Payne, S. C., O. Burns, M. J. Stebbing, R. Thomas, A. C. de Silva, A. Sedo, F. Wiessenborn, T. Hyakumura, M. Huynh, C. N. May, R. A. Williams, J. Furness, J. Fallon, and R. Shepherd. 2019. Vagus nerve stimulation to treat inflammatory bowel disease: a chronic, preclinical safety study in sheep. *Bioelectronics in Medicine*. **1**(4): 235-250.

doi: https://doi.org/10.2217/bem-2018-0011

Re-submitted
16/11/2018

Vagus nerve stimulation to treat Inflammatory Bowel Disease: A chronic, pre-clinical safety study in sheep

Abstract

Background: Electrical stimulation of the left cervical vagus nerve is a feasible therapy for inflammatory bowel disease (IBD). However, due to the location of the electrode placement, stimulation is often associated with side effects. **Methods**: We developed a cuff electrode array, designed to be implanted onto the vagus nerve of the lower thorax or abdomen, below branches to vital organs, to minimize off-target effects to stimulation. **Results**: Following chronic implantation and electrical stimulation, electrodes remained functional and neural thresholds stable, while there were minimal off-target affects to stimulation. No nerve damage or corrosion of stimulated electrodes was observed. **Conclusion**: This novel electrode array, located on the vagus nerve below branches to vital organs, is a safe approach for the treatment of IBD.

Summary Points

1. Electrical stimulation of the left cervical vagus nerve above the heart and lungs can result in stimulation-induced side effects
2. We developed a peripheral nerve array that was implanted on the vagus nerve, below branches to the heart and lungs, in order to minimize off-target effects
3. We evaluated safety and maintained effectiveness of the electrode array by continuous stimulation (2 months: 30 Hz, 30 s ON, 150 s OFF) over 3 months.
4. The array recorded electrically-evoked neural activity, thereby confirming that fibres were stimulated
5. There were very minor off-target effects of stimulation
6. There was no adverse histopathological effects, failed nerve activation or electrode deterioration observed following chronic implantation and stimulation of the vagus nerve
7. This novel peripheral nerve array may be implanted onto the abdominal vagus nerve of humans, as an alternative treatment of IBD

Reference Annotations

1. ** Bonaz et al., 2016. Neurogastroenterol. Motil.

This reference describes the pivotal clinical trial showing efficacy of vagal nerve stimulation in Crohn's patients.

2. ** Castoro et al., 2008. Exp. Neurol.

This reference describes the fibre composition of the cervical vagus nerve, and the different neural threshold properties of A, B and C fibres.

3. * Martelli et al., 2014. Auton. Neurosci. This paper reviews the mechanisms and experimental evidence of the anti-inflammatory effects of vagal nerve stimulation

4. * Tyler and Durand 2003. Ann. Biomed. Eng.
The design of our electrode array was adapted from the flat interface nerve electrode described in this paper.

5. * Hoffman and Schnitzlein 1961. Anatomical Record
This paper describes the anatomy of the human vagus nerve, including fiber types, in detail.

6. * Payne et al., 2018 Nat. Rev. Gastrolenterol & Heptol.
This paper describes the mechanisms and clinical evidence of the anti-inflammatory effects of vagal nerve stimulation to relieve inflammatory bowel disease

Key words

Inflammatory bowel disease, vagus nerve stimulation, peripheral nerve, electrode array

1. Introduction

Neurotechnology has improved the lives of millions of patients worldwide *via* the stimulation of, or recording from, target neural populations [1]. Neural stimulators provide sensory feedback in deprived systems (deafness), motor control to effect movement disorders (Parkinson's disease), or treatment to relieve disease symptoms (epilepsy) [2]. The peripheral nervous system has extensive, surgically accessible, neural connections with the central nervous system and end organs [3]. A number of peripheral nerve stimulation sites have been utilised for a range of different disorders, including targeting sacral (pelvic) nerves for the treatment of faecal incontinence [4], constipation [5] and urinary dysfunction [6, 7]. Applications for bioelectric neuromodulation of the cervical vagus nerve to treat disease are diverse. Cervical vagus nerve stimulation (VNS) is an FDA approved treatment for epilepsy [8] and depression [9]. Following the discovery that stimulation of the vagus nerve inhibits acute inflammation [10], promising pilot open label clinical studies show that stimulation of the left cervical vagus nerve has therapeutic efficacy in the treatment of inflammatory diseases such as rheumatoid arthritis [11] and inflammatory bowel disease (IBD) [12]. However, there are limitations and adverse side effects associated with stimulation of this target. A pivotal efficacy and safety study of left cervical VNS in epileptic patients (n=95) reported that at 3 months post-surgery, 63% of patients had experienced dysphonia, 44% coughing, 37% pain and 24% dyspnea during stimulation [13]. These issues were not resolved after 12 months, with over half of the patients (55%) continuing to experience voice alterations, while 15% of patients still experienced pain, and coughing [14]. Similarly, during an open label pilot clincal trial Crohn's disease patients implanted with a similar device on the left cervical vagus nerve experienced dysphonia [12]. To minimise these off-target effects, the intensity and the duration of stimulation can be reduced; however, this may reduce the therapeutic efficacy.

Anatomical and physiological studies provide insight into the off-target effects observed during stimulation of the left cervical vagus nerve. The vagus nerve in the cervical region is composed of fast conducting myelinated A- and B-fibers, and slow conducting unmyelinated C-fibers. A- and B-fibers have lower electrical thresholds than the target C-fibers [15]. Electrical activation of slow conducting unmyelinated vagal C-fibers likely drives the anti-inflammatory effects in inflammatory diseases such as rheumatoid arthritis and IBD [16, 17]. Because of their higher thresholds, activation of cervical vagal C-fibers will also activate A-and B-fibers, resulting in off-target effects that have been reported clinically.

