the period 5–15 post-natal days might be explained, in part, by the withdrawal of these neurosuppressive mechanisms. In addition, it is likely that post-natal 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 post-natal age may be a result of post-natal cortical development, combined with the gradual withdrawal of neurosuppressive mechanisms.

Regardless of whether, or for how long, neurosuppressive mechanisms remain active in the period following birth, information regarding the long-term consequences of noxious stimulation in the neonatal pig must also be considered before any recommendations are made. In humans, noxious stimulation in the very early post-natal period has been associated with increased reactivity to later painful stimuli (Taddio *et al* 1997; Grunau 2013). A similar phenomenon was observed in lambs, whereby those castrated at one day of age exhibited greater behavioural responses to subsequent tail-docking than those castrated at ten days (McCracken *et al* 2010). The presence and extent of any such phenomenon in pigs should be investigated.

In addition to the acute pain associated with tissue damage itself, piglets may experience short-term post-procedural pain (hours to days) after tail-docking without analgesia, as a result of peripheral and central sensitisation (Woolf 2011; Pogtzki-Zahn *et al* 2017). Such sensitisation can still occur when pain perception is prevented, eg by the use of general anaesthesia, which is why multimodal analgesia, in addition to general anaesthesia, is recommended for animals undergoing painful surgical procedures in veterinary practice (Flecknell 2008). As such, the absence of acute EEG responses to noxious stimulation in one day old pigs in the current study does not preclude the development of subsequent short-term pain. The development of post-procedural pain after tail-docking was not investigated in the present study.

In conclusion, we identified an increase in cerebral responsiveness to the noxious stimulus of tail-docking with increasing post-natal age in pigs. This may be due to both the persistence of fetal neurosuppressive mechanisms in the first days of post-natal life, along with rapid cerebrocortical development after birth. These findings suggest there may be qualitative differences in pain perception as a function of post-natal age in pigs during the first two weeks of life. Although cortical responses to acute noxious stimulation were not observed at one day of age, the precise implications of this finding, in terms of pain perception, requires further investigation. Furthermore, investigation of the potential longer-term consequences of early noxious stimulation on later pain perception in the pig is needed.

Animal welfare implications

Whilst the absence of an acute nociceptive response to tail-docking at one day of age suggests that concerns about painful husbandry practices may be greater when their application is delayed after birth, analgesia is, given our current state of knowledge, advisable at any age.

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