

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

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13
14 **Running title:** Post-natal development of nociception in pigs

15
16 **Abstract**

17 *This study examined electroencephalographic (EEG) indices of acute nociception in*
18 *pigs (Sus scrofa) aged 1, 5, 7, 10, 12 and 15 days, post-natal. Ten pigs per age were*
19 *anaesthetised with halothane in oxygen and maintained at a light plane of*
20 *anaesthesia. EEG was recorded bilaterally using a five-electrode montage. Following*
21 *a 10-min baseline period, tails were docked using side-cutter pliers and recording*
22 *continued for a further 5 min. Changes in the median frequency (F50), 95% spectral*
23 *edge frequency (F95) and total power (P_{TOT}) of the EEG were used to assess*
24 *nociception. Tail-docking at one day of age induced no significant changes in the*
25 *EEG spectrum. A typical nociceptive response, characterised by an increase in F50*

26 *and decrease in P_{TOT}, was evident at ten days of age, with five and seven day old pigs*
27 *exhibiting responses in either F50 or P_{TOT} only. Pooling of data into ≤ 7 days of age*
28 *and > 7 days of age revealed F50 was higher overall in the older group. Whilst P_{TOT}*
29 *decreased after docking in both groups, this response was larger and more prolonged*
30 *in the older group. F95 increased after docking in the older pigs only. Overall, these*
31 *data provide evidence of an increase in cortical responsiveness to noxious stimulation*
32 *with increasing post-natal age, suggesting there may be qualitative differences in pain*
33 *perception between age groups. Further, the data provide some support for current*
34 *recommendations that tail-docking and other painful husbandry procedures be*
35 *performed within seven days of birth in order to minimise their impact on animal*
36 *welfare.*

37

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

39

40 **Introduction**

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

47 Current animal welfare guidelines recommend that tail-docking, along with other
48 potentially painful husbandry procedures, be performed at a young age, reflecting a
49 desire to minimise any associated pain. For example, in New Zealand, it is

50 recommended that tail-docking of pigs be performed within 72 h of birth and
51 minimum standards require the provision of analgesia for pigs aged seven days or
52 over (Anonymous 2018). Similarly, both Australia and the UK recommend that tail-
53 docking of pigs be performed within seven days of birth, with the latter stipulating
54 that analgesia be provided for pigs > 7 days of age (Council of the European Union
55 2008; Commonwealth Scientific and Industrial Research Organisation [CSIRO]
56 2008). Despite such recommendations, there is little scientific evidence to support
57 these age thresholds and little research has been undertaken comparing the effects of
58 piglet age on pain responses.

59 The subjective nature of pain makes its assessment in animals complicated. Animals'
60 inability to report their experiences necessitates the use of indirect indices of pain,
61 including metabolic, endocrine and behavioural measures (Livingston & Chambers
62 2000). However, the interpretation of these is confounded by the fact that they are
63 non-specific and may alter in response to non-painful stressors, such as handling and
64 restraint. In addition, these indices represent responses to noxious stimulation, rather
65 than pain perception (Johnson *et al* 2005) and have been shown to correlate poorly
66 with reports of pain in people (Chapman *et al* 1985). More recently,
67 electroencephalographic (EEG) indices of nociception have been used to infer pain
68 and test analgesic efficacy in a range of mammals. The EEG provides a summation of
69 electrical activity arising from the cerebral cortex. In man, changes in the frequency
70 spectrum of the EEG mirrored changes in cortical activity relating to the cognitive
71 perception of pain (Bromm 1984). Changes in the EEG frequency spectra under light
72 anaesthesia have been used to assess nociception in a range of adult mammals,
73 including horses (Murrell *et al* 2003), cattle (Gibson *et al* 2007), sheep (Johnson *et al*
74 2005) and pigs (Haga & Ranheim 2005). These studies all reported transient increases

75 in median frequency (F50) and decreases in total power (P_{TOT}) of the EEG following
76 application of a known noxious stimulus. Furthermore, prior administration of
77 effective analgesia has been shown to obtund spectral EEG responses to noxious
78 stimuli (Haga & Ranheim 2005; Johnson *et al* 2005b; Murrell *et al* 2005; Kongara
79 *et al* 2014). In sheep, the magnitude of changes in the EEG frequency spectrum
80 correlated well with behavioural responses to noxious stimuli (Ong *et al* 1997) whilst,
81 in man, the magnitude of changes correlated with reports of pain intensity in response
82 to graded noxious stimuli (Chen *et al* 1989).

83 In a previous study, we investigated the EEG responses of two and 20 day old pigs to
84 tail-docking (Kells *et al* 2017b) and identified differences in the magnitude and
85 duration of EEG responses to tail-docking between the two ages. This led us to
86 question how responses to noxious stimulation develop over the early post-natal
87 period in pigs.

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

90

91 **Materials and methods**

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

96 Sixty commercial white line (Large white \times Landrace) entire male pigs aged 1, 5, 7,
97 10, 12 or 15 days ($n = 10$ per age), were obtained from a commercial pig farm on the
98 day of testing and housed in a temperature-controlled (30°C) indoor facility on deep

99 straw litter with *ad libitum* access to water. A sample size of ten animals per age
100 group was selected based on previous studies using the same methodology, whereby
101 statistically significant differences were obtained using groups of ten pigs (Kells *et al*
102 2017a,b), ponies (Murrell *et al* 2005) and calves (Gibson *et al* 2007). Pigs had not
103 previously undergone any potentially painful husbandry procedures (eg castration,
104 tooth-trimming, ear-tagging, iron injection) and had intact tails. Within each age
105 group pigs were sourced from three separate litters, with each litter being tested at
106 only a single age. The duration of travel from the farm to the laboratory was
107 approximately 30 min. The maximum interval between time of collection from the
108 farm and induction of anaesthesia was 320 min.