The left vagus nerve, which is the target site for depression and epilepsy, branches to innervate the larynx, pharynx, heart and lungs. The nerve traverses through the thoracic cavity into the abdominal cavity and becomes what is known as the anterior abdominal vagus nerve, and forms the hepatic, celiac and gastric branches to innervate visceral organs [18]. The anterior abdominal vagus nerve consists of 97% C-fibers [19] and is below vagal branches to the larynx, heart and lungs. Therefore, we hypothesised that stimulating the nerve at this location will reduce the potential off-target affects that are experienced with left cervical VNS.

We hypothesised that stimulating the anterior (i.e. left) vagus nerve below branches to the larynx, heart and lungs would result in the recruitment of C-fibers that have anti-inflammatory effects, while minimizing stimulus-induced off-target effects. In order to demonstrate that stimulation levels employed are above C-fiber thresholds, we developed an electrode array with multiple electrode pairs designed to both stimulate and record electrically-evoked neural activity. In order to improve neural recordings of C-fibers, the cuff electrodes were designed to reshape the nerve slightly for an optimal electrode-neural interface, similar to methods reported by Tyler and Durand [20]. Taken together, we aimed to develop a multi-bipolar cuff electrode array that was able to stimulate and record evoked neural activity.

The potential for electrical stimulation-induced tissue damage is a limiting factor when selecting stimulation parameters [21]. The Shannon limit for current intensity describes the boundary between damaging and non-damaging stimulation levels [22-24]. If this limit is exceeded, stimulus-induced neuronal damage can occur due to the production of irreversible electrochemical reactions at the electrode-neural interface. Furthermore, damage from high stimulus intensities can be induced by fatiguing the neurons. Continuous metabolic demands on stimulated neurons may lead to over activation of mitochondria and the generation of free radicals, oxidative stress and tissue damage [25]. Therefore, testing of stimulus regimes using electrode arrays and the target neural population intended for clinical application is essential for the evaluation of safety.

Here, we sought to evaluate the safety of chronic implantation and electrical stimulation of the anterior vagus nerve below the branches to the larynx, heart and lungs in a large

animal model (sheep). Stimulus parameters well above those required to evoke neural activity were used and the nerve was assessed for changes in function or damage. Moreover, we monitored potential off-target effects of stimulation.

2. Methods:

Animals and housing

This study was performed using five Merino ewes. All procedures were conducted with approval from the animal ethics committee of the Florey Institute of Neuroscience and Mental Health (No. 16-073) under the guidelines provided by the Australian Code of Practice for the Care and Use of Animals for scientific purposes. All procedures were also approved by the United States Army Medical Research and Material Command Animal Care and Use Review Office (approval SSC-7486). Animals were housed individually in metabolic cages (n=4) or in a larger free-ranging pen (n=1; 4 m x 4 m). Sheep were provided with 800 g of oaten chaff and given 2L of water per day. The intrarectal temperature was measured for up to 1 week prior to surgery (to establish a baseline), and daily following surgery. Temperature of the room was kept between 20 - 24 ºC and the light-dark cycle depended on natural day-night light.

Design of the vagus nerve electrode array

The electrode array was designed to facilitate ease of surgical application, conformability to the target nerve, chronic stability and quality of neural recording. The cuff array featured three bipolar platinum (Pt, 99.95%) electrode pairs embedded in a medical grade silicone elastomer carrier (**Figure 1**). Bipolar pairs were used to stimulate or record neural activity. Each Pt electrode had an exposed surface area of 1.5 mm^2 (2.5 x 0.6 mm). Each bipolar pair was separated by 2 mm (edge-to-edge), adjacent electrode pairs were separated by 10 mm, and the distance between E1-E2 and E5-E6 was 20 mm. This allowed the recording of both slow and fast conducting electrically-evoked compound action potential (ECAP) responses without significant masking of the response by the electrical artefact (see below). The cuff was sized and shaped to slightly deform the nerve, thereby ensuring good contact between the electrode and nerve whilst maintaining blood flow and nerve health [20] (**Figure 1**). An elongated silicone tab extending on each half of the cuff, proving easy surgical handling when positioning the array around the nerve. The electrodes were connected to a cable that transitioned to a percutaneous connector providing electrical connection to a portable battery powered stimulator.

