Techniques for chronic monitoring of brain activity in freely moving sheep using wireless, longitudinal EEG recording

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Keywords: ovine, large animal, neurodegenerative disease, behaviour, sleep, pig, epilepsy, Huntington's disease

Running title: Methods for chronic brain implants in sheep

Acknowledgements: This work was funded by CHDI *Inc*. (AJM).

18 ## Abstract

19 **Background:** Large mammals with complex central nervous systems offer new
20 possibilities for translational research into basic brain function. Techniques for
21 monitoring brain activity in large mammals, however, are not as well developed as
22 they are in rodents.

23 **New method:** We have developed a method for chronic monitoring of
24 electroencephalographic (EEG) activity in unrestrained sheep. We describe the
25 methods for behavioural training prior to implantation, surgical procedures for
26 implantation, a protocol for reliable anaesthesia and recovery, methods for EEG data
27 collection, as well as data pertaining to suitability and longevity of different types of
28 electrodes.

29 **Results:** Sheep tolerated all procedures well, and surgical complications were
30 minimal. Electrode types used included epidural and subdural screws, intracortical
31 needles and subdural disk electrodes, with the latter producing the best and most
32 reliable results. The implants yielded longitudinal EEG data of consistent quality for
33 periods of at least a year, and in some cases up to 2 years.

34 **Comparison with existing methods:** This is the first detailed methodology to be
35 described for chronic brain function monitoring in freely moving unrestrained sheep.

36 **Conclusions:** The developed method will be particularly useful in chronic
37 investigations of brain activity during normal behaviour that can include sleep,
38 learning and memory. As well, within the context of disease, the method can be used
39 to monitor brain pathology or the progress of therapeutic trials in transgenic or natural
40 disease models in sheep.

41 ## Introduction

42 *In vivo* experimentation in neuroscience research benefits from the use of a variety of
43 different mammalian species, both large and small. A critically important determinant
44 for species selection is the degree of desired translatability. While it is easy to
45 translate findings from non-human primate studies to humans, this may not
46 necessarily be the case when using smaller animals with simpler nervous systems.

47 Rodents are arguably the most widely used laboratory animals in neuroscience
48 research and their value is undisputed. Indeed, many of the most advanced
49 experimental tools, for example *in vivo* optogenetics, are optimised for use in rodents
50 (Arenkiel et al., 2007). Furthermore, rodents offer the possibility of conducting
51 experiments using large numbers of animals, thus allowing studies to be sufficiently
52 well powered statistically. However, the short life span and small brain size of rodents
53 make them less useful when translational investigations are considered. Species that
54 sacrifice statistical power for increased translatability include predominantly non-
55 human primates, but recently other less costly alternatives are being considered such
56 as sheep or mini pigs (for a review, see Morton and Howland, 2013).

57 In the past, sheep have been widely used for physiological studies of many different
58 organ systems and disease states such as arthritis and cancer (Fraser et al., 1993;
59 Palmarini et al., 1999). They have also been used in studies of brain function such as
60 brain development (Petit et al., 2015; Williams et al., 1992), sleep apnoea
61 (Letourneau, 2003), visual processing (Clarke and Whitteridge, 1976; Peirce and
62 Kendrick, 2002), epilepsy (Stypulkowski et al., 2014). However, the capability of
63 chronic monitoring of EEG activity in freely moving animals has not been shown
64 until recently (Perentos et al., 2015). Sheep offer a number of attractive features as

65 model organisms for studying brain function. First, they have a long lifespan, making
66 them suitable for investigations into prodromal stages of late onset disorders. Second,
67 their brain anatomy is much more similar to that of the primate than is that of rodents.
68 Both of these features make findings in sheep more 'translational' than those in
69 rodents. Third, in comparison to primates, sheep are easier and safer to manage in an
70 experimental setting. Fourth, sheep have very rudimentary and well-established
71 housing demands, making them a practical laboratory species that can be kept in
72 natural groups. Fifth, sheep have large brains and thick skulls that are well suited for
73 investigations requiring skull fixation of complex large-scale neural recording
74 interfaces. Finally, sheep are large mammals without hands. As such, they cannot
75 easily interfere with recording equipment and can carry relatively heavy equipment
76 unimpeded, making experimental paradigms in large naturalistic environments
77 possible.

78 The efficient use of an animal model requires reliable and easy-to-implement methods
79 for monitoring relevant physiological variables. Electroencephalography (EEG) is a
80 brain activity-monitoring tool that has found wide applications in both research and
81 clinical domains. In humans, it is easily and non-invasively applicable, requiring
82 minimal infrastructure. It is used routinely for clinical diagnosis of sleep disorders
83 (Shneerson, 2009), epilepsy (Smith, 2005) and diagnosis of neonatal brain function
84 abnormalities (Holmes and Lombroso, 1993). It is also a widely used experimental
85 research tool (for a recent example, see Carhart-Harris et al., 2016). Its application in
86 animals is particularly attractive due to the translational potential. It would therefore
87 constitute a useful tool when utilising large animal models of central nervous system
88 function.

89 Here we describe in detail the techniques we developed that allow for the use of sheep
90 in chronic studies of brain activity through EEG. We describe the methods associated
91 with animal training, anaesthesia, surgery, implantations, instrumentation and data
92 collection from freely moving animals in naturalistic environments. We suggest future
93 improvements that can further expand the uses of this animal model in systems
94 neuroscience.

95 Methods

96 Animals

97 All procedures were conducted in accordance with the UK Animals Scientific
98 Procedures Act (1986) and the University of Cambridge Animal Welfare and Ethical
99 Review Bodies (AWERB). Data from 29 sheep are included in this study, of which 15
100 were of the Borderdale breed. Nine Borderdale sheep were homozygous for the CLN5
101 gene ($CLN5^{-/-}$) and six were heterozygous ($CLN5^{+/-}$). This mutation leads to neuronal
102 ceroid lipofuscinosis, a genetic condition with a multiple behavioural abnormalities
103 and severe degeneration of the cerebral cortex. Therefore, these sheep were
104 considered as a good candidate in which to validate the usability of our EEG methods
105 in detecting changes associated with neurodegenerative processes. Data from these
106 sheep shown here have been previously published in other studies (Nicol et al., 2016;
107 Perentos et al., 2016, 2015). As described in (Perentos et al., 2015) Borderdale sheep
108 were bred and reared in New Zealand under procedures approved by the Lincoln
109 University Animal Ethics Committee in compliance with the NZ Animal Welfare Act
110 (1999) and in accordance with National Institutes of Health guidelines imported from
111 New Zealand (Lincoln University). Sheep were then imported to the UK for further
112 procedures. Borderdale sheep were implanted in two groups: (group 1, age at

113 implantation 14.3 ± 0.5 months and weight 42.5 ± 3.2 kg; group 2, age at implantation
114 7.8 ± 0.3 months and weight 35.9 ± 5.8 kg). The remaining 14 sheep were of Welsh
115 mountain breed (age at implantation ~18 months and weight 42.3 ± 7.3 kg). All sheep
116 were housed in an indoor barn with natural light illumination, supplemented with
117 artificial light between the hours of 6 am and 6pm. Sheep were grouped-housed but in
118 some cases, where skull-fixed electrode chambers were used, sheep were housed
119 individually. In the latter sheep we anticipated a stress response but we found that
120 they quickly habituated to their new environment. At all times, sheep had visual,
121 auditory and olfactory access to conspecifics. Stress levels were monitored through
122 behavioural assessment. No bleating, agitation, or repetitive behaviours were
123 observed. Recordings lasted for ~24 h and typically started between 9-11 AM.

124 **Sheep training prior to implantation**

125 To allow for efficient and safe procedures, all sheep underwent behavioural training
126 aimed at habituating them to the human handlers and experimental procedures. Using
127 training tools such as halters and leads, sheep were habituated to follow confidently
128 any handler (Fig. 1A). Sheep were trained to tolerate being restrained using a
129 Gambrel device (p/n: GAMJIPV851 Chilvers Country Supplies, UK; Fig. 1B), and
130 were also habituated to being supported for up to 1.5 h within a sling in which their
131 feet kept elevated above ground (Fig. 1D). Other devices used included Elizabethan
132 collars, which were utilised to protect the implants from mechanical strain (Fig. 1C),
133 and jackets for housing equipment such as electrophysiological amplifiers (Fig. 1E).
134 Throughout training intermittent reinforcement was provided in the form of food
135 pellets.

136 **Anaesthesia**

137 General anaesthesia in ruminants necessitates particular care in securing the airway,
138 as there is considerable risk of fluid aspiration into the lungs from the rumen and
139 continuous parotid duct salivation. Although the rumen cannot be completely emptied
140 by starvation, pre-anaesthetic starvation of 18-24 hours is recommended to reduce
141 both fluid volume and the amount of gas produced from fermentation (White and
142 Taylor, 2000). Further, it is critical to maintain stress-free preoperative conditions in
143 order to facilitate smooth anaesthesia induction and recovery (Kain et al., 2000; Zhou
144 et al., 1993). Therefore, sheep were food-deprived for at least 12 hours prior to
145 surgery in a familiar environment with visual access to conspecifics and *ad libitum*
146 water.

147 An endotracheal tube with inflatable cuff was inserted in all sheep before they
148 underwent general anaesthesia. Intermittent positive pressure ventilation (IPPV) was
149 used throughout anaesthesia to prevent the hypercapnia that would otherwise develop
150 as a result of anaesthetic-induced respiratory depression. When invasive procedures
151 are performed in close proximity to the brain it is important to manage brain volume
152 and allow working space. During anaesthesia, brain volume can be reduced by
153 hyperventilation to produce hypocapnia which decreases the cerebral blood volume
154 (Raichle and Plum, 1972) thus maximising the distance of the cerebral cortex from
155 the cranium. In order to reduce the chance of damaging the cortical surface during
156 drilling or dural incision, we used hyperventilation (increased tidal volume) during
157 IPPV to produce hypocapnia (end tidal CO_2 at 25 - 30 mmHg). During the rest of the
158 procedure, IPPV was adjusted to maintain normocapnia.