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

113


114 ***Anaesthesia***

115 An established minimal anaesthesia model (MAM) was followed (Murrell & Johnson
116 2006). Pigs were anaesthetised with halothane (Halothane-Vet, Merial NZ Limited,
117 Manukau City, New Zealand) vaporised in oxygen (4 L min^{-1}) delivered via
118 facemask. Halothane concentration was maintained at 3–4% during induction and
119 instrumentation and at 0.95–1.05% during the data acquisition period. End tidal
120 halothane and CO_2 tension, SpO_2 and heart rate were monitored throughout using an
121 anaesthetic agent monitor (Hewlett Packard M1025B, Hewlett Packard, Hamburg,
122 Germany). Rectal temperature was monitored using a digital thermometer (Q 1437,

123 Dick Smith Electronics, New Zealand) and maintained at 38–40°C with the aid of a
124 circulating warm-water heating blanket (T pump, Gaymar Industries Inc, NY, USA).

125 *Electrophysiology*

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

132 EEG signals were fed via breakout boxes to separate amplifiers (Iso-Dam isolated
133 biological amplifier, World Precision Instruments Inc  u: Location?]). The signals
134 were amplified with a gain of 1,000 and a band-pass of 1.0–500 Hz and digitised at a
135 rate of 1 kHz (Powerlab 4/20, ADInstruments Ltd, Colorado Springs, CO, USA). The
136 digitised signals were recorded on an Apple Macintosh personal computer for off-line
137 analysis at the conclusion of the experiment.

138 *Experimental procedure*

139 Once end tidal halothane tension was stable at 0.95–1.05%, 10 min of baseline EEG
140 was recorded. Tail-docking was then performed by severing the tail approximately
141 2 cm from the base using a pair of clean, disinfected side-cutter pliers, taking care to
142 sever between adjacent vertebrae. EEG recording was continued for 5 min after
143 docking. As the pigs in this study were not yet weaned and could not be returned to
144 the farm of origin due to biosecurity restrictions, the experimental protocol dictated
145 that they be euthanased at the conclusion of data collection. This was carried out via
146 I/P injection of sodium pentobarbital (250 mg kg⁻¹, Pentobarb 500, Provet NZ Pty

147 Ltd, Auckland, New Zealand) administered whilst pigs were still under general
148 anaesthesia.

149

150 *Data analysis*

151 EEG data from only the right cerebral cortex were analysed. Although EEG was
152 recorded bilaterally, previous studies using the MAM have demonstrated equivalency
153 in spectral EEG between hemispheres (Murrell *et al* 2007, 2010; McIlhone 2011),
154 suggesting data from either hemisphere alone are suitable for analysis. Data from the
155 left cortex were collected for use in the event that right cortex data were unsuitable for
156 analysis due, for example, to electrode displacement or the presence of extensive
157 artefact confined to a single channel. Raw EEG recordings were inspected manually
158 and any artefacts, such as over-scale, under-scale, nystagmus or other muscular
159 activity, were excluded from subsequent analysis. The total power (P_{TOT}), median
160 frequency (F50) and 95% spectral edge frequency (F95) were calculated for
161 consecutive 1-s epochs, using purpose-written software (Spectral Analyser, CB
162 Johnson, Massey University, Palmerston North, New Zealand). Fast Fourier
163 transformation was applied to each epoch, generating sequential power spectra with
164 1 Hz frequency bins.

165

166 *Statistical analysis*

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

171 approximate normal distribution and, thus, were considered suitable for parametric
172 analysis.

173 *Comparison of baseline EEG*

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

179

180 *Analysis of all ages combined*

181 Analysis of baseline EEG demonstrated a significant age effect on EEG variables. In
182 order to account for differences in baseline EEG between pigs of different ages, data
183 were standardised to a percentage of pre-stimulus baseline for statistical analyses as
184 follows: values for F50, F95 and P_{TOT} generated over consecutive 1-s epochs were
185 transformed to a percentage of baseline mean, by dividing each variable by the mean
186 F50, F95 or P_{TOT} calculated over the final 60 s of the baseline recording period and
187 multiplying the product by 100. EEG data for consecutive 15-s blocks (up to 120 s)
188 after tail-docking were then compared to those from the final 15 s of baseline. A
189 single mean value for each EEG variable was calculated for each time-period in each
190 pig, generating a total of nine data-points per pig per variable (one before and eight
191 after tail-docking; Figure 1). Analysis of variance was performed using the MIXED
192 procedure in SAS to compare post-docking means for each variable to baseline mean
193 within-age and to compare means between ages at each time-period after docking.
194 The model included age, litter (age) [Au: OK] and order of testing as fixed effects,

195 pig as a random effect, and time as a repeated measure. Statistical significance was set
196 at $P < 0.05$. Where significant main or interactive effects were identified, *post hoc*
197 tests were carried out to identify group differences with Bonferroni correction for
198 multiple comparisons.