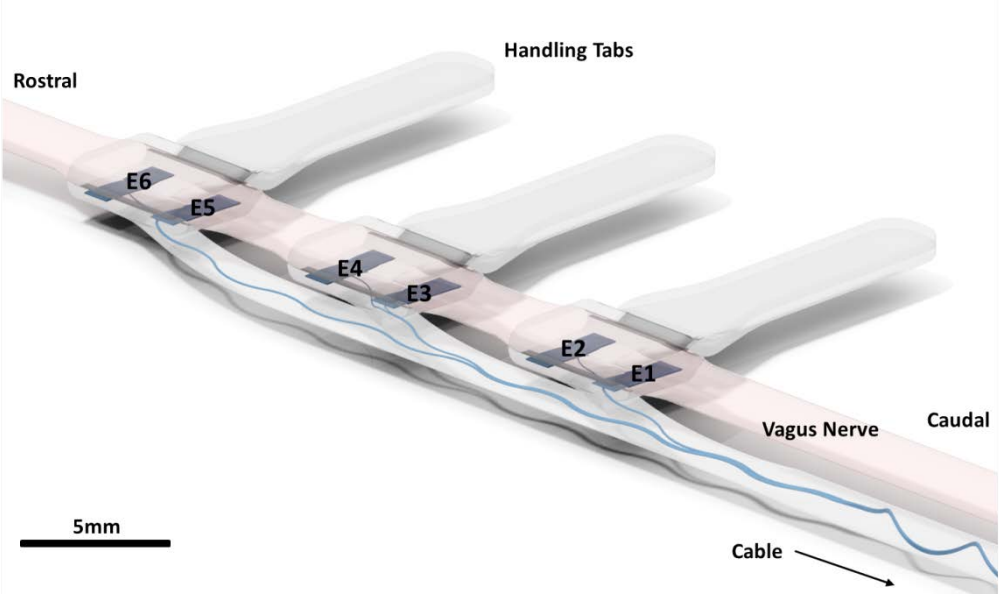

Figure 1. Schematic of the multi-cuff electrode array and cable. The array contained 6 Pt electrodes each with a surface area of 1.5 mm^2. Electrode pair E1-E2 was used to chronically stimulate the nerve. All electrode pairs were able to stimulate and record electrically-evoked compound action potentials from the vagus nerve. Electrode pair E3-E4 (10 mm from the stimulating electrodes E1-E2) were used to record slow conducting ECAPs, while electrode pair E5-E6 (20 mm from the stimulating electrodes E1-E2) were used to record fast conducting ECAPs.

Surgical implantation of the vagus nerve array

Animals were anaesthetised (Time, T = week 0; sodium thiopentone: 15 mg/kg, intravenous) and intubated with an endotracheal tube (cuff size 10). Anaesthesia was maintained (isoflurane 2-2.5%), fractional inspired oxygen altered to maintain partial oxygen (PaO_2) pressure at 100 mmHg, and ventilation regulated to maintain $PaCO_2$ at 40 mmHg [26]. Analgesia was given (Flunixin 1 mg/kg intramuscular) immediately before surgery, and 4 hours and 24 hours after surgery. Intramuscular antibiotics (900 mg, procaine penicillin) were given before surgery and for 2 days post-operatively. The vagus nerve in the lower thorax was selected as the implantation site because of the difficulty in surgically accessing the abdominal vagus in the sheep. Using sterile surgical techniques, the skin and muscle of the left side of the animal was incised at the region of the 6^{th} rib and a thoracotomy performed. Following removal of the 6^{th} rib, the lungs were gently retracted to allow access to the anterior (left) vagus nerve of the lower thorax. The vagus nerve was carefully dissected away from the oesophagus and surrounding fat, and the three pairs of cuff electrodes were placed and secured around the nerve. The array was mechanically stabilised by suturing the cable to superficial connective tissue on the oesophagus. The cable traversed intercostal muscles to exit between the 5^{th} and 7^{th} rib and was tunnelled subcutaneously to the exit location on the dorsal region of the thorax. Overlying muscle and skin layers were sutured closed and

the animals were allowed to recover. Implantation of the vagus nerve array (from induction of anesthesia to the final suturing procedure) in typically took 90 – 120 minutes.

Stimulator design and chronic stimulation program

One month following the implantation of the electrode array, four of the five animals commenced a chronic stimulation program using a custom built programmable back pack stimulator [27]. Charge balanced biphasic current pulses of 0.4 μC/phase were continuously delivered generating a charge density of 27 $\mu C/cm^2$/phase. The stimulus was delivered at 30 pulses per second (pps) using a duty cycle similar to that used in a recent clinical VNS trial (30 s ON, 150 s OFF, Table 1) [12]. Charge recovery was achieved *via* electrode shorting between stimulus pulses [28]. The animal's behavioural responses to the stimulation were monitored carefully.

Electrode impedance and electrically-evoked compound action potentials

Electrodes were monitored daily by measuring their common-ground impedance. The peak electrode voltage developed at the end of the first phase of a 200 μs pulse (0.65 mA current) was measured and the impedance calculated using Ohm's law [29]. ECAPs were recorded weekly in order to monitor neural function. Biphasic current pulses (10 pps, 200 μs per phase, 50 μs interphase gap) were used to stimulate electrodes E1-E2. Neural responses were recorded from bipolar electrodes 10 mm (E3-E4) and 20 mm (E5-E6) from the site of stimulation. Two sets of recordings were made at currents from below threshold to 2.0 mA in 0.1 mA steps. Recordings were sampled at a rate of 100 kHz, filtered (high pass: 200 Hz; low pass: 2000 Hz), amplified ($x10^3$) and 50 presentations were averaged for each response. The ECAP threshold was defined as the minimum stimulus intensity producing a response amplitude of at least 1 μV in both recordings within a latency window of 4-8 ms following the stimulus.

Monitoring off-target effects to stimulation

Food intake and faecal output were measured at the same time each day to assess off-target effects of the stimulation on gut motility. These measurements commenced one week prior to implantation surgery and continued until the day of termination. The median value for each week was analysed. Respiration patterns were recorded in conscious, freely moving sheep by placing a piezo-electric respiration band (UFI model 1132 Pneumotrace II) around the abdomen. Care was taken to place the sensor over the point of largest excursion during respiration. Animals were habituated to the respiration band for at least 1 day before continuous baseline recordings were made with the experimenter out of the room. Respiration rate was then measured within 4 days following the onset of chronic stimulation (n=3 sheep;) and again after 4 – 8 weeks of continuous stimulation (n=4 sheep;). Electrocardiograms (ECGs) were simultaneously recorded to measure heart rate using two vagus nerve electrode pairs. A

breathing and heart rate trial consisted of: 30 seconds OFF (a non-stimulation baseline), 30 seconds 'ON' (2 mA, 200 μs, 30 pps) and 30 seconds OFF (recovery). Trials were repeated up to 21 times in one session. On the day of termination, stimulus induced changes to heart rate were also assessed while the animal was anaesthetised by recording the potential differences between three subcutaneous needle electrodes placed in the fore and hind limbs.