159 *Induction and maintenance of anaesthesia:* On the surgery day, sheep were positioned
160 on their hindquarters (caudo-cranial axis perpendicular to the floor, allowing easy
161 access to forelimbs while keeping them calm (Fig. 2A and B). Anaesthesia was

162 induced using Alfaxalone (Alfaxan®, Jurox, UK) at 3mg/kg, given intravenously (IV)
163 through a catheter (Intraflon 20 SWG; Vygon, UK) placed in the cephalic vein on the
164 anterior aspect of the thoracic limb (Fig. 2A). Alfaxalone is a $GABA_A$ receptor
165 agonist that is fast-acting and has a short half-life (Ferré et al., 2006). Alfaxalone has
166 been found to have favourable properties as an anaesthetic induction agent, such as
167 minimal cardiorespiratory suppression in sheep (Andaluz et al., 2013), and no adrenal
168 suppression (Gil et al., 2012). Upon loss of consciousness, the sheep's head and neck
169 were extended upwards and away from the sternum, allowing unimpeded
170 visualisation of the larynx (Fig. 2B). For intubation, an endotracheal tube (Portex®,
171 Smiths Medical International Ltd., UK; size 9-11) was then inserted with the aid of a
172 laryngoscope (20 cm Miller blade) and an endotracheal tube stiffener (Fig. 2C and D).
173 The cuff of the endotracheal tube was inflated and the tube secured in place using a
174 tie around the lower jaw, avoiding the tongue. The sheep was immediately transferred
175 into lateral recumbency on a surgical table and anaesthesia continued with isoflurane
176 in oxygen using a Magill circuit and spontaneous respiration.

177 With the sheep stable under anaesthesia the head was clipped of all wool, the eyes
178 lubricated with Lacri-lube® cream (Allergan Ltd., UK), and topical anaesthetic cream
179 (EMLA™, UK) was applied into both ear canals. The latter was required to reduce
180 stimulation from ear bar placement and to alleviate potential postoperative pain
181 induced from the ear bars used for stereotaxic fixation. To allow blood gas sampling
182 during surgery, an arterial catheter (Intraflon 23 SWG; Vygon, UK) was placed
183 medially in the metacarpal artery of one of the forelimbs, or occasionally medially in
184 the saphenous artery of the hind limb. Finally, the sheep was given procaine penicillin
185 (Depocillin; MSD Animal Health, UK; 15mg/kg) intramuscularly, a non-steroidal
186 anti-inflammatory drug, carprofen (Rimadyl, Zoetis UK Ltd, 4mg/kg)

187 subcutaneously, dexamethasone (Dexadreson; MSD Animal Health, UK; 2mk/kg)
188 and the opioid buprenophrine (Vetergesic; CEVA Animal Health Ltd, UK;
189 0.01mg/kg) intramuscularly.

190 The sheep was transferred to the operating theatre and anaesthesia was then
191 maintained throughout the procedure with isoflurane in oxygen and nitrous oxide (1:2
192 ratio) and IPPV using a Manley ventilator. Vital function was monitored throughout
193 and recorded at five-minute intervals. Target values for physiological variables were a
194 rectal temperature of 37-39°C (Impact III, Vetronic Veterinary Services, UK)
195 (supported with blankets and a warm air heater), end-tidal CO_2 tension of 35-40
196 mmHg, except during procedures in close proximity to the brain when the target was
197 25-30 mmHg (Impact III or VM-2500-M, Viamed, UK) and a mean arterial pressure
198 of 70-90 mmHg (anaeroid manometer, Accoson, UK, and oscillometric "wrist" blood
199 pressure monitor, Lloyds, UK), supported through IV fluid administration (lactated
200 Ringers, Hartmann's Solution 11, Aquapharm, UK).

201 *Recovery:* Once surgical procedures were completed, the sheep was carefully
202 detached from the stereotaxic frame. Isoflurane, nitrous oxide and intermittent
203 positive pressure ventilation were discontinued, while oxygen flow was maintained
204 and respiration supported manually until spontaneous breathing commenced. The
205 sheep was then removed from the sling trolley, placed in a recovery area with padded
206 walls, and supported by a handler with the head in an upright position. When the
207 sheep was sufficiently alert, the endotracheal cuff was deflated and the trachea
208 extubated. Thereafter the sheep was observed until it was able to stand unaided, at
209 which point it was allowed to eat pelleted feed, and was then transferred to the home
210 environment.

211 **Surgery**

212 *Sheep positioning and stereotaxic fixation:* Once stable under anaesthesia, the sheep
213 was transferred onto a sling trolley (hammock trolley Item# 720058, Harvard
214 Bioscience, Inc., USA) in which it remained for the duration of the surgery. This body
215 position (Fig. 2E) was found to be optimal for this type of surgery. It maintained a
216 natural body position while avoiding any pressure or blood flow restriction to the
217 limbs, gave easy access to the dorsal aspect of the cranium, and did not impeded
218 blood drainage from jugular veins, thereby minimizing bleeding through the scalp
219 wound. Limbs were supported in a slightly flexed position with the hooves resting on
220 foam padding support (Fig. 2E).

221 We used a large animal stereotaxic frame (Model 1530 Large Animal Stereotaxic
222 Frame, Kopf Instruments), modified to allow a better fit to the sheep's head (Fig. 3).
223 Modifications included elevating the ear bars above the main bars of the frame and
224 lowering the jaw attachment below the main bars, and elevating the ear socket
225 attachments above the level of the main ear bars (Fig. 3). This head position improved
226 saliva drainage as well as access by the anaesthetist to the mouth area. This allowed
227 monitoring of vital signs without interfering with the aseptic cranial area and ensured
228 excessive pressure was not inadvertently applied to any mouth parts. Additional
229 components used and their dimensions are shown in Supplementary Fig. 1.

230 *Implants:* All sheep were implanted with electroencephalographic (EEG),
231 electromyographic (EMG) and electrooculography (EOG) electrodes. Three types of
232 EEG electrodes were used. These were
233 *1. Skull screws* (Micro Cheese Head Slotted Machine Screws DIN 84A, M2, length 6-
234 9 mm (1 mm increments), New Star Fastenings Ltd, UK, threaded through a stainless
235 steel wire loop).
236 2. *Intracortical needles* (Watkins and Doncaster UK, p/n E6912 diameter of .31 mm

237 and length of 11 mm, soldered at right angles onto insulated stainless steel wire).

238 *3. Subdural circular disk electrodes* (silver disks of 3 mm diameter and 1mm
239 thickness were formed using silver wire annuli fused together with a silver core
240 forming the socket for a pin connector). Dorsal and lateral surfaces of the disk along
241 with the connector pin were insulated with enamel while the ventral conducting
242 surfaces were coated with pure granular silver and a layer of electrolytically formed
243 AgCl. These electrodes were fabricated by NDimension (Science & Engineering) Ltd,
244 Cambridge, UK. The electrodes were placed between the dura and pial membranes,
245 thereby ensuring good contact with the surface of the cortex. These disk electrodes
246 were not rigidly fixed to the skull, thus allowing small movements within the skull.

247 Two types of EMG electrodes were used. The first consisted of two staggered length
248 stainless steel wires with 2-3 mm exposed tips (Cooner wire p/n AS 633-1SSF); the
249 second was a stainless steel spring (PlasticsOne®, p/n 303-017/SPC) that was
250 tunnelled intramuscularly through a 14 gauge, 3 cm long needle cannula. EOG
251 electrodes were made with stainless steel wire (Cooner wire p/n AS 633-1SSF) with
252 an uninsulated tip or stainless steel wire terminated with a silver electrode in pellet
253 form (3mm long, 1mm diameter) constructed in the same manner as the circular disk
254 EEG electrodes (NDimension (Science and Engineering) Ltd, Cambridge, UK).

255 All electrodes from the implant were bundled together and terminated with either a
256 Nano-circular or Nano-strip connector (Omnetics Connector Corporation,
257 Minneapolis, MN). In some cases, the electrode bundles were exteriorised at the
258 occiput, while in others they were housed within a 3D-printed chamber that was
259 secured on the dorsal aspect of the skull using stainless steel brackets and dental
260 acrylic. A schematic of the chamber design is shown in (Fig. 4).

261 *Surgical equipment*: Apart from standard sterile surgical instruments, other essential
262 equipment included a high speed hand drill with adjustable speed for drilling
263 craniotomies, an aspiration pump for removing fluids from the surgical area, and an
264 electrocautery device used to stem excessive bleeding and a fibre optic cold light
265 source (KL1500 LCD, Leica, UK). In some cases, a surgical microscope was also
266 used to visualise deep craniotomies.

267 *Implantation technique:* With the sheep in place in the stereotaxic frame (Fig. 2E), the
268 cephalic area was cleaned thoroughly with 4% w/v chlorhexidine gluconate solution
269 (HiBiScrub®) and pure ethanol solutions. The sheep was then covered with sterile
270 drapes. Sterile drapes were also used to cover the sling trolley so that only the
271 cephalic area remained exposed though a fenestrated drape.

272 A midline incision was created, extending from the midpoint of the interaural line to
273 the occipital end of the skull. Using blunt dissection, the cranial skin was retracted
274 from the skull and the periosteum was scraped to reveal bone landmarks. The skull
275 was cleaned with 3% hydrogen peroxide solution and rinsed with sterile saline spray.
276 As previously described (Perentos et al., 2015) we defined electrode positions relative
277 to 'bregma', which we defined as the point of intersection of the midline skull suture
278 separating the frontal bones, and the transverse suture between the frontal and parietal
279 bones i.e. where frontal and posterior cranial bones meet (Fig. 3A). Approximate
280 electrode positions with respect to the cortex were over the postcruciate gyrus (20-25
281 mm anterior to bregma), the ansatus sulcus (10 mm anterior to bregma), along the
282 front third of the ectolateral sulcus (0 mm anterior to bregma) and on the lateral sulcus
283 near the anterior part of the entolateral sulcus (10mm posterior to bregma). All
284 electrodes were placed ~10 mm lateral to the midline on each hemisphere.