199

200 **Figure 1 Schematic diagram illustrating the consecutive non-overlapping time**
201 **periods used for statistical analyses of transformed data. B = baseline. Mean F50,**
202 **F95 and P_{TOT} were calculated for each period in each individual.**

203

204 *Analysis of each age separately*

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

213

214 *Analysis of age-blocked data*

215 Given various industry recommendations that tail-docking be performed within the
216 first week of life, data were combined into two age blocks for comparison: ≤ 7 days
217 (data from 1, 5 and 7 day old pigs) and > 7 days (data from 10, 12 and 15 day old

218 pigs). Analysis of variance was performed using the MIXED procedure in SAS to
219 compare post-docking EEG variables to baseline within-age and to compare values
220 between ages at each time-period after docking. The model included age block, litter
221 within age block and order of testing as fixed effects, pig as a random effect, and time
222 as a repeated measure.

223

224 **Results**

225 EEG data were successfully collected from all 60 pigs. Twenty-one pigs (35%)
226 exhibited brief movement responses to tail-docking, in the form of leg or tail stump
227 twitches, hind limb extension or, in some instances, a brief ‘running’ motion of the
228 hind limbs. Of these, 17 resulted in discrete movement artefacts in the EEG recording.
229 Periods affected by artefact ranged from 1–8 (mean = 4.6) consecutive epochs. Within
230 each individual recording, periods containing artefact were excluded from subsequent
231 analyses. Movement responses to tail-docking were observed in seven five day old,
232 one seven day old, six ten day old, three 12 day old and four 15 day-old pigs. No
233 movement response to tail-docking was observed in one day old pigs.

234

235 *Comparison of baseline EEG*

236 Pig age significantly influenced baseline F50 ($F = 4.18$; $P < 0.01$) and F95 ($F = 2.82$;
237 $P = 0.03$), but not P_{TOT} ($F = 1.72$; $P = 0.15$). Baseline F50 was lower in one day old
238 than ten, 12 or 15 day old pigs, but did not differ between other age groups (Figure 2).
239 Baseline F95 was lower in one day old pigs than seven day old pigs but did not differ
240 between other age groups (Figure 2). Test order had a significant effect on baseline
241 F50 ($F = 3.66$; $P = 0.02$), with F50 higher in pigs that were tested first on a given day

242 compared with those tested second ($P = 0.02$), with no difference between those
243 tested first and third, first and fourth, second and third, second and fourth, or third and
244 fourth.

245

246 **Figure 2 Comparison of baseline (a) F50 and (b) F95 of the EEG of pigs aged**
247 **one, five, seven, ten, 12 and 15 days of age. Data are presented as mean (\pm SEM).**
248 **Superscripts denote significant differences between means (Bonferroni adjusted**
249 **$P < 0.05$).**

250

251 *Combined analysis*

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

255

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

260

261 *Median frequency (F50)*

262 Overall, mean F50 was lower in one day old pigs than five or 15 day old pigs
263 (102.31 [\pm 1.63] versus 110.18 [\pm 1.63]% ($P = 0.006$) and 108.8 [\pm 1.63]%
264 [$P = 0.037$], respectively) and lower in seven than five day olds (102.63 [\pm 1.76]

265 versus 110.18 [\pm 1.63]% [$P = 0.003$]). There was a significant effect of time on F50,
266 with F50 being elevated, relative to baseline, from 15–105 s after docking ($P \leq 0.02$).
267 Mean F50 did not differ significantly between ages at any individual time-point (no
268 interaction between age and time).

269

270 *Spectral edge frequency (F95)*

271 F95 was elevated relative to baseline in ten day old pigs from 15–75 s after docking
272 ($P < 0.01$). Comparison at individual time-points revealed that mean F95 was higher
273 in ten day old pigs than one, five, seven or 12 day old pigs, 30 and 45 s after tail-
274 docking (Figure 3[a]). Test order (1st, 2nd, 3rd or 4th) significantly influenced F95.
275 Piglets tested first on a given test day exhibited lower F95 (99.68 [\pm 0.15]%) than
276 those tested second (100.72 [\pm 0.15]%; $P < 0.001$) or third (100.76 [\pm 0.16]%;
277 $P = 0.001$), but did not differ from those tested fourth (100.26 [\pm 0.25]%; $P = 0.30$).
278 Despite the overall effect of litter within age, *post hoc* tests revealed no significant
279 differences in F95 between the three litters tested at each age.

280

281 *Total power (P_{TOT})*

282 A reduction in P_{TOT} after docking was observed in five, ten, 12 and 15 day old pigs.
283 P_{TOT} was lower than baseline 15 s after docking in five, 12 and 15 day old and from
284 15–45 s after docking in ten day old pigs ($P < 0.01$). Comparison at individual time-
285 points revealed that P_{TOT} was lower in ten and 12 day old pigs than one day old pigs
286 45 s after docking (Figure 3[b]). P_{TOT} did not differ between age groups over the
287 period 60–120 s after docking. Despite the overall effect of litter within age, *post hoc*
288 tests revealed no significant differences in P_{TOT} between the three litters tested at each
289 age.

290 **Figure 3 Comparison of the changes in mean (\pm SEM) (a) F95 and (b) P_{TOT} of the**
291 **EEG following tail-docking (time 0) in pigs aged one, five, seven, ten, 12 and 15**
292 **days. Data are shown as percentages of baseline mean. Means at the same time-**
293 **points with different superscripts differed significantly (Bonferroni adjusted**
294 **$P < 0.05$).**

295

296 *Analysis of each age separately*


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

301

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

305

306 *One day old pigs*

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

310

311

312

313 *Five day old pigs*

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

317 *Seven day old pigs*

318 Mean F50 was lower than baseline 15 s after docking ($P < 0.01$) but did not differ [Au:
319 from?] to baseline from 30 s onward. Neither F95 nor P_{TOT} differed to [Au: from?]
320 baseline at any time after docking.