ECGs were recorded over a two minute period without stimulation followed by two minutes of continual stimulation (2 mA, 200 μs, 30 pps), and then a second two minute period of stimulation. This procedure was repeated at 10 and 2 pps. The ECG signal was amplified using a WPI Iso-80 bioamp (Gain: $x10^3$; high pass: 5Hz; low pass 10kHz) and all signals digitized using a CED Micro3 1401 A/D interface and Spike 2 software. Heart rate was calculated from the ECG waveform using a spike detection algorithm in the Spike2 software.

Histological processing and analysis

On completion of the experimental period, animals were euthanized with sodium pentobarbitone (100 mg/kg, I.V). The anterior vagus nerve of the lower thorax together with the entire implanted electrode array (E1-E6) was exposed and images of the array in situ were generated and macroscopic observations made. Next, the array with the nerve and surrounding tissue in place was rapidly removed from the carcass and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) for 48 hours at 4ºC. On completion of the fixation, tissue was thoroughly washed (3 x 20 minutes in phosphate buffered saline). Electrode pair E3 – E4 and the implanted vagus nerve were removed whole and processed separately for assessment of the electrode-neural interface (see Methods section: Electrode neural interface processing). Tissue taken 20 mm rostral and caudal from the electrode array was taken as an intra-animal control. Electrode pairs E1 - E2 and E5 - E6 were carefully dissected from the nerve and the region of the nerve adjacent to the electrodes were labelled using tissue dye (Davidson's Marking system, Bradley Products) [30]. Neural tissue was processed for paraffin wax embedding, sectioned (5 μm), representative sections stained with hematoxylin and eosin (H&E) and mounted with DPX. Electrodes were cleaned (sonication in pyroneg solution and rinsed in water), and examined for evidence of Pt corrosion using a scanning electron microscope (sem, see below). The anterior vagus nerve adjacent to the stimulated electrodes (E1, E2) and unstimulated electrodes (E5, E6) was examined as were regions of nerve just caudal and rostral to the electrode array. Total fascicle area at these locations were quantified and compared statistically. Light microscope images of the nerve sections were taken (Zeiss Axioplan microscope), and these data analysed using Image J.

Clinical pathology examination:

Histological sections of the vagus nerve from all implanted animals (n=5) and a non-implanted control nerve were examined by a clinical pathologist for evidence of neural

damage, the extent and nature of the inflammatory response and any evidence of infection. The examination was performed using appropriate blinded techniques.

Scanning electron microscopy imaging and analysis

Following dissection, both stimulated (E1, E2) and unstimulated (E5, E6) electrode pairs from each animal were examined for evidence of Pt corrosion (FEI QUANTA 200 sem).

Each electrode was examined over a range of magnifications (x 600 – x10,000). The surface condition of each Pt electrode was evaluated by an investigator blinded to the experimental groups. Surface features, including mechanical damage, pitting corrosion, intergranular corrosion and surface deposits, were recorded. The severity of Pt corrosion was graded from 0 (no corrosion); 1 (no evidence of corrosion but electrode at least partially coated with organic material); 2 (localized minor corrosion); 3 (localized moderate corrosion); 4 (widespread corrosion); 5 (severe and extensive corrosion) [31].

Electrode neural interface processing

Electrode pair E3 and E4 and the underlying vagus nerve were collected for processing to enable visualisation of the electrode-neural interface. The specimens were dehydrated in ethanol and immersed in degassed epoxy resin. After embedding, the specimens were sectioned using a grinding technique [32]. Every 300 μm the surface was polished, stained with toluidine blue and photographed under a light microscope. These digital images provided records of the position of the anterior vagus nerve relative to the Pt electrode.

Statistical analysis

Differences in evoked neural thresholds, food intake, faecal output and heart rate data over time were examined statistically using an ordinary or repeated measures (RM) one-way analysis of variance and appropriate *post-hoc* comparisons. Differences between commonground impedance of stimulated and unstimulated electrodes over time was assessed using a two-way ANOVA. Statistical differences in histological measurements between implanted and non-implanted tissue was assessed using a one-way ANOVA or paired two-tailed T-Test). Statistical comparison of Pt electrode surfaces was determined using a Mann-Whitney rank sum test (stimulated and control electrodes). Significance was accepted if $P \leq 0.05$.

3. Results

Implantation period

The mean ± standard error of the mean (S.E.M) vagus nerve array implantation period for all sheep (n = 5) was 11.9 ± 1.0 weeks. Four sheep were successfully implanted for >12 weeks, all of which were electrically stimulated, while one animal (VN_c5), randomly selected as an unstimulated control, was sacrificed at 7.9 weeks post implantation due to complications not related to the implantation of the vagus nerve array (**Table 1**).

309

310 **Table 1**. Summary of sheep and stimulation used in the safety cohort.

Sheep	Implantation duration (weeks)	Stimulated electrodes	Charge per phase (nC)	Total switch on time (hours)	Adverse events	Electrode region at autopsy
VNS1	12.8	E1-E2	400	984	None	Benign tissue fibrosis; no infection
VNS2	13.6	E1-E2	400	1524	None	Benign tissue fibrosis; no infection
VNS3	13.1	E1-E2	400	1800	None	Benign tissue fibrosis; no infection
VNS4	12.1	E1-E2	400	1404	None	Benign tissue fibrosis; no infection
VN_c5	7.9	N/A	N/A	N/A	Yes - event not related to device	Less mature tissue fibrosis; no infection

311 N/A, not applicable.

312

313 *Post-surgical monitoring*

314 During the implantation period, the rectal temperature of sheep ranged from 39.0 to
315 39.8 ºC, which is considered normal. Surgical incision sites healed quickly and remained
316 free from infection. The skin in proximity with the percutaneous connector and
317 subcutaneous cable remained free from infection and irritation. Furthermore, no
318 adverse responses (e.g. coughing, agitation or dyspnea) were observed during the
319 chronic stimulation program.