285 When stainless steel screws or needles were used, 2 mm craniotomies were drilled at
286 the desired locations. Stainless steel screws were screwed in place and stainless steel
287 needles were secured in place using a plastic wedge. Where disk electrodes were
288 used, large (~5 mm diameter) craniotomies were drilled. These allowed visualisation
289 and accurate incision of the dura membrane followed by electrode placement below
290 the dura. Dura incisions were achieved using either a micro dissection tool (WPI Inc.
291 Item #14135) or a 23 gauge needle whose tip was bent backwards to form a hook. A
292 cut was performed after inspection of the dura to ensure that no large vessels were
293 near the site of incision. Where there was bleeding after incising the dura, Gelfoam
294 was applied. Once bleeding ceased and visualisation of the dura was possible, the
295 electrodes were then placed under the dura as normal. With all cranial EEG electrodes
296 in place, the skull was dried thoroughly and the craniotomies were first blocked with
297 absorbable haemostat (Fibrillar™, Surgicel®, Ethicon, Johnson & Johnson) and/or
298 bone wax (Ethicon, Johnson & Johnson, p/n ETHW31G) and finally sealed using
299 dental acrylic containing Gentamicin (Depuy, Johnson & Johnson, p/n 3325020,
300 CMW1). The cement was allowed to thicken before application so as to avoid cement
301 entering the intracranial space. In cases where the electrode connector was housed in
302 a skull-fixed chamber (Fig. 4), the chamber was attached to the cranium using skull
303 screws. Subsequently all electrodes and wires were secured in place using dental
304 acrylic.

305 EOG electrodes were tunnelled subcutaneously, using blunt dissection to the inner
306 and outer canthi of one of the eyes. To implant EMG electrodes, the splenius muscle
307 on the posterior neck area was exposed by expanding the main incision in the
308 posterior direction and parting underlying connective tissue. The spring EMG
309 electrodes were tunnelled intramuscularly through a 14-gauge cannula into the

310 splenius muscle. The cannula was then removed from within the muscle by pushing it
311 through the distal side from the point of entry. In an identical manner, a second EMG
312 electrode was implanted into the contralateral splenius muscle. A twisted-pair EMG
313 electrode was also implanted unilaterally on the same muscle by securing the tips of
314 the wires into a 17-gauge needle, pushing the needle and wire through the muscle and
315 retracting the needle while applying pressure onto the muscle. With all electrodes in
316 place, the wound was washed thoroughly with sterile saline and then closed using the
317 horizontal mattress suture technique (Zuber, 2002). The skin around the point of
318 electrode exteriorisation (electrode bundle or skull-fixed chamber) was closed using
319 the purse string suture technique (Teitelbaum, 1998). Finally, the wound was sealed
320 using wound plaster spray (Kruuse Wound Plast, Denmark). After recovery, a
321 transparent Elizabethan collar was fitted around the sheep's head in order to protect
322 the wound (Fig. 1C, 4A and 4C).

323 **Post-operative care and housing conditions**

324 After surgery, sheep were returned to their normal home pen. To reduce the chance of
325 mechanical damage to the implant, the pen had smooth transparent walls (height of
326 1m). Following surgery, all sheep received a four-day course of procaine penicillin
327 (Depocillin, 15mg/kg), carprofen (Rimadyl, 4mg/kg) and buprenophrine (Vetergesic,
328 0.01mg/kg) once per day for 2-3 days. If any signs of pain or discomfort persisted
329 beyond 2-3 days, carprofen and buprenophrine administration was continued until
330 symptoms subsided. Signs of pain might include teeth grinding (Fell and Shutt, 1988),
331 reduced appetite (Gigliuto et al., 2014), abnormal head posture (Molony and Kent,
332 1997), or long periods of immobility (Ley et al., 1989). The wound was checked
333 regularly for any signs of infection and sutures were removed 1-2 weeks after surgery.
334 In cases where a housing chamber was implanted, the purse string suture was left in

335 place for at least 3 weeks after surgery. To ensure adequate food intake, feeding
336 troughs were checked daily and the sheep were weighed weekly.

337 **EEG recording equipment and data collection**

338 Three recording systems were used (Supplementary Table 1). These were:
339 1. Compumedics Siesta amplifier (Compumedics® Ltd, Australia). This 32-channel
340 amplifier was set to 256 Hz sampling rate per channel and was housed in a specially
341 designed jacket (EMKA canine jackets, Lomir Biomedical Inc., France). These
342 jackets were developed for use with dogs but sizes were adapted to fit the sheep, (Fig.
343 1E). The exteriorised electrode bundle cable was channelled to the amplifier through a
344 longitudinal pouch on the dorsal aspect of the jacket. As such, the cables were
345 protected from any interference from conspecifics.
346 2. Neurologger devices (NewBehaviour, TSE Systems, Germany). These devices
347 feature four recording channels and on-board data storage. Sampling rate was set to
348 256 Hz for each channel. They were enclosed in a plastic waterproof case and
349 connected near the sheep's occiput using adhesive Velcro.
350 3. Multichannel systems (MCS wireless W2100 HS-16) wireless transmitters.
351 Sampling rate was set to 512 Hz for each channel and the devices were housed in a
352 similar manner to the Compumedics Siesta devices. In those sheep that were
353 implanted with skull-mounted chambers, the MCS amplifiers were completely housed
354 within the chamber (Fig. 4).

355 All three configurations were well tolerated by the sheep and their conspecifics. Since
356 all of these devices have hard-wired reference and ground electrodes, we adapted their
357 connectors to allow hot swapping of electrodes in case the intended reference or
358 ground electrodes were damaged. As sheep are curious creatures and would take

359 interest in novel objects, where possible we tried to camouflage any exteriorised
360 equipment or cables using the sheep's wool on the back or the neck area.

361 The EEG setup allowed continuous recording across several consecutive days from
362 unrestrained sheep. 24 h recordings were collected from all sheep at multiple time
363 points throughout the lifespan of the implants. These recordings were used primarily
364 for quantitative EEG (qEEG) assessments of sleep wake cycles. Where the
365 Compumedics and the Neurologger devices were used, data were stored on-board the
366 devices until completion of the experiment. Subsequently, data were transferred on to
367 a computer for analysis. Where the MCS system was used, data were wirelessly
368 transmitted to a host PC and stored there for further processing. Acute recordings
369 were also collected that involved investigations into drug effects on the EEG (data not
370 shown).

371 **Analysis**

372 *Anaesthesia:* Throughout each surgery, vital signals were monitored at regular time
373 intervals. We present these data as a function of time into surgery and discuss the
374 anaesthesia method and its applicability to long-duration surgeries.

375 *EEG analysis:* Data were analysed in Mathworks MATLAB R2015a. Custom scripts
376 were written using functions from the EEGLAB, Chronux and Fieldtrip toolboxes
377 (Bokil et al., 2010; Delorme and Makeig, 2004; Oostenveld et al., 2011). Sample
378 EEGs obtained from the different electrode types are shown and a qualitative
379 assessment of the traces is described. Non-rapid eye movement (NREM) sleep was
380 used for quantitative comparisons of electrode types. We chose periods of NREM
381 because this vigilance state is that which is least contaminated by movement artefacts,
382 and the synchronous nature of cortical activity results in large EEG oscillations of

383 known waveform characteristics, which spread throughout most of the cortex and thus
384 can be compared easily. We computed the degree of variability in spectral power
385 across all electrodes for each sheep, with the hypothesis being that a method that leads
386 to reproducible electrode placements across the cortex should also be associated with
387 smaller variability between the spectral power of electrodes. This was achieved by
388 taking 60 seconds of NREM sleep from each sheep, z-scoring all waveforms and
389 computing spectra powers for each channel, then computing the average spectral
390 power in the 0.5 to 5 Hz range, and finally computing the standard deviation of these
391 spectral powers across all electrodes for each sheep. Statistical significance was tested
392 through a one-way analysis of variance. For further analysis (principal components
393 analysis and connectivity analysis) we used wake and NREM epochs. We chose to
394 focus on these two states only as they are associated with markedly different EEG
395 signatures and thus could be used to easily demonstrate the analysis methods that can
396 be applied to these data. Thus spectral analysis, principal components analysis were
397 performed as a function of state. In addition, a connectivity analysis using the
398 weighted phase lag index (wPLI) was also performed. wPLI is a phase
399 synchronisation measure whereby a constant phase relationship for certain parts of the
400 spectrum, is taken to imply function connectivity between the associated brain
401 regions. wPLI, unlike the coherency spectrum, is not susceptible to volume
402 conduction effects and is therefore considered an unbiased estimator of connectivity
403 (Vinck et al., 2011).

404 ## Results

405 ### Anaesthesia

406 Twenty-nine EEG implantation surgeries were conducted. Anaesthesia induction and
407 maintenance were uneventful in all cases. Induction was achieved with an intravenous
408 administration of Alfaxalone at an average dose of 2.5 ± 0.5 mg/kg. On average the
409 sheep spent 4±1.2 h under anaesthesia and vitals throughout this time were stable.
410 Anaesthesia monitoring variables are summarised in (Fig. 5). Core temperature was
411 found to be sensitive to surgery duration, but this was easily counteracted using an air
412 heater. All other vital signals were easily maintained within target ranges. If
413 physiological signs of increased alertness (such as increased blood pressure or heart
414 rate) were observed, isoflurane flow was increased by 0.1-0.3 % and then gradually
415 returned back to the target range of 2.1-2.3%.

416 **Recovery and adverse effects**

417 *Recovery:* All sheep recovered unremarkably. Within 30 minutes after surgery they
418 were able to eat and stand unaided, at which point they were returned to their home
419 pens. No behavioural abnormalities were observed and in general, after recovery from
420 anaesthetic, implanted sheep were behaviourally indistinguishable from non-
421 implanted conspecifics. Two exceptions were encountered. One was in a sheep where
422 anaesthesia recovery was complicated and return of full consciousness delayed due to
423 apparent suppression of spontaneous respiration whilst the endotracheal tube was in
424 place while the sheep regained consciousness. Manual ventilation was maintained
425 during this period but normal spontaneous respiration did not commence until the
426 tube was removed. Thereafter recovery was normal. The other case was a sheep that
427 recovered normally from anaesthesia, but two days after surgery it developed
428 difficulties in eating/swallowing. This sheep was euthanized. It is possible that
429 muscular or nerve damage was induced from stereotaxic fixation, although a post
430 mortem examination failed to reveal any cause for the observed abnormalities.