321 *Ten day old pigs*

322 Mean F50 was lower than baseline 15 s after docking ($P = 0.03$) and showed a tendency
323 toward being higher than baseline 45 and 60 s after docking ($P = 0.08$ and 0.07 ,
324 respectively). F95 was higher than baseline 15, 30, 45 (all $P < 0.01$) and 60 ($P = 0.01$) s
325 after docking. P_{TOT} was lower than baseline 15, 30 (both $P < 0.01$) and 45 ($P = 0.04$) s
326 after docking.

327 *Twelve day old pigs*

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

331 *Fifteen day old pigs*

332 Mean F50 was elevated relative to baseline 30 and 45 s after docking ($P = 0.02$ and
333 0.04 , respectively), whilst P_{TOT} was lower than baseline 15 and 30 s after docking
334 ($P < 0.01$ and $P = 0.01$, respectively). F95 did not differ to [Au: from?] baseline at any
335 time.

336 *Analysis of age-blocked data*

337 Statistical results are presented in Table 3.

338

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

343

344 *Median frequency (F50)*

345 There were significant effects of age and time on piglet F50 (Table 3). F50 was lower
346 overall in pigs aged ≤ 7 days than those aged > 7 days ($103.63 [\pm 0.16]$ versus
347 $108.71 [\pm 0.14]\%$). F50 was elevated relative to baseline from 30–105 s after docking
348 (all $P \leq 0.01$). Although there was an overall effect of litter within age, F50 did not
349 differ between litters within each age group following correction for multiple
350 comparisons.

351

352 *Spectral edge frequency (F95)*

353 There was a significant age \times time effect, and a significant effect of test order on F95
354 (Table 3). F95 did not differ from baseline at any point after docking in pigs ≤ 7 days,
355 whereas in pigs > 7 days, F95 was greater than baseline from 15–75 s after docking
356 ($P < 0.05$) (Figure 4). F95 was higher in pigs aged > 7 days than those aged ≤ 7 days
357 30 ($P < 0.001$) and 45 ($P = 0.014$) s after docking but did not differ between age
358 groups over the period 60–120 s after docking (Figure 4). F95 was lower in pigs
359 tested first on a given day than those tested second or third, ($P < 0.001$). Although

360 there was an overall effect of litter within age, F95 did not differ between litters
361 within each age group following correction for multiple comparisons.

362

363 **Figure 4 Comparison of mean (a) F95 and (b) P_{TOT} of the EEG in pigs**
364 **aged ≤ 7 days (comprised of data from one, five and seven day old pigs)**
365 **or > 7 days (comprised of data from ten, 12 and 15 day old pigs) following tail-**
366 **docking at time 0. Asterisks indicate mean differed from baseline within age**
367 **group (Dunnett's $P < 0.05$). Superscripts indicate differences between age groups**
368 **at common time-points (Bonferroni adjusted $P < 0.05$).**

369

370 *Total power (P_{TOT})*

371 There was a significant age × time effect, and a significant effect of litter within age
372 block on P_{TOT}. P_{TOT} was lower than baseline from 15–30 s after docking in pigs
373 aged ≤ 7 days, and from 15–45 s after docking in pigs aged > 7 days ($P < 0.05$). P_{TOT}
374 was lower in pigs aged > 7 days than those aged ≤ 7 days 30 ($P = 0.014$) and
375 45 ($P < 0.001$) s after docking, but did not differ between groups over the period 60–
376 120 s after docking (Figure 4). Of the nine litters aged >7 days, P_{TOT} was lower in
377 pigs from litter 12 than from litter 13 ($P = 0.007$).

378

379 **Discussion**

380 Previously, we identified differences between the EEG responses of two and 20 day old
381 pigs to the noxious stimulus of tail-docking (Kells *et al* 2017a). The aim of the present
382 study was to examine EEG responses to tail-docking in pigs aged between one and
383 15 days of age, to determine the manner in which cortical responses to acute noxious
384 stimulation develop over this period.

385 The cerebral cortex remains responsive to noxious stimuli at a minimal plane of
386 halothane anaesthesia (Murrell *et al* 2003). Using the minimal anaesthesia model, prior
387 studies have consistently demonstrated that noxious stimuli elicit an increase in F50
388 and decrease in P_{TOT} of the EEG of adult mammals (Murrell *et al* 2003; Johnson *et al*
389 2005b; Murrell *et al* 2005; Gibson *et al* 2007; Kongara *et al* 2010). In lambs, the degree
390 of responsiveness of the cerebral cortex to noxious stimulation was shown to vary with
391 postnatal age (Johnson *et al* 2005a, 2009). The present study demonstrated that the EEG
392 responses of pigs to the noxious stimulus of tail-docking also varied with post-natal
393 age, with an overall pattern emerging of increased responsiveness with increasing post-
394 natal age.