320 *Electrode impedance during implantation*

321 All electrodes from each animal in this study remained functional throughout the
322 implantation/stimulation period (n = 30 electrodes; n = 5 sheep; **Figure 2**). There was
323 no significant difference between the impedance of chronically stimulated and
324 unstimulated electrodes over the implantation period (P = 0.37, RM two-way ANOVA,
325 time x stimulation; **Figure 2**).

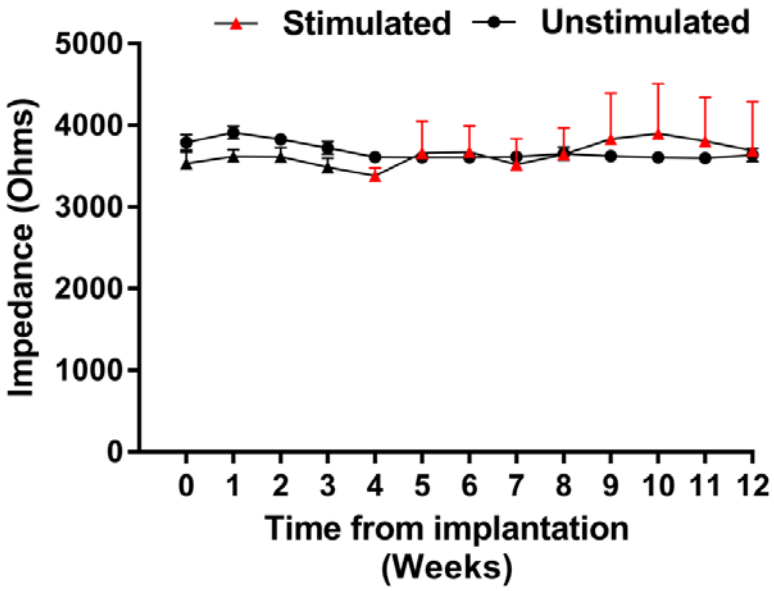

Figure 2. Electrode impedance recorded weekly during the implantation period for all electrodes in this study. All electrodes (n = 30) remained functional during this period, and there were no significant differences between impedance of stimulated (red; n=8) and unstimulated (black; n=22) electrodes (P = 0.37). Data show mean common-ground impedance +S.E.M. While all error bars have been plotted, some are obscured by the symbols.

Electrically-evoked neural responses

ECAPs were successfully recorded from all implanted animals (n=5) over the duration of the implantation period. Representative ECAP waveforms recorded at 20 mm from the stimulation site are shown in **Figure 3A** (indicated by arrow) while the recruitment of slower neural populations, recorded at 10 mm from the stimulation site, is displayed in **Figures 3B-C** (indicated by arrow). Although there was a trend for an increase in ECAP threshold over the implantation period, this difference was not statistically significant (n = 4; P = 0.08; RM one-way ANOVA; **Figure 3D**). The average latency of the ECAP recorded 10 mm from the stimulation site was 7.0 ± 1.2 ms (T = 0; estimated conduction of 1.4 ± 0.2 m/s; Figure **3B-C**) did not change during the implantation period (P = 0.49, n = 4). Furthermore, neural thresholds remained well below the stimulus levels used for chronic stimulation (2 mA, 200 μs pulse width), thereby confirming that the vagus nerve was stimulated at suprathreshold levels for the duration of the stimulus program.