431 Nevertheless, with subsequent sheep we ensured that stereotaxic fixation did not exert
432 excessive pressure on the ear canal, jaw and inferior orbital rim. Other post-operative
433 adverse effects were limited to four instances of wound infections that developed
434 several weeks after surgery. Interestingly these were only observed in sheep that were
435 implanted during the summer. All instances of wound infections were treated with a
436 three-day course of procaine penicillin (Depocillin, 15mg/kg) while the wound was
437 washed and dried daily using a diluted chlorhexidine solution. Finally, in one sheep
438 whose implant was still operational 24 months after surgery, the cranial skin retracted
439 from the purse string suture opening. This resulted in exposure of part of the surface
440 of implant. However, this did not lead to any infections or other perceptible
441 behavioural abnormalities. In all, the surgical techniques were successful.

442 The degree of difficulty of implantation varied as a function of electrode type.

443 1. *Epidural and subdural screw electrodes*: While these were relatively easy to place,
444 the appropriate length of screw was difficult to estimate given the small size of the
445 craniotomy and the variable skull thickness. Average surgery time was 3:49 ± 0:59 h.
446 2. *Intracortical needles*: These needles were sufficiently long to penetrate the cortex
447 and were therefore more reliable than the screw electrodes. However, the exact
448 electrode depth within the cortex was variable and depended on skull thickness.
449 Average surgery time was 3:22 ± 0:22 h.
450 *3. Subdural circular disk electrodes*: For their placement, these electrodes required
451 larger craniotomies and a small incision of the dura mater. As such, they were
452 technically more difficult to implant and were associated not only with longer surgery
453 times but also occasional bleeding upon dura incision. Such bleeding could be easily
454 stemmed through the brief (1-2 minutes) application of absorbable gelatine sponge
455 (Gelfoam®, Pfizer). Importantly, while taking longer, the larger craniotomies allowed

456 for better visualisation of the brain, which greatly facilitated accurate placement of the
457 electrodes. In the latest 8 disk implantation procedures, which we performed on
458 Merino sheep (data not shown), the average surgery time was 5:08 ± 0:30 h.

459 **Recording device and electrode performance**

460 Multichannel sample traces and frequency spectra from one sheep implanted with
461 disk electrodes are shown for each vigilance state in (Fig. 6 and 7), respectively.
462 Examples of recordings obtained from all electrode types during NREM sleep
463 periods, are shown in (Fig. 8). Although the degree of difficulty of electrode
464 placement increased progressively from screws to needles to disks, the quality of the
465 recordings also improved, with the disk electrodes giving the best results. Z-scored
466 disk electrode spectral powers were less variable that other electrode types (one way
467 ANOVA, $F_{(2,26)} = 4.959$, $p < 0.0295$; screws [mean spectral power variability across
468 electrodes (μ) = 1.534] versus disks [$\mu = 0.557$]: $p = 0.0685$ and needles [$\mu = 1.534$]
469 versus disks [$\mu = 0.557$]: $p = 0.0324$ Fig. 8C). Furthermore, the average success rate
470 measured by counting the fraction of usable electrodes during the first recordings was
471 found to be higher in disk electrodes (96.3 ± 3 %) when compared with needle
472 electrodes (74.5 ± 5 %) or screws (74.8 ± 6 %). A confounding factor of this
473 assessment is that disk electrodes were used in combination with an electrode
474 chamber in all cases except one, so a direct comparison cannot be made. The other
475 electrode types were exteriorised at the occiput in a wire bundle, which was more
476 susceptible to mechanical damage. However, even at earliest recordings (first or
477 second recording post-surgery) where electrodes were mechanically intact, the
478 spectral power variability across electrodes on a per subject basis was still smaller for
479 disk electrodes. Therefore, disk electrodes provide more consistent signals and the
480 implant durability was further improved due to the use of an electrode chamber (Fig.

481 8). Because we were interested in developing methods that could be used to produce
482 electrophysiological markers of disease, the longitudinal applicability of the method
483 was of particular interest. It was therefore important for electrodes to be functional for
484 at least several months. This was easily achieved, especially in the case where a
485 combination of disks electrodes and electrode chambers were used (Fig. 8D). Of all
486 animals carrying screw implants, one reached 24 months with 4 out of 14 electrodes
487 functional, one reached 8 months with 8 out of 14 electrodes, while the rest lasted less
488 than 5 months. Animals carrying needle implants lasted for 9.3 ± 3.8 months.
489 However, some of these were animals that were euthanized before the electrodes
490 failed due to progressed disease phenotype. Animals implanted with disk electrodes
491 lasted on average for 11.8 ± 6.3 months. However, at the time of writing 5 out of 7 of
492 these animals still have more than 14 out of 16 of their electrodes working. Therefore,
493 longevity of disk electrode implants is expected to be longer than 11.8 months, and
494 we consider to be far superior than the other two methods for longitudinal recordings.

495 Examples of multichannel data obtained from disk electrodes are shown in Figs. 6 and
496 9). During wakefulness, the EEG is desynchronised and occasionally accompanied by
497 movement related contaminations (Fig. 9A and B). During NREM sleep, prominent
498 slow wave oscillations can be observed throughout all electrode sites (Fig. 9C and D).
499 With electrodes covering from frontal to occipital areas of the cortex, such recordings
500 allow for methods such as principal components analysis (PCA), or connectivity
501 analysis to be performed. PCA can be applied on multichannel EEG data for signal
502 'denoising' purposes, dimensionality reduction, or simply to capture the signal
503 variability across all electrodes, thereby facilitating interpretation of the data (Fig 9E-
504 H). In this particular example, (Fig. 9F), the first principal component captures most
505 of the slow wave activity during NREM sleep while the second principal component

506 seems to identify a second slow wave component which is temporally not correlated
507 with the first. These two components would not have been identifiable through
508 analyses of the raw data in time or spectral domains. Therefore, additional
509 information about underlying brain activity can be extracted through this method.
510 Connectivity analysis (Fig. 9I and J) permits investigation of brain-wide dynamics
511 and could be particularly useful when analysing interactions between different brain
512 areas or overall connectivity measures as a function of vigilance state, or
513 pharmacological treatments. Here, (Fig. 9I and 9J), it can be seen that the weighted
514 phase lag index is decreased from wakefulness to sleep in the delta, theta and alpha
515 bands. This effect is consistent with reduced connectivity during NREM sleep,
516 possibly due to the synchronous oscillations that occupy most of the cortex during this
517 state (Massimini et al., 2005).

518 While all three devices we used performed well, and permitted recordings from
519 multiple animals at the same time, each was associated with some disadvantages. The
520 Compumedics Siesta amplifier was the most reliable of the three devices with no data
521 loss from any recording and a capability for 24 h recordings. On this device data
522 storage on board as well as wireless transmission to a computer were possible. We
523 made use of the wireless feature at the beginning of the recording to ensure good
524 quality EEG and subsequently switched to on-board storage for the rest of the
525 recording. The bulkiness of the device, however, rendered it more susceptible to
526 physical damage especially since a long cable is required to connect the device on the
527 animals back to the electrode connector on the occiput. The Neurologger devices were
528 physically small, storing all data on board and with battery life exceeding 24 h which
529 made them compatible with recordings in sheep. However, with these devices it was
530 impossible to check data integrity without disconnecting the device first and

531 downloading the data. On some occasions data loss was also experienced due to
532 battery disconnections. This device had a high-pass filter that was limited to 1 Hz,
533 which led to some information loss associated with the slow wave oscillations during
534 slow wave sleep. The MCS devices were small in size and due to their wireless
535 transmission feature they provided the opportunity for monitoring signals remotely
536 for lengths of time exceeding 24 h. On this device data are amplified, filtered and
537 digitised on the head stage and the digitised data transmitted wirelessly. These
538 devices were associated with occasional intermitted signal loss. Although we were not
539 able to confirm the reason for this loss, it is likely that this resulted from interference
540 with the wireless transmission. Therefore, it is important to restrict the use of other
541 wireless devices (such as mobile phones) in the vicinity of the recording setup.
542 Finally, the only one of these devices that supported precise marking of external
543 stimuli onto the EEG traces was the MCS device.

544 Discussion

545 There has been a surge of interest in using large animals as models for human brain
546 function, in particular in the context of disease. Sheep compare favourably to other
547 large animal alternatives such as pigs and cattle and, within some contexts, non-
548 human primates. In some respects, sheep are superior for studying the EEG when
549 compared to other large animal species. For example, although suitable for many
550 other experiments, pigs have large sinuses that extend throughout the dorsal skull and
551 these continue to grow as the animal ages. These sinuses make fixation of implants
552 onto the skull of pigs considerably more challenging and the surgeries riskier than
553 they are in sheep. The use of cattle in behavioural studies is also restrictive, given
554 their size and the associated dangers to the experimenters. While the use of non-
555 human primates would be more translational, the ethical restrictions and the capability

556 these animals have of interfering with recording equipment limit the scenarios of their
557 use. Goats are evolutionarily closely related to sheep and they are likely to be as
558 useful as sheep as a model. Surprisingly, artefacts associated with rumination pose
559 little challenge to the EEG recording technique and, as we have shown in previous
560 studies, may be a useful measure in diseases which affect motor control (Nicol et al.,
561 2016; Perentos et al., 2015). The presence of horns on goats, however, would be an
562 impediment to the EEG implantation. While our report has made use of sheep breeds
563 of smaller size, we have also conducted implantations in Merino breed sheep with
564 weights up to 100 kg (data not shown). Therefore, out technique is suitable for use in
565 breeds of sheep of all sizes. Note that we specifically avoided the use of horned sheep
566 as this was expected to add further variance in skull thickness and possible bleeding
567 from skull craniotomies.