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

405 Whilst, at one day of age, the cerebral cortex did not respond to noxious stimulation,
406 pigs aged between five and 15 days exhibited at least some elements of a characteristic
407 nociceptive response. From ten post-natal days onward, EEG responses to tail-docking
408 were broadly consistent with those previously reported in 20 day old pigs following
409 castration (Haga & Ranheim 2005) and tail-docking (Kells *et al* 2017a) and in other

410 adult mammals in response to noxious stimulation (eg Johnson *et al* 2005b, 2009;
411 Kongara *et al* 2010); ie an increase in F50 and decrease in P_{TOT}. Notably, at 12 days
412 old, there was no significant increase in F50 after docking, although P_{TOT} decreased.
413 This was unexpected, given that F50 increased after docking in ten and 15 day old pigs
414 in the present study, and in 20 day old pigs in previous studies (Kells *et al* 2017a,b).
415 The lack of a significant increase may have been due to the high degree of individual
416 variation among this age group.

417 Seven day old pigs exhibited a reduction in F50 in response to docking, whilst ten day
418 olds exhibited a brief reduction prior to a sustained increase. A reduction in F50
419 represents an atypical response to noxious stimulation, which has previously been
420 reported in two day old pigs following tail-docking (Kells *et al* 2017b). Whilst
421 nociception typically elicits EEG desynchronisation, characterised by a shift toward
422 lower amplitude, higher frequency activity (Otto 2008) with corresponding increases in
423 F50 and F95 (Johnson *et al* 2012), paradoxical arousal, or synchronisation,
424 characterised by a shift toward higher amplitude, lower frequency activity and
425 corresponding decreases in F50 and F95, has also been reported. In a study of
426 isoflurane-anaesthetised sheep undergoing orthopaedic surgery, both synchronisation
427 and desynchronisation of the EEG were observed, with responses differing according
428 to depth of anaesthesia and stimulation intensity (Otto & Mally 2003). In a study of
429 EEG responses to skin incision in anaesthetised people, adult patients demonstrated
430 desynchronisation following skin incision, whereas EEG synchronisation
431 predominated in infants and young children, suggesting this may be an age-dependent
432 effect (Oshima *et al* 1981).

433 Age-related differences in anaesthetic requirements have previously been identified in
434 human patients. The inspired concentration of inhalational agent required to maintain

435 anaesthesia is up to four times higher in paediatric patients than in older adults (Gregory
436 *et al* 1969; Nickalls & Mapleson 2003). If such an effect were present in pigs across
437 the relatively small age range examined in this study, it might be expected that younger
438 pigs would have higher anaesthetic requirements than older pigs and maintaining end-
439 tidal halothane at a constant concentration across age groups may have resulted in
440 lighter anaesthesia in younger pigs. If this were the case, we would have expected to
441 see lower EEG amplitude and higher EEG frequency of the EEG, resulting in lower
442 baseline P_{TOT} and higher baseline F50 and F95 in younger pigs. However, baseline EEG
443 data do not support such an effect across the age range examined. Total EEG power did
444 not differ between age groups and, whilst baseline F50 did vary between one and 10–
445 15 day old pigs, it was lower in the one day olds, thus not indicative of a reduced state
446 of anaesthesia. Nevertheless, there would be value in determining age-specific
447 halothane MAC for pigs in future studies.

448 Based on previous data collected from two and 20 day old pigs undergoing tail-docking
449 (Kells *et al* 2017b), we anticipated seeing significant changes in two or more EEG
450 variables in pigs docked at all interim ages. This was not the case. In particular, the
451 failure to see any changes in F50 at either five or 12 days of age was unexpected. The
452 observed decrease in F50 at seven days old was consistent with previously observed
453 decrease in F50 in pigs docked at two days old (Kells *et al* 2107b). The biphasic
454 response seen at ten days (initial decrease, followed by an increase) suggests a transition
455 toward the characteristic increase in F50 observed in 15 and 20 day old pigs and other
456 adult mammals. Similarly, given the reductions in P_{TOT} seen after docking at five, ten,
457 12 and 15 days of age, the absence of such a response in seven day old pigs was
458 surprising. Again, there is no obvious explanation for this — no changes in diet,

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

461 Although there were few statistically significant differences between age groups in
462 EEG responses to tail-docking over time, there were some qualitative differences in
463 the pattern of responses, which may be of biological significance. In terms of F50,
464 two different response patterns emerged: pigs aged five, seven and ten days exhibited
465 a decrease in mean F50 relative to baseline (Time 0) immediately after docking,
466 followed by a subsequent increase above baseline mean, peaking 45–60 s after
467 docking (Figure 5). However, pigs aged 12 and 15 days differed, in that no initial
468 decrease but a similarly timed increase in mean F50 was observed. In terms of F95,
469 pigs aged ten and 15 days exhibited an increase in F95, peaking 30 s after docking,
470 whereas all other ages showed little change (Figure 5). P_{TOT} decreased to relative to
471 baseline to varying degrees after docking in all age groups, with the duration of this
472 appearing to be more prolonged in pigs aged ten days and older (Figure 5). Thus,
473 overall EEG responsiveness to tail-docking appeared to increase with increasing age,
474 with pigs aged ten days and over exhibiting characteristic nociceptive response
475 patterns.