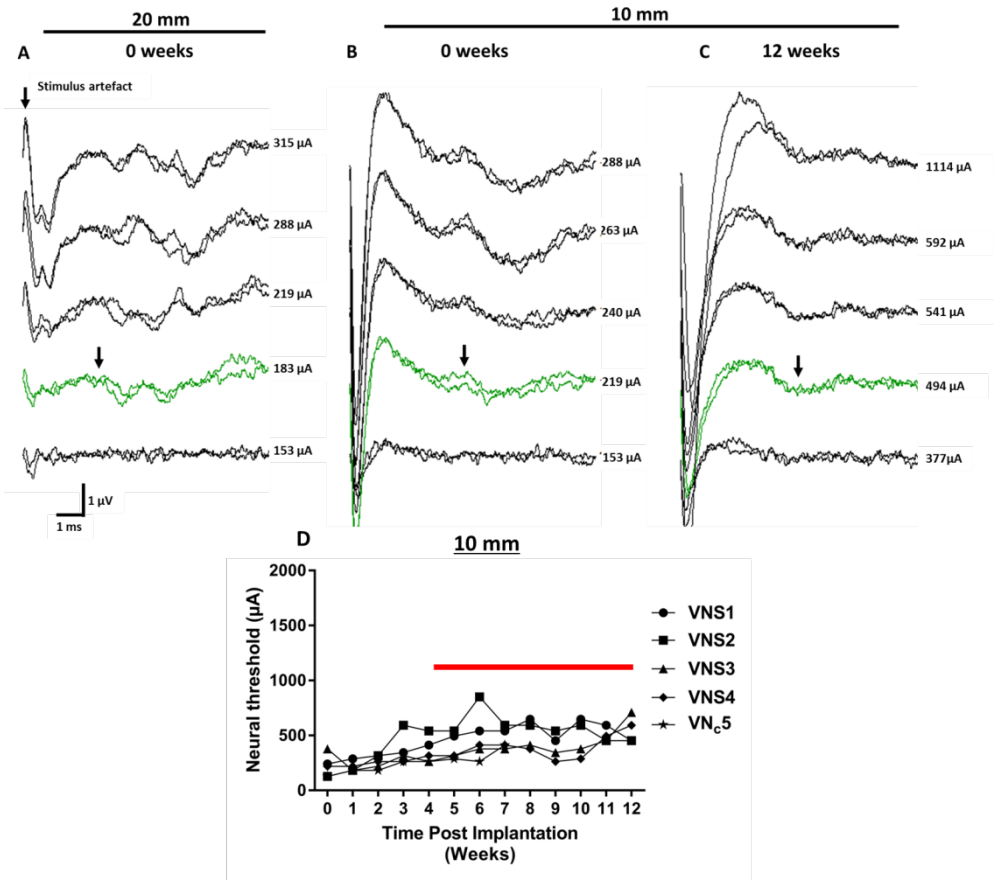

Figure 3. Electrophysiological recordings of the vagus nerve during the implantation period. **A:** ECAPs (indicated by arrow) of faster conduction fibers were detected using recording electrodes 20 mm from the stimulating electrodes pairs at 0 weeks (latency: 4.4 ms, conduction: 4.5 m/s). **B-C**: ECAPs of slower conduction fibers were recorded at a site 10 mm from stimulating electrodes at 0 weeks (**B**, latency: 4.1 ms, conduction: 1.4 m/s) and 12 weeks (**C,** latency: 5.0 ms, conduction: 2 m/s) from the same animal (VNS4). **D**: Quantification of neural response thresholds (indicated by the green recordings in A-C) from 10 mm electrode configuration (T = 0) show no changes (P = 0.08) in thresholds over the implantation period. The red bar indicates the stimulation period). In **A-C**, the arrows indicate the times of the ECAP peak at the recording electrode.

Off-target effects pre- and post- the onset of stimulation

The sheep that were stimulated did not display any adverse behaviour indicative of pain, alarm or discomfort at either the initial onset of stimulation or for the duration of the stimulation program. Food intake and faecal output (per day, **Figure 4A, B)** was analysed (RM one-way ANOVA, Dunnett's *post hoc* test using T = -1 as control) for stimulated animals maintained in metabolic cages (VNS1, 2, 4). Food intake remained similar to baseline following implantation surgery (T = 0 to 12 weeks; n = 3; P = 0.12; **Figure 4A**). Daily faecal output was reduced during the first week after surgery (T = 0; P = 0.02) compared with pre-surgical baseline values. However, between 1 and 12 weeks

faecal output remained similar to pre-surgical baseline values (P = 0.25; n = 3; **Figure 4B**).

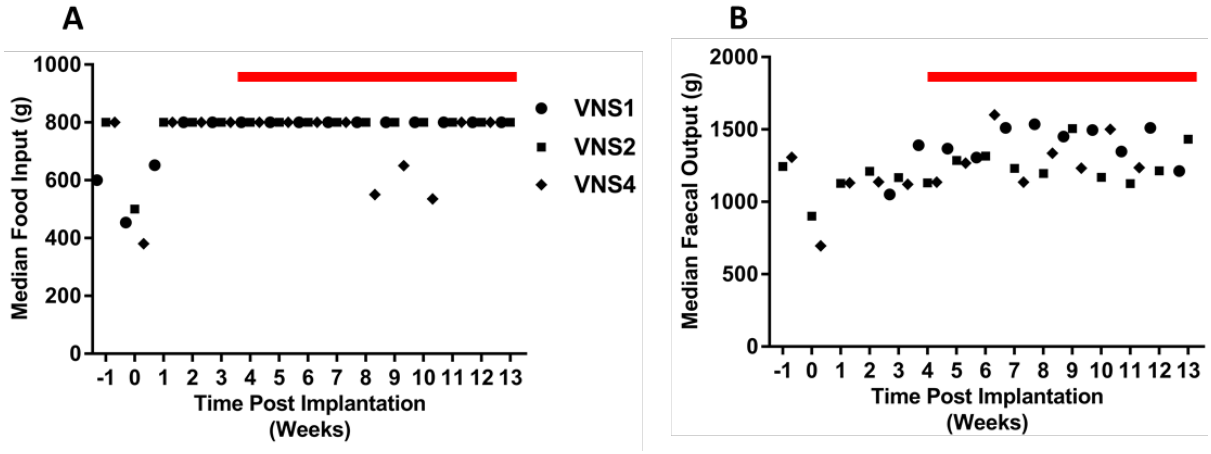

Figure 4. Effect of stimulation on food intake and faecal output. **A**: Food intake (maximum 800 g) per day remained similar to pre-surgery levels (T=-1) following implantation surgery and during the chronic stimulation (indicated by red bar) (T=0 to 12 weeks, P = 0.12). **B**: Although faecal output (per day) decreased during the first week post-surgery (T=0, P = 0.02), no change was seen from baseline (T=-1, P = 0.25) in subsequent weeks.

During the period in which the vagus nerve was being stimulated, we assessed respiration and heart rate in awake, freely moving sheep within 4 days of first switching on the chronic stimulation (n = 3 sheep) and after 4 weeks of continuous stimulation (n = 4 sheep). Respiration varies greatly over time in conscious sheep. We inspected the recordings for any abrupt change in respiration pattern that coincided with the start of the stimulus period over multiple stimulus trials. Within 4 days of the onset of the chronic stimulation program there were no observed changes to respiration rate in one animal, while two animals exhibited a transient slowing of respiration that coincided with the onset of stimulation in 15/21 and 8/12 trials respectively. A stimulus induced reduction in respiration rate became less evident during the course of the chronic stimulation program. At 4 weeks of stimulation, two animals showed no stimulus induced effects on respiration (**Figure 5A**) and the third animal exhibited only small changes in respiration during the onset of stimulation (observed in 6/11 trials).