568 Surgery incorporating intricate work in close proximity to the brain (skull drilling and
569 electrode placement) was facilitated by employing hyperventilation to induce
570 hypocapnia to reduce brain volume. Hyperventilation to produce hypocapnia is a
571 widely accepted and easily controlled technique used to reduce the brain volume in
572 order to improve surgical access and limit trauma to the brain (Gelb et al., 2008). In
573 primate neurosurgery surgical access is vastly improved by hyperventilation (Murphy
574 et al., 2012). Manipulation of carbon dioxide tension is precise and rapid leading to
575 near ideal control of brain volume with minimal hysteresis as would be the case using
576 infusion of hypertonic solutions. We used hyperventilation to ensure sufficient space
577 below the cranium to allow screws and electrodes to be placed and fixed without
578 trauma to the dura and brain. The actual brain volume was not measured, but a degree
579 of hypocapnia was first induced, based on current values reported in human and non-
580 human primates, and ventilation was then adjusted according to the effect produced.

581 End tidal carbon dioxide was not reduced below 25 mmHg as this produces no further
582 decrease in brain volume. The duration of hypocapnia was kept to the minimum
583 required for the surgical procedure since prolonged hypocapnia potentially leads to
584 ischaemia. During this period perfusion was impaired as little as possible through
585 maintaining adequate perfusion pressure by ensuring that systemic hypotension did
586 not develop.

587 To leverage the possibilities of using the sheep models of disease that are available or
588 being developed, suitable measures of disease state are desirable. The EEG method
589 was applied in unrestrained freely moving animals and was found to be suitable for
590 monitoring brain activity across vigilance states, during sensory evoked response as
591 well as during drug administration. The method has proved to be highly reliable and
592 safe, with only one out of twenty-nine sheep suffering prolonged post-surgery
593 complications.

594 The three types of electrodes that we used all proved to be adequate for chronic
595 monitoring of vigilance states and were able to distinguish wakefulness, rumination,
596 rumination with concurrent NREM sleep, NREM sleep and REM sleep. While screw
597 electrodes were suitable for quantitative EEG analysis, due to the thickness of the
598 sheep skull precise placement of the screw tip with respect to the brain's surface was
599 not possible. Recording quality and yield were improved considerably when
600 intracortical needles were used. Further improvements were obtained when using disk
601 electrodes. All three methods are useful, and we have outlined the trade-offs in terms
602 of data integrity, quality and longevity versus ease of implantation. For example,
603 where the interest is in vigilance state assessment epidural and subdural screw
604 electrodes or needle electrodes would be sufficient. If the focus of a future study were
605 to be on cortical dynamics across different states and behavioural tasks, disk

606 electrodes would be more suitable. Despite the fact that the disk electrode technique is
607 more time consuming and difficult, when combined with an electrode chamber, it
608 provides better data quality and increased implant longevity. In fact, both of these two
609 latter reasons make the disk electrode technique especially suited to longitudinal
610 investigations.

611 Our work is in line with the three core principles of humane animal experimentation
612 (Reduce, Replace, Refine; Russel and Burch, 1959). In some cases, sheep models may
613 provide a means of reducing or replacing non-human primates in animal
614 experimentation. We facilitate this possibility by providing methods for measuring
615 brain activity *in vivo*. We also expect that reduction of the numbers of animals needed
616 for any one experiment will be possible. Because the methodology described in this
617 paper lends the possibility of recording EEG longitudinally, in some cases it should
618 be possible to replace a cross-sectional study design using multiple cohorts with a
619 longitudinal design using a single cohort. The refinement of electrodes and
620 implantation technique will also lead to better data quality and therefore more
621 efficient use of implanted animals. Finally, as the sheep do not need to be restrained
622 during recordings, they can behave naturally in the absence of stress that might arise
623 from restraining.

624 Future improvements to the method will include the capacity to record brain activity
625 from other brain regions, such as the striatum, thalamus and hippocampus
626 simultaneously with EEG recordings from cortices. Such recordings would be highly
627 valuable in models of diseases such as Huntington's disease or Alzheimer's disease
628 where psychiatric, emotional and behavioural symptoms generated from multiple
629 parts of the brain are features of the disease. Such recordings would prove valuable

when investigating aberrant neuronal networks in various central nervous system disorders.

In conclusion, we have developed a reliable technique that allows the measurement of brain activity longitudinally, in freely moving, unrestrained sheep. The method has already been used for studying naturally occurring models of neurodegenerative disease in sheep and therefore holds great promise in other models of central nervous system diseases in sheep and goats.

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759 ## Figure Legends

760 Figure 1. Photographs of sheep training prior to implantation. (A) Sheep were trained
761 to follow the handler on and off a halter. (B) Sheep were trained through
762 reinforcement to remain calm within a Gambrel restrainer (inset B'). (C) Elizabethan
763 collars were used to protect implants and skin incisions following surgery. (D) Sheep
764 were trained to be calm when suspended in a sling, for up to 1.5 h at a time. (E) An
765 implanted sheep wearing a jacket that housed the ambulatory EEG amplifiers.

766 Figure 2. Illustration of procedures that are part of anaesthesia induction and
767 maintenance. (A) While one operator restricts venous blood flow (arrow 1), a second
768 operator cannulates the cephalic vein of the thoracic limb (arrow 2). The catheter is
769 then used for intravenous induction of anaesthesia. (B) One operator extends the head
770 and neck of the anaesthetised sheep while the other supports the sheep and intubates
771 the trachea. (C) With the neck extended, a laryngoscope (arrow 3) is used to visualise
772 the larynx. (D) The trachea is then intubated with an endotracheal tube and stiffened
773 with a temporarily-placed stylet (arrow 4). (E) The sheep is shown in a sling in the
774 surgery where it is connected to the anaesthetic supply via a breathing circuit and
775 Manley ventilator (arrow 5) for maintenance of anaesthesia. Note the head position
776 that permits saliva drainage (not shown) as well as access to mouth and endotracheal
777 tube (arrow 6). The stereotaxic frame is also visible (arrow 7). The sheep’s feet are
778 supported by foam pads in a slightly flexed position to avoid tension and numbness
779 post recovery (arrow 8). Sterile drapes have been omitted from the figure for clarity.

780 Figure 3. Photographs showing the stereotaxic frame and in-house modifications to
781 the frame. A large animal frame was adapted for use in sheep (a skull is shown in the
782 frame for illustration purposes). (A) Shows a dorsal view of stereotaxic frame and a

783 skull. The box in A, is shown at higher magnification in (A′) showing skull features

784 (●: electrode locations; *: Bregma point marking the intersection of skull sutures). (B)

785 shows a side view of the stereotaxic frame and (C) shows the frame modifications. To

786 achieve the appropriate head angle, auditory fixation points (1) were elevated above

787 the main bars of the stereotaxic frame. Ocular fixation onto the orbital bone is also

788 modified by two additional components that elevate the fixation height while

789 providing additional degrees of movement (2). To allow for stereotaxic fixation of

790 sheep breeds with larger head size, a machined fitting that expands the distance

791 between the frame's main bars was also incorporated (3). The jaw fixation was

792 lowered with respect to stereotaxic frame bars (4).

793 Figure 4. Illustrations of sheep implanted with EEG electrodes and skull chambers.

794 (A) An implanted sheep in an Elizabethan collar (1) is shown with the cover of the

795 electrode chamber visible (blue). The magnification shows a schematic of the implant.

796 When not recording, a low profile screw top is used (blue). (B-D) During recording

797 the low profile screw-top is replaced with the extended chamber that houses the

798 battery (2) and the EEG amplifier/transmitter (3). (C) Illustration of wireless

799 recording while the sheep is freely moving within its home environment. (D) During

800 drug experiments, the sheep is restrained comfortably in a sling. In (C,D) the wireless

801 base station receiver is shown (4), but it could be placed up to 5 m from the sheep.

802 Figure 5. Average physiological variables across all surgical implants are shown as a

803 function of time under anaesthesia. (A) Average O_2 (solid line) and N_2O flow (dashed

804 line). (B) Isoflurane vaporizer setting (C) end tidal CO_2 (solid line) and O_2 saturation

805 (dashed line). (D) Core body temperature, (E) Arterial blood pressure (solid line:

806 systolic, dotted line: mean, dashed line: diastolic). (F) Heart rate (dashed line) and

807 respiration rate (solid line). Data are means (lines) ± S.E.M (shaded grey areas). The
808 arrow (a) shows the start of surgery and surgeries generally lasted between 3-4 h.

809 Figure 6. Example EEG traces from each vigilance stage are shown from a sheep
810 implanted with subdural disk electrodes. For each plot, electrodes from top to bottom
811 correspond to: Fr1L and Fr1R, on the postcruciate gyrus; Fr2L and Fr2R on the
812 ansatus sulcus; CL and CR, on the front third of the ectolateral sulcus; OL and OR, on
813 the lateral sulcus near the anterior part of the entolateral sulcus; EMG is the
814 differential neck muscle recording and EOG is the differential eye movement
815 recording.

816 Figure 7. Example EEG spectral powers are shown from a sheep implanted with
817 subdural disk electrodes for each vigilance stage. The same EEG traces used in
818 Supplementary Figure 2 were used to derive these data. For each electrode location
819 the spectral power is shown for each identified vigilance state. Electrodes Fr1L and
820 Fr1R were on the postcruciate gyrus; Fr2L and Fr2R were on the ansatus sulcus; CL
821 and CR, were on the front third of the ectolateral sulcus; OL and OR were on the
822 lateral sulcus near the anterior part of the entolateral sulcus. Differential neck EMG
823 and differential EOG are also shown at the top of the figure.

824 Figure 8. Data derived from the different electrode types are shown. (A) Illustrations
825 of electrode types are shown. Screw electrodes were either epidural or subdural,
826 depending on skull thickness. Needle electrodes were invariably intracortical. Disk
827 electrodes were placed beneath the dura. (B) Representative NREM sleep EEG
828 records from two electrodes obtained from different screws (sheep 1 and 2), needles
829 (sheep 3 and 4) and disks (sheep 5 and 6). (C) Boxplots of normalised spectral powers
830 for all electrodes are show for each sheep. (D) An example longitudinal dataset from a
831 sheep that was implanted with disk electrodes is shown. The inter-electrode

832 variability in z-scored spectral powers across 11 months of recordings was always
833 within 1 standard deviation of the average.