476

477 **Figure 5 Qualitative comparison of the changes in mean F50, F95 and P_{TOT} of the**
478 **EEG following tail-docking (time 0) in pigs aged one, five, seven, ten, 12 and 15**
479 **days. For ease of distinguishing between ages, non-transformed data are**
480 **presented, and standard errors omitted.**

481

482 Pooling of data into two age blocks (≤ 7 and >7 days of age) revealed significant
483 differences in EEG responses to docking between age groups. Overall, the older group
484 exhibited heightened responses to tail-docking compared with the younger group. An
485 increase in F95 was observed in the older group only, and although P_{TOT} decreased
486 after docking in both groups, the magnitude and duration of the decrease was greater
487 in pigs > 7 days. Changes in the responsiveness of the cerebral cortex to noxious
488 stimulation are thought to reflect changes in the degree to which noxious stimuli are
489 perceived (Johnson *et al* 2009). This is supported by data from sheep, in which the
490 magnitude of changes in the EEG frequency spectrum correlated well with
491 behavioural responses to noxious stimuli (Ong *et al* 1997) and in man, where the
492 magnitude of EEG changes correlated with reports of pain intensity in response to
493 graded noxious stimuli (Chen *et al* 1989).

494 Therefore, the greater magnitude of EEG responses observed in the older group
495 suggests that the qualitative perception of tail-docking is different between the two age
496 groups and that tail-docking without analgesia may be perceived as more noxious to
497 pigs aged > 7 days compared with those aged ≤ 7 days. This finding is important, given
498 current recommendations regarding the age at which husbandry procedures, such as
499 tail-docking should be performed, along with requirements for provision of analgesia.
500 These data provide some support for New Zealand and Australian recommendations
501 that if tail-docking is to be performed without analgesia, this should occur within the
502 first week of life (CSIRO 2008; Anonymous 2018) and for UK and New Zealand policy
503 requiring the use of anaesthesia and analgesia in pigs aged seven days and older
504 (Council of the European Union 2008; Anonymous 2018).

505 In the present study, order of testing had a significant effect on baseline F50. Test order
506 reflects the elapsed time between piglet collection from the farm and induction of

507 anaesthesia, therefore also reflecting the length of time since the last feed from the sow.
508 As a result, piglet nutritional status might have varied across test order. However, the
509 effect of test order on F50 was non-linear, ie did not show a consistent increase or
510 decrease with increasing test order. As such, it is likely that the observed effect was
511 random, rather than a consequence of time off feed. This is further supported by the
512 absence of order effects on baseline F95 or P_{TOT}.

513 The findings of this study are consistent with other animal studies that have identified
514 age-related differences in cortical responses to noxious stimuli. A recent study of
515 noxious-evoked EEG activity in rats aged 21 or 40 post-natal days identified age-
516 specific changes in the frequency spectrum of the EEG recorded from the rat primary
517 somatosensory cortex (Devonshire *et al* 2015). Whilst post-stimulus total EEG power
518 did not differ between 21 and 40 post-natal days of age, the authors identified an
519 increase in theta power (4–8 Hz), which correlates with F50, in the older rats only. The
520 authors concluded the differences were due to alterations in the cortical processing of
521 nociceptive inputs as a result of post-natal maturation of the cerebral cortex. Although
522 the rats used in the study were older than the pigs in the present study, the neurological
523 immaturity of rats at birth may explain the absence of an increase in theta power at
524 21 post-natal days. It is well known that cortical development continues post-natally in
525 humans, with maturation not complete until adolescence. Importantly, one-third of the
526 total cortical development over the first six years of life takes place within six weeks of
527 birth, in response to both intrinsic and sensory-driven neuronal inputs (Shankle *et al*
528 1998). In pigs, the most rapid neural development occurs between 50 days pre-natal
529 and 40 days post-natal (Dickerson & Dobbing 1967).

530 An earlier study investigating the effects of post-natal age on EEG responses to
531 castration in lambs which, like pigs, are born neurologically mature, identified an

532 increase in cerebral responsiveness to noxious stimulation over the first 7–10 days of
533 life (Johnson *et al* 2009). In this case, the authors concluded that the lingering effects
534 of fetal neurosuppressive mechanisms might have been responsible for the lesser
535 responsiveness in younger lambs. In mammals, a number of circulating factors act to
536 maintain the fetus in a permanent sleep-like state and are thought to be responsible for
537 the observed absence of fetal responses to noxious and nociceptive stimuli (Mellor *et al*
538 2005). In sheep, plasma concentrations of the neurosuppressive agents pregnanalone
539 and allopregnanalone were found to be significant up to three days after birth (Nguyen
540 *et al* 2003), leading to the suggestion that these chemicals may continue to exert some
541 cerebral effects in the early post-natal period (Mellor & Diesch 2006). Whether
542 significant concentrations of these chemicals are present in the plasma of pigs at birth
543 has not been investigated.

544 In the present study, the lack of nociceptive response at one day post-natal supports the
545 presence of neurosuppressive mechanisms acting to inhibit cerebral processing of
546 nociceptive stimuli. The increasing cortical responsiveness observed over the period 5–
547 15 post-natal days might be explained, in part, by the withdrawal of these
548 neurosuppressive mechanisms. In addition, it is likely that post-natal maturation of pain
549 processing pathways, including cortical components, contributed to the observed
550 increase in responsiveness. Thus, the observed pattern of increasing cerebral
551 responsiveness to nociception with increasing post-natal age may be a result of post-
552 natal cortical development, combined with the gradual withdrawal of neurosuppressive
553 mechanisms.

554 Regardless of whether, or for how long, neurosuppressive mechanisms remain active
555 in the period following birth, information regarding the long-term consequences of
556 noxious stimulation in the neonatal pig must also be considered before any

557 recommendations are made. In humans, noxious stimulation in the very early post-natal
558 period has been associated with increased reactivity to later painful stimuli (Taddio *et al*
559 1997; Grunau 2013). A similar phenomenon was observed in lambs, whereby those
560 castrated at one day of age exhibited greater behavioural responses to subsequent tail-
561 docking than those castrated at ten days (McCracken *et al* 2010). The presence and
562 extent of any such phenomenon in pigs should be investigated.