Heart rate was also measured in awake, freely moving sheep. Heart rate data from multiple stimulation trials before, during and after each 30 s stimulation period was analysed in each sheep separately using a RM one-way ANOVA. When tested within 4 days of the onset of stimulation, there was no significant effect of stimulation on heart rate in three sheep ($P \geq 0.05$) and only a small increase in one animal (VNS3; 73 to 78 beats per minute; P = 0.01). This effect was not seen in any of the four stimulated sheep tested at a later time point ($P \geq 0.05$; **Figure 5B**). At the time of termination, changes in heart rate under anaesthesia before, during and after each 30 s stimulation period was

measured. Under these conditions, there were no stimulus induced changes in heart rate in any tested animal (n=4).

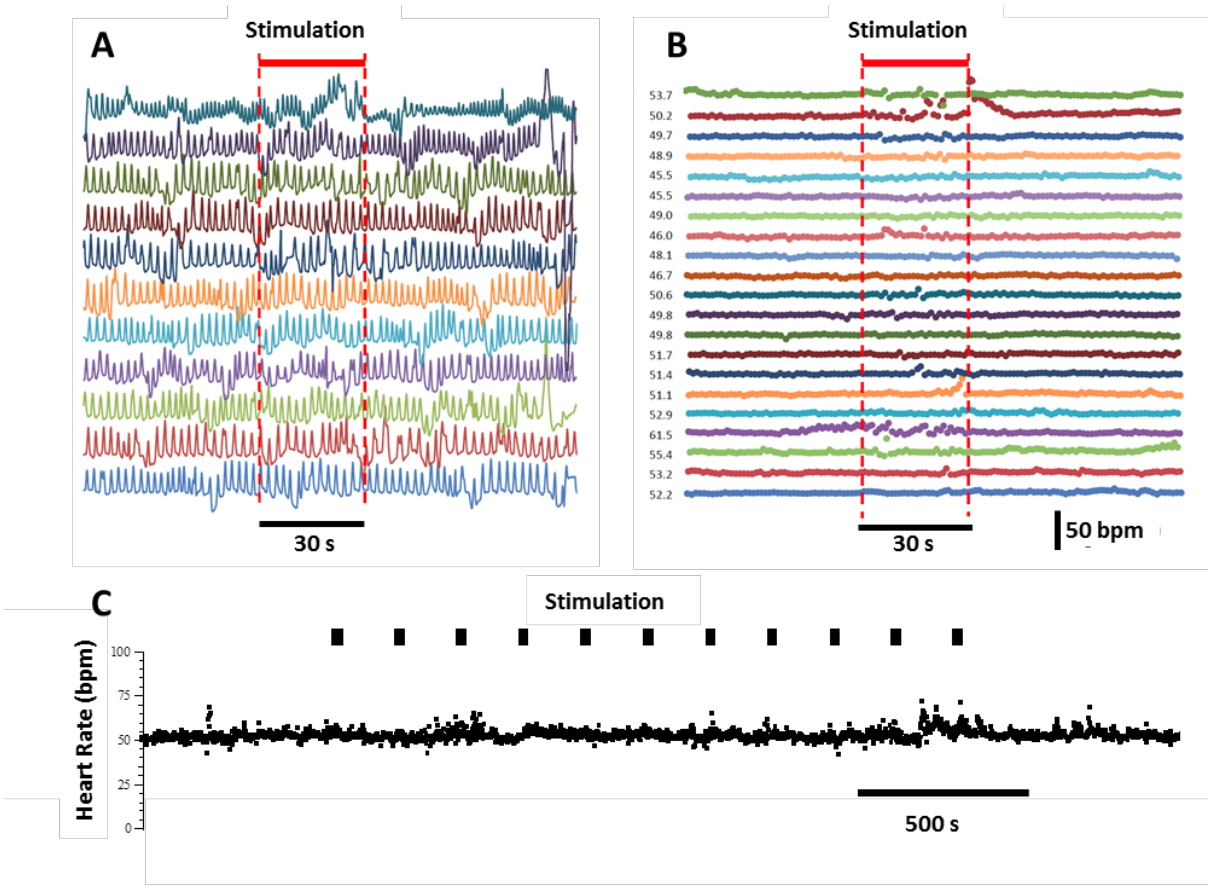

Figure 5. Assessment of potential off-target effects in response to anterior vagus nerve stimulation. **A:** Representative respiration traces (VNS4) recorded at 4 weeks following onset of chronic stimulation illustrating the baseline variation in the recordings; there was no evidence of a stimulus induced change in respiration. **B:** A trace from one sheep (VNS4) is an example of the heart rate recording trials (n = 21) taken within the first 4 days of the onset of stimulation. No consistent changes to heart rate were observed within the 30 s stimulation period. Mean heart rate for each recording is also illustrated to the left of each recording trial. **C:** Longitudinal recording of heart rate in one sheep (VNS4) recorded at 4 weeks following onset of stimulation. Again, no changes to heart rate were consistently observed within the 30 s stimulation period.

Autopsy observations of array in vivo

On the day of euthanasia, macroscopic examination of the status of the tissue surrounding the percutaneous connector, cable and vagus nerve electrode array was undertaken (Table 1). In all implanted animals (n = 5), fibrous tissue surrounding the percutaneous connector and the subcutaneous cable was free from infection and no signs of irritation or inflammation were observed macroscopically. The cable transitioning point through the intercostal muscle layer into the thoracic cavity was free

from infection and had healed well. There was minimal scar tissue, and the intercostal muscle tissue around the cable was unencumbered. Examination of the multi-cuff array *in vivo* showed that a thin fibrous tissue encapsulation had formed around the array (**Figure 6**), and there was no distortion (i.e. twisting) of the vagus nerve. The tissue encapsulation was restricted to the vicinity of the array and cable, and did not spread from this area and affect adjacent tissues (**Figure 6**). Attachment of the electrode array had not caused any macroscopic damage to the nerve, and blood vessels were typically observed running longitudinally along the implanted vagus nerve. No irritation, haemorrhaging or haematomas were observed within adjacent tissue (oesophagus, lung and mediastinum).