834 Figure 9. Multichannel EEG data are shown, obtained from a sheep implanted with
835 disk electrodes. (A) Desynchronised EEG activity during wakefulness with slow
836 potential drifts correspond to movement artefacts. (B) Spectral powers for electrodes
837 shown in (A) are similar across electrodes. (C) EEG Data from the same recording
838 during NREM sleep. Clear slow wave oscillations can be seen and these are present in
839 all electrodes. (D) The increased delta band power is reflected in the power spectra
840 that are consistent across electrodes. (E, F) The data from A and B were subjected to
841 PCA. (E) shows the first two principal components during wakefulness and (F) shows
842 the same principal components during NREM sleep. (G) The first two principal
843 components account for ~50% of the variability. (H) When the principal components
844 are compared across states through a 3D projection of the first three principal
845 components, distinct phase-space excursions in the principal component space are
846 clearly seen. (I,J) Weighted phase lag index, a directional measure of connectivity,
847 reveals reduced connectivity across electrodes in the delta, theta and alpha bands as
848 the sheep transitions from wakefulness (I) to sleep (J).

849 Supplementary Figure 1.

850 Modifications to stereotaxic frame are shown. Three components were added to the
851 stereotaxic frame to achieve optimal head positioning during surgery. (A) Jaw
852 fixation extension constructed out of a malleable threaded brass bar allowing for the
853 jaw fixation height to be adjusted downwards. (B) Swivel clamp for extension of
854 ocular fixation points allowing for an additional degree of movement as well as
855 elevation of the orbit fixation point by 2 cm. (C) Raised ear bar fixing clamp for
856 elevation of the fixation points position by 5 cm from the default position.