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

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

581 of the potential longer-term consequences of early noxious stimulation on later pain
582 perception in the pig is needed.

583

584 *Animal welfare implications*

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

589

590 **References**

591 **Anonymous** 2018 *Animal Welfare (Pigs) Code of Welfare 2018*. National Animal

592 Welfare Advisory Committee: Wellington, New Zealand

593 **Bromm B** 1984 *Pain Measurement in Man: Neurophysiological Correlates of Pain*.

594 Elsevier: New York, USA

595 **Chapman CR, Casey KL, Dubner R, Foley KM, Gracely RH and Reading AE**

596 1985 Pain measurement: an overview. *Pain* 22: 1-31

597 **Chen ACN, Dworkin SF, Haug J and Gehrig J** 1989 Topographic brain measures

598 of human pain and pain responsivity. *Pain* 37: 129-141

599 **Council of the European Union** 2008 Council Directive 2008/120/EC Laying down

600 minimum standards for the protection of pigs. *Official Journal of the European Union*

601 *L47*: 5-13

602 **Commonwealth Scientific and Industrial Research Organisation (CSIRO)** 2008

603 *Model Code of Practise for the Welfare of Animals: Pigs*. CSIRO: Collingwood, VIC,

604 Australia

605 **Devonshire IM, Greenspon CM and Hathway GJ** 2015 Developmental alterations
606 in noxious-evoked EEG activity recorded from rat primary somatosensory cortex.
607 *Neuroscience* 305: 343-350

608 **Dickerson JWT and Dobbing J** 1967 Prenatal and postnatal growth and
609 development of the central nervous system of the pig. *Proceedings of the Royal*
610 *Society of London B: Biological Sciences* 166: 384-395

611 **Flecknell P** 2008 Analgesia from a veterinary perspective. *British Journal of*
612 *Anaesthesia* 101: 121-124


613 **Gibson TJ, Johnson CB, Stafford KJ, Mitchinson SL and Mellor DJ** 2007
614 Validation of the acute electroencephalographic responses of calves to noxious
615 stimulus with scoop dehorning. *New Zealand Veterinary Journal* 55: 152-157

616 **Gregory G, Eger E and Munson E** 1969 The relationship between age and
617 halothane requirement in man. *Anesthesiology* 30: 488-491


618 **Grunau RE** 2013 Neonatal pain in very preterm infants: long-term effects on brain,
619 neurodevelopment and pain reactivity. *Rambam Maimonides Medical Journal* 4:
620 e0025


621 **Haga H and Ranheim B** 2005 Castration of piglets: the analgesic effects of
622 intratesticular and intrafunicular lidocaine injection. *Veterinary Anaesthesia and*
623 *Analgesia* 32: 1-9


624 **Johnson CB, Gibson TJ, Stafford KJ and Mellor DJ** 2012 Pain perception at
625 slaughter. *Animal Welfare* 21: 113-122

626 **Johnson CB, Stafford KJ, Sylvester S, Ward R, Mitchinson S and Mellor DJ**
627 2005 Effects of age on electroencephalographic responses to castration in lambs
628 anaesthetised using halothane in oxygen. *New Zealand Veterinary Journal* 53: 433-
629 437 [Au: 2005a or 2005b, see text?] 

630 **Johnson CB, Sylvester SP, Stafford KJ, Mitchinson SL, Ward RN and Mellor DJ**
631 2009 Effects of age on the electroencephalographic response to castration in lambs
632 anaesthetized with halothane in oxygen from birth to 6 weeks old. *Veterinary*
633 *Anaesthesia and Analgesia* 36: 273-279

634 **Johnson CB, Wilson P, Woodbury M and Caulkett N** 2005 Comparison of
635 analgesic techniques for antler removal in halothane-anaesthetised red deer (*Cervus*
636 *elaphus*): electroencephalographic responses. *Veterinary Anaesthesia and Analgesia*
637 32: 61-71 [Au: 2005a or 2005b, see text?] 

638 **Kells NJ, Beausoleil NJ, Sutherland MA et al** (2017a) Electroencephalographic
639 assessment of oral meloxicam, topical anaesthetic cream and cautery iron for
640 mitigating acute pain in pigs (*Sus scrofa*) undergoing tail docking. *Veterinary*
641 *Anaesthesia & Analgesia* in press [Au: Please include all authors' names and
642 clarify volume/page numbers 

643 **Kells NJ, Beausoleil NJ, Sutherland MA et al** (2017b) Electroencephalographic
644 responses of anaesthetised pigs (*Sus scrofa*) to tail docking using clippers or cautery
645 iron, performed at two or twenty days of age. *Veterinary Anaesthesia & Analgesia* in
646 Press [Au: Please include all authors' names and clarify volume/page numbers 

647 **Kongara K, Chambers JP and Johnson CB** 2010 Electroencephalographic
648 responses of tramadol, parecoxib and morphine to acute noxious electrical stimulation
649 in anaesthetised dogs. *Research in Veterinary Science* 88: 127-133

650 **Kongara K, Johnson L, Kells N, Johnson C, Dukkipati V and Mitchinson SL**
651 2014 Alteration of electroencephalographic responses to castration in cats by
652 administration of opioids. *Journal of Veterinary Science* 1: 38-42