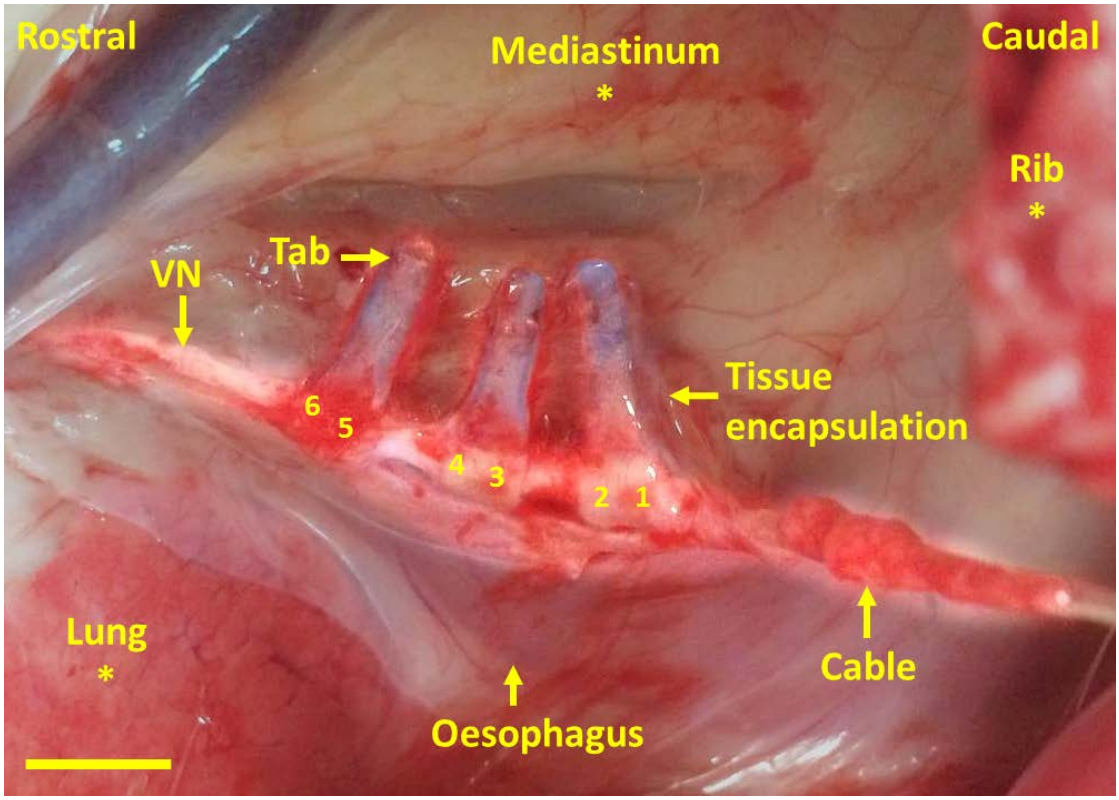

Figure 6. *In vivo* macroscopic observation of the implanted array. Autopsy image shows the typical gross tissue response around the electrode array following 12 weeks of implantation (VNS1), and illustrates the anterior vagus nerve of the lower thorax (VN) and the electrode array (with each of the six electrodes identified numerically), the three handling tabs and the cable. Scale bar = 10 mm.

No histological damage seen in implanted vagus nerves

Representative micrographs of the tissue response of the vagus nerve following chronic implantation and electrical stimulation are illustrated in **Figure 7**. The vagus nerve from a non-implanted control animal is included in this figure for comparison (**Figure 7A**).

A clinical pathologist (author: RAW) reported no damage to nerve fascicles due to the implant and/or electrical stimulation, and no evidence of granulation tissue or the infiltration of acute inflammatory cells (**Figure 7C-F**). There was a mature, but benign, fibrous tissue response around the epineurium that was not observed to have chronic inflammatory cells within it. There was no observed histological difference between stimulated (E1 and E2) and unstimulated (E5 and E6) regions of the nerve. However, a foreign body and histiocytic response around the periphery of the tissue capsule had developed along the boundary of the nerve with the cuff electrode array in both stimulated and unstimulated tissue (**Figure 7C-F,** indicated by arrows). The distribution and appearance of blood vessels within the fascicles appeared normal in both stimulated and unstimulated tissue. The fascicle shape was distorted transversely due to the design of the cuff electrode, similar to that described previously [20]. In a few sections, there was evidence of small regions of chronic inflammation inside the fibrotic tissue capsule. These inflammatory pockets rarely encroached into the boundary of the epineurium, and mostly remained within the confines of the fibrotic tissue encapsulation.

Tissue taken rostral (**Figure 7B**) and caudal (**Figure 7G**) to the implanted electrode array did not have a fibrous tissue response around the epineurium. Typically, these rostral and caudal sites had less or no fibrous tissue present between fascicles, when compared with implanted regions of nerve (**Figure 7C-F**). The tissue response in control animal VN_c5 (implanted for 2 months) was less mature than the response in the animals implanted for 3 months (VNS1-4) as evidenced by the greater number of fibroblasts in the histology of VN_c5 (data not shown). Small deposits of hemosiderin were occasionally observed in some sections of the implanted tissue, implying that some localized bleeding had occurred at the time of surgery.

Fascicle quantification was conducted by measuring both fascicle area and the total cross-sectional area of the nerve from histological sections taken from tissue 20 mm rostral and caudal to the implant, adjacent to stimulated electrodes (E1 and E2) and adjacent to unstimulated electrodes (E5 and E6). The control animal (VN_c5) was not included in analysis as it had been implanted for 7 rather than 12 weeks. There was no significant difference in the total fascicle area