Figure 1

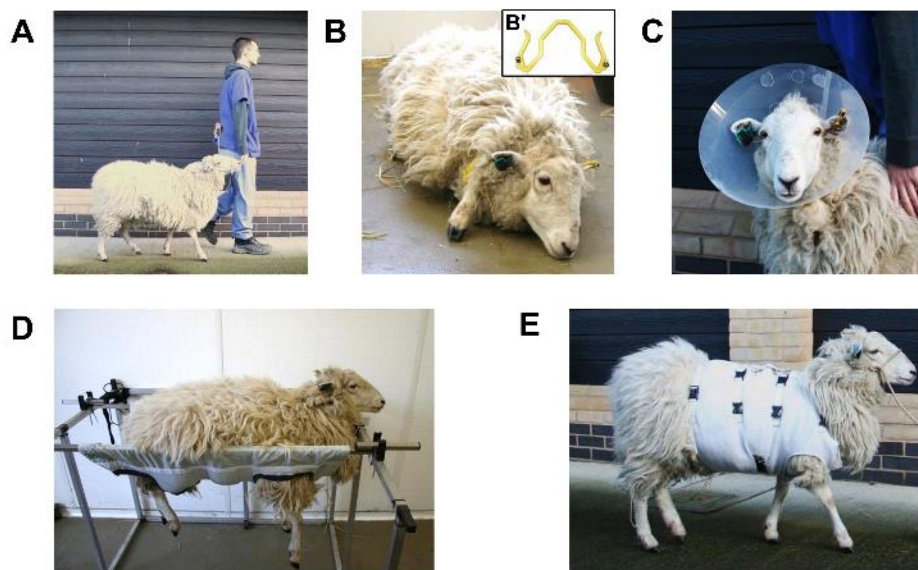

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Figure 2

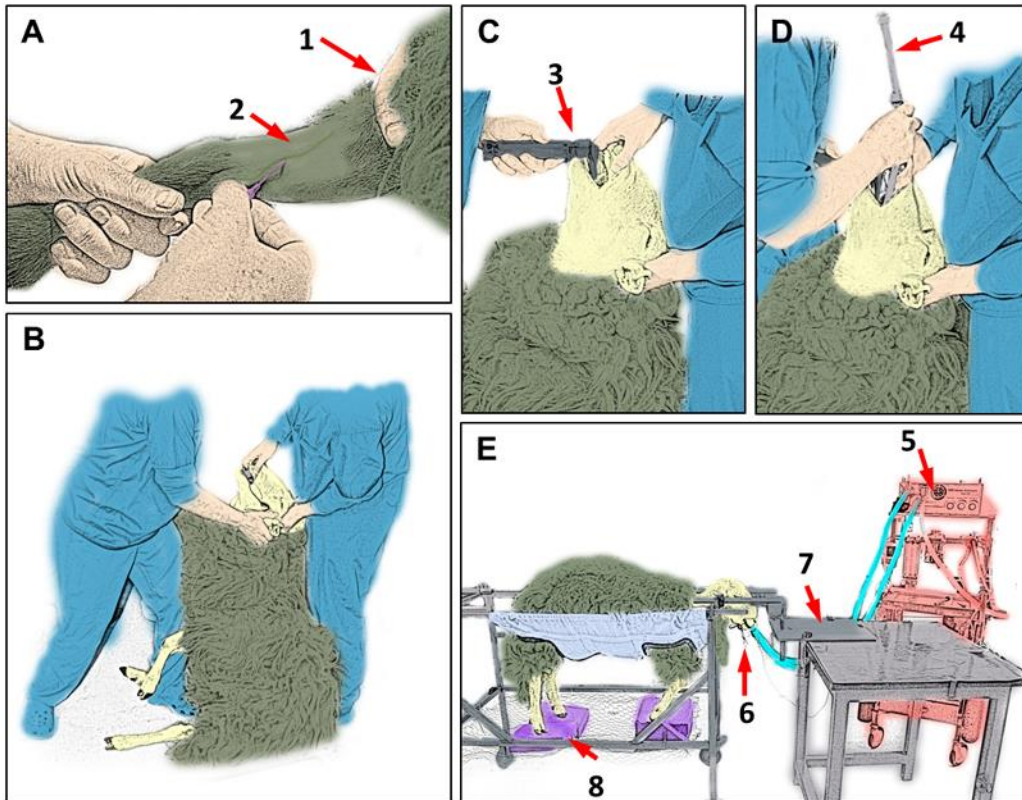

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Figure 3

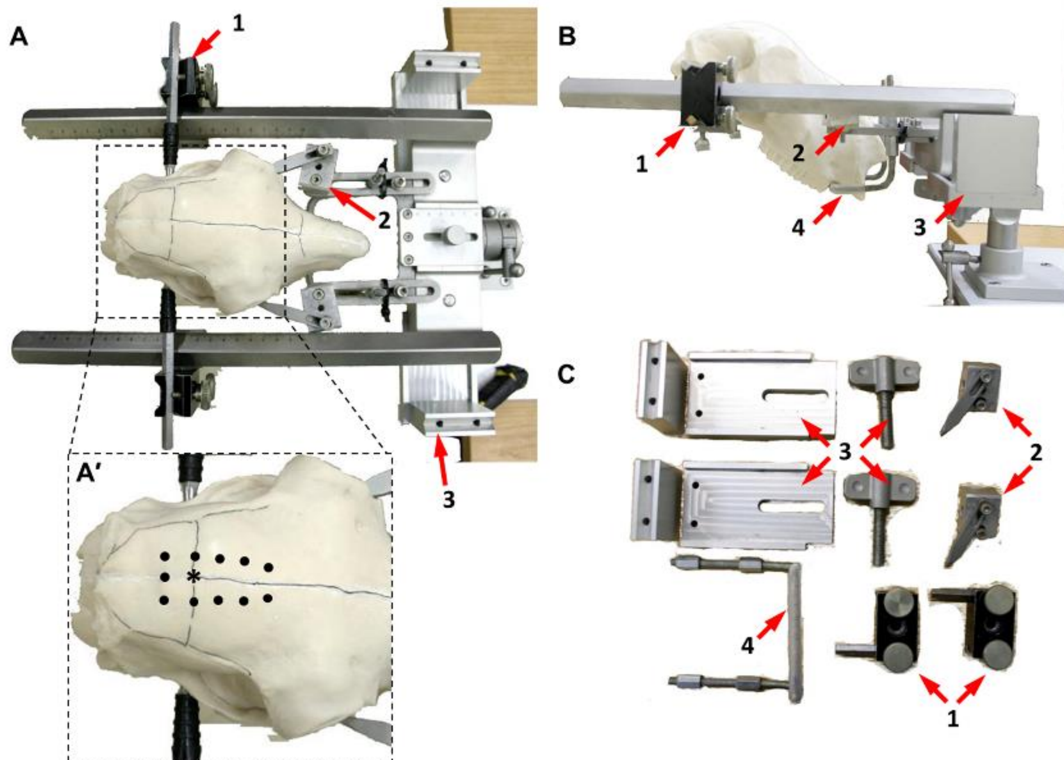

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Figure 4

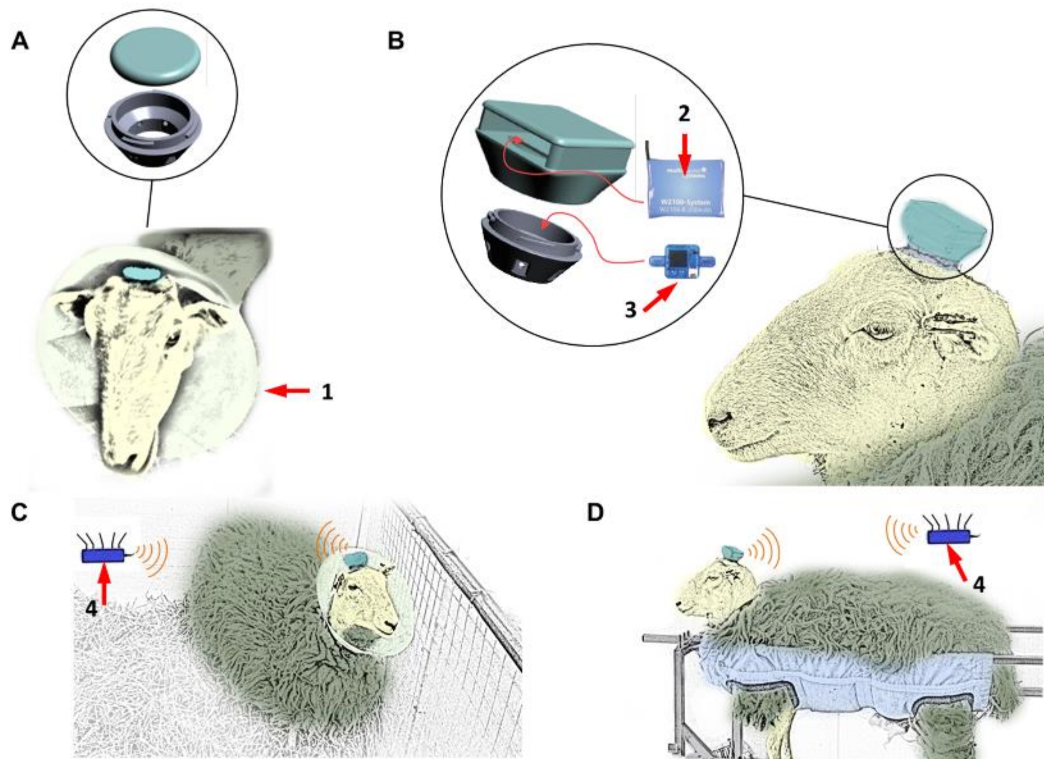

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Figure 5

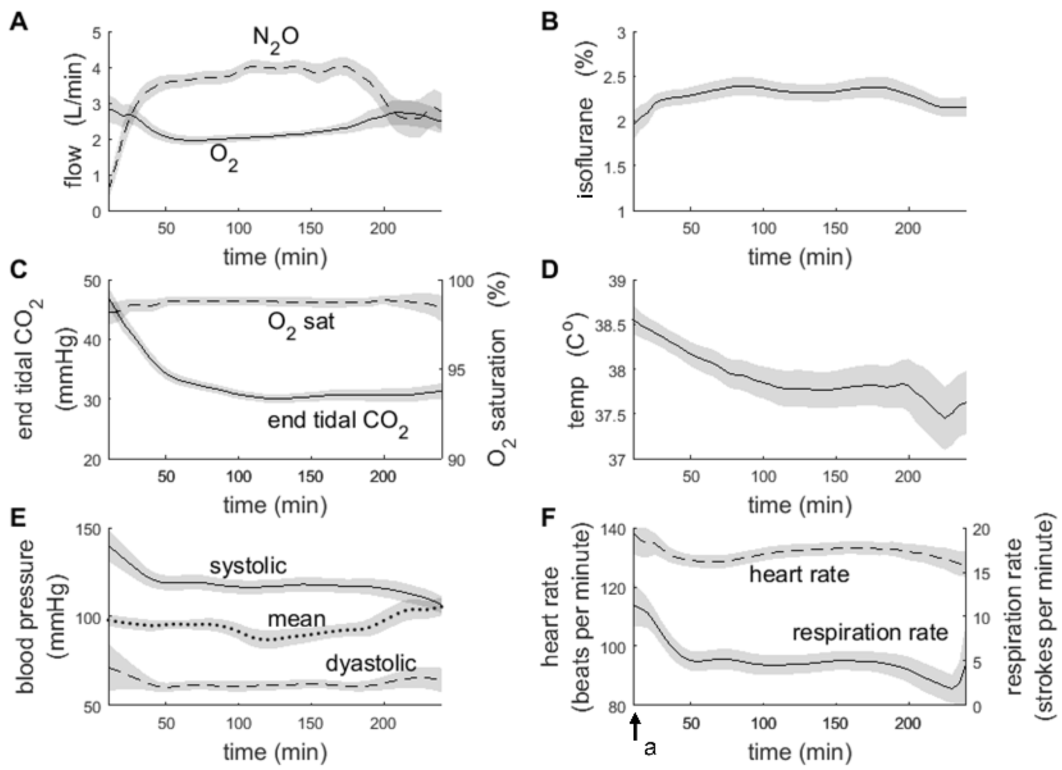

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Figure 6

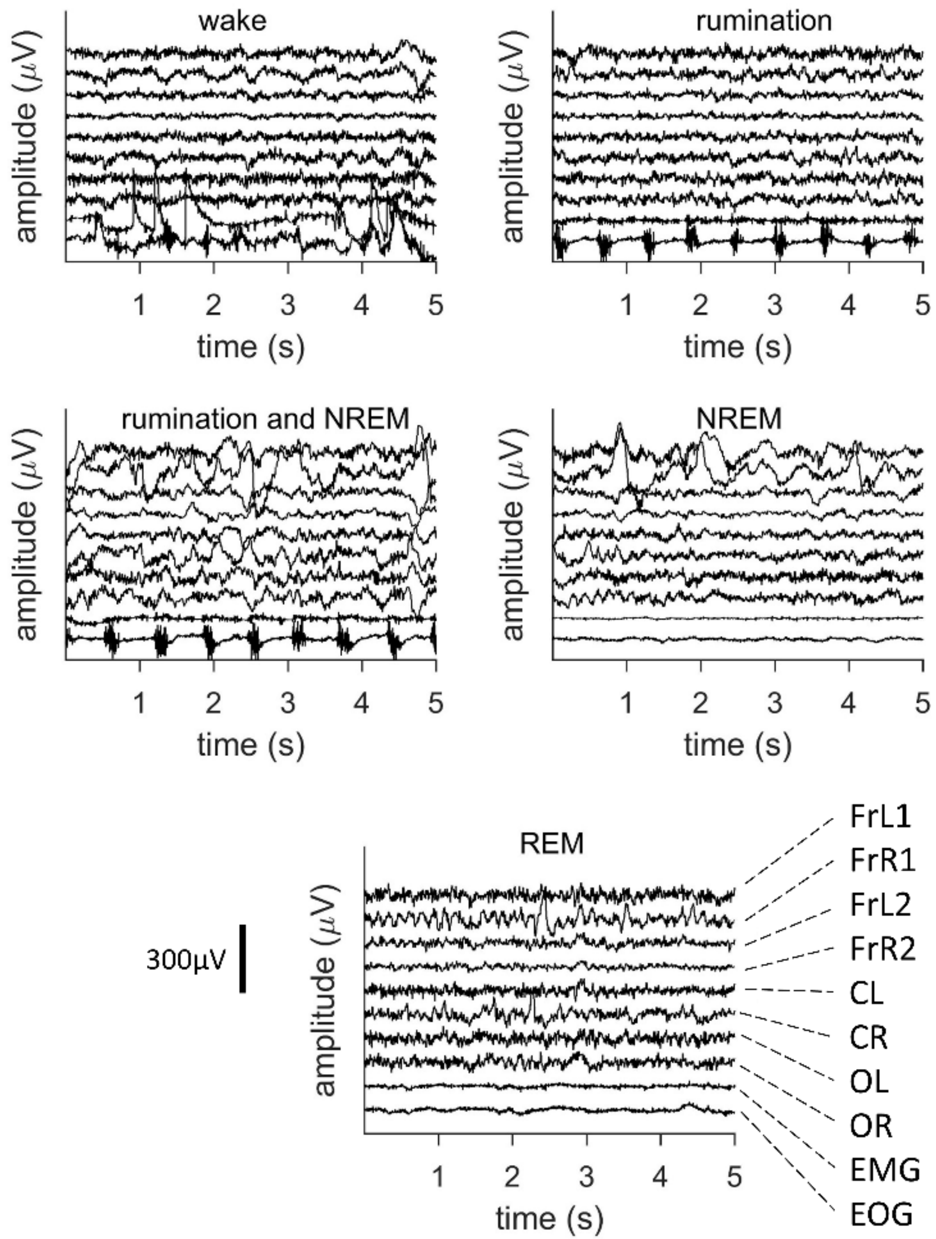

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Figure 7

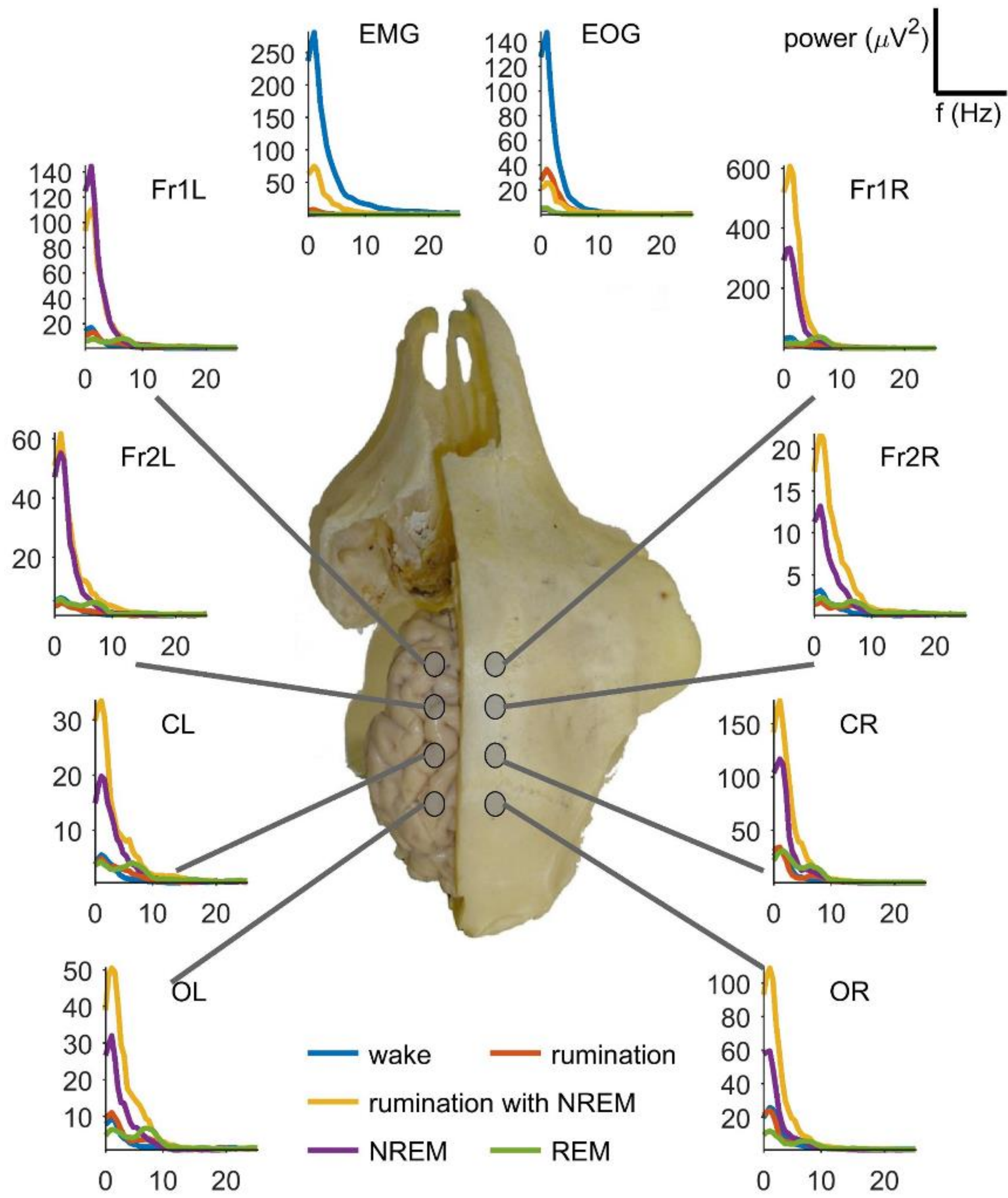

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Figure 8

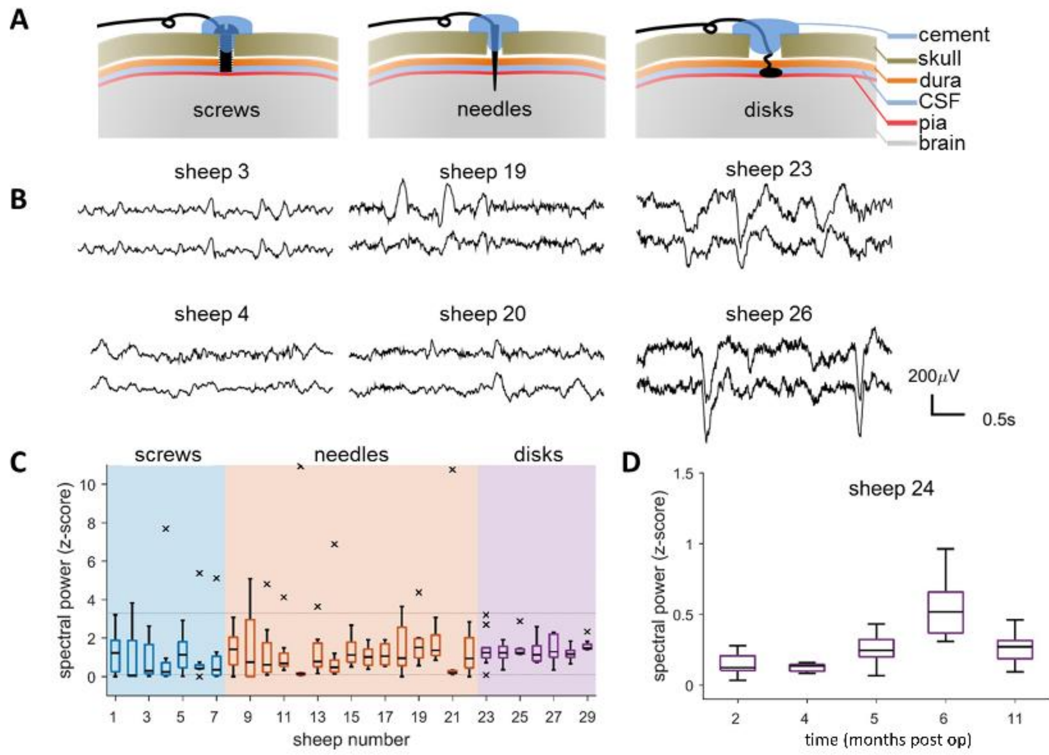

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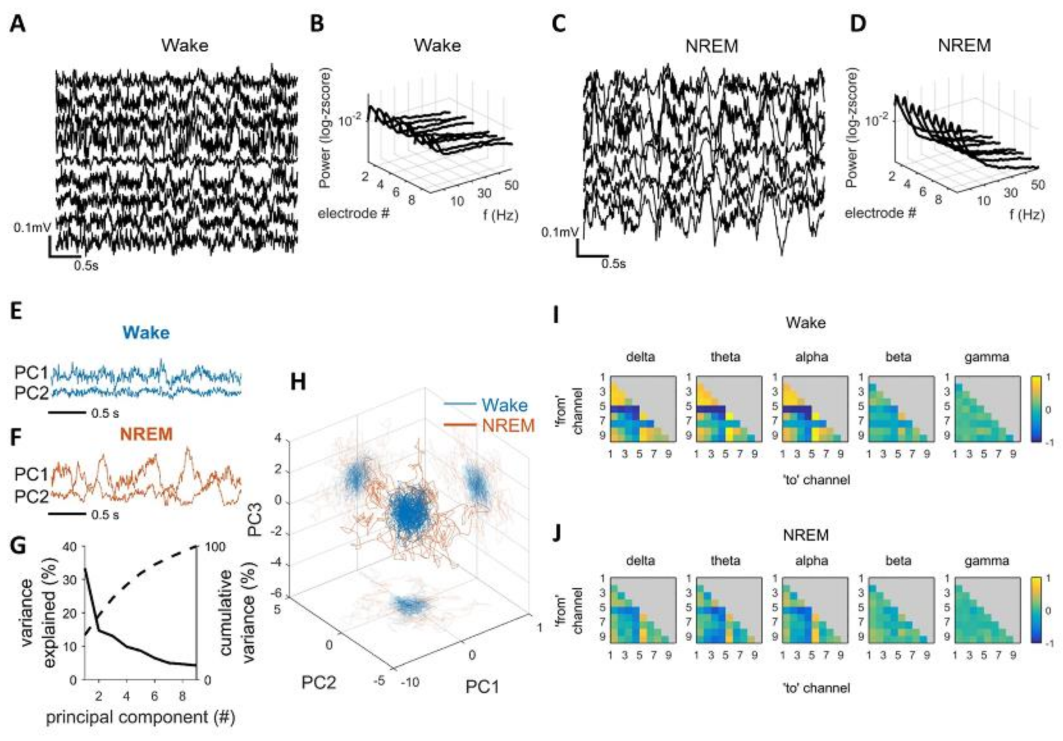