653 **Livingston A and Chambers P** 2000 Physiology of pain. In: Flecknell P and
654 Waterman-Pearson AE (eds) *Pain Management in Animals*. WB Saunders: London,
655 UK

656 **Marchant-Forde JN, Lay DC, McMunn KA, Cheng HW, Pajor EA and**
657 **Marchant-Forde RM** 2009 Postnatal piglet husbandry practices and well-being: The
658 effects of alternative techniques delivered separately. *Journal of Animal Science* 87:
659 1479-1492

660 **McCracken L, Waran N, Mitchinson SL and Johnson CB** 2010 Effect of age at
661 castration on behavioural response to subsequent tail docking in lambs. *Veterinary*
662 *Anaesthesia and Analgesia* 37: 375-381

663 **McIlhone A** 2011 *Some characteristics of brain electrical activity in the domestic*
664 *chicken*. PhD thesis, Massey University, Palmerston North, New Zealand

665 **Mellor DJ and Diesch TJ** 2006 Onset of sentience: The potential for suffering in
666 fetal and newborn farm animals. *Applied Animal Behaviour Science* 100: 48-57

667 **Mellor DJ, Diesch TJ, Gunn AJ and Bennet L** 2005 The importance of ‘awareness’
668 for understanding fetal pain. *Brain Research Reviews* 49: 455-471

669 **Murrell JC and Johnson CB** 2006 Neurophysiological techniques to assess pain in
670 animals. *Journal of Veterinary Pharmacology and Therapy* 29: 325-335

671 **Murrell JC, Johnson CB, White K, Taylor P, Haberham Z and Waterman-**
672 **Pearson A** 2003 Changes in the EEG during castration in horses and ponies
673 anaesthetised with halothane. *Veterinary Anaesthesia and Analgesia* 30: 138-146

674 **Murrell JC, White K, Johnson CB, Taylor P, Doherty T and Waterman-Pearson**
675 **A** 2005 Investigation of the EEG effects of intravenous lidocaine during halothane
676 anaesthesia in ponies. *Veterinary Anaesthesia and Analgesia* 32: 212-221

677 **Murrell J, Mitchinson SL, Waters D and Johnson CB** 2007 Comparative effect of
678 thermal, mechanical, and electrical noxious stimuli on the electroencephalogram of
679 the rat. *British Journal of Anaesthesia* 98: 366-371

680 **Murrell JC, Mitchinson SL, Lesperance L, Sivakumaran S and Johnson CB**
681 2010 Electroencephalography during ovariohysterectomy in rats anaesthetized with
682 halothane. *Veterinary Anaesthesia & Analgesia* 37: 14-24

683 **Nguyen PN, Billiards SS, Walker DW and Hirst JJ** 2003 Changes in 5-alpha-
684 pregnane steroids and neurosteroidogenic enzyme expression in the perinatal sheep.
685 *Pediatric Research* 53: 956-964

686 **Nickalls RWD and Mapleson WW** 2003 Age-related iso-MAC charts for isoflurane,
687 sevoflurane and desflurane in man. *British Journal of Anaesthesia* 91: 170-174

688 **Noonan GJ, Rand JS, Priest J, Ainscow J and Blackshaw JK** 1994 Behavioural
689 observations of piglets undergoing tail docking, teeth clipping and ear notching.
690 *Applied Animal Behaviour Science* 39: 201-213

691 **Ong R, Morris J, O'Dwyer J, Barnett J, Hensworth P and Clarke I** 1997
692 Behavioural and EEG changes in sheep in response to painful acute electrical stimuli.
693 *Australian Veterinary Journal* 75: 189-193

694 **Oshima E, Shingu K and Mori K** 1981 EEG activity during halothane anaesthesia
695 in man. *British Journal of Anaesthesia* 53: 65-72

696 **Otto KA** 2008 EEG power spectrum analysis for monitoring depth of anaesthesia
697 during experimental surgery. *Laboratory Animals* 42: 45-61

698 **Otto KA and Mally P** 2003 Noxious stimulation during orthopaedic surgery results
699 in EEG arousal or paradoxical arousal reaction in isoflurane-anaesthetised sheep.
700 *Research in Veterinary Science* 75: 103-112

701 **Pogatzki-Zahn EM, Segelcke D and Schug SA** 2017 Postoperative pain: from
702 mechanisms to treatment. *PAIN Reports* 2: e588

703 **Prunier A, Mounier A and Hay M** 2005 Effects of castration, tooth resection, or tail
704 docking on plasma metabolites and stress hormones in young pigs. *Journal of Animal*
705 *Science* 83: 216-222

706 **Shankle WR, Romney AK, Landing BH and Hara J** 1998 Developmental patterns
707 in the cytoarchitecture of the human cerebral cortex from birth to 6 years examined by
708 correspondence analysis. *Proceedings of the National Academy of Sciences* 95: 4023-
709 4028

710 **Sutherland MA, Bryer PJ, Krebs N and McGlone JJ** 2008 Tail docking in pigs:
711 acute physiological and behavioural responses. *Animal* 2: 292-297

712 **Taddio A, Katz J, Ilersich AL and Koren G** 1997 Effect of neonatal circumcision
713 on pain response during subsequent routine vaccination. *The Lancet* 349: 599-603

714 **Woolf CJ** 2011 Central sensitization: implications for the diagnosis and treatment of
715 pain. *Pain* 152: S2-S15

718