Figure 9



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Supplementary Figure 1

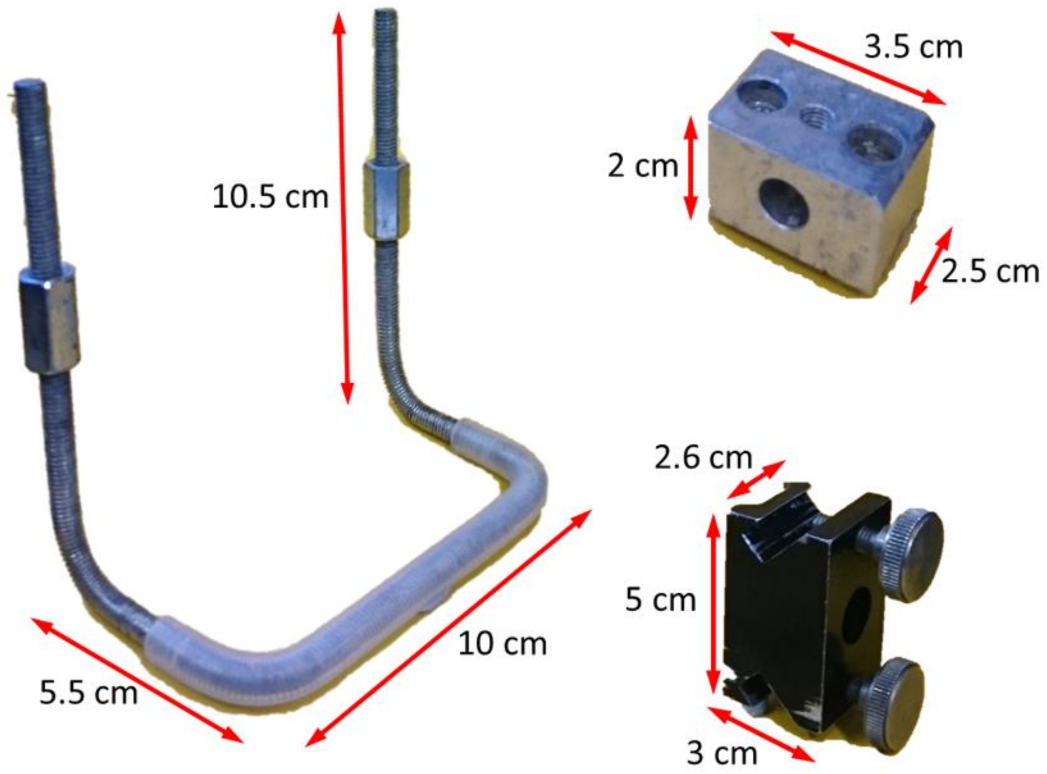

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Table 1. Properties of data acquisition devices

EEG acquisition device	No of channels	Recording type	Acquisition rate/Hz per channel	Filtering/Hz	Maximum recording time	Total weight/g	Ease of fitting*	Time to setup /min	Reliability*	Data management*
Compumedics Synamps Siesta	32	wireless or onboard storage available	256	0.15 - 128	> 24 hours	1200	3	15	3	3
Neurologger	4	onboard storage only	256	1 – 115[#]	> 24 hours	27	1	10	1	1
MCS wireless	16	wireless only	1000	0.5 - 250	> 24 hours	29	2	10	2	2

* Qualitative performance on these variables ranked from best to worst (3 > 2 > 1); # As defined in (Vyssotski et al., 2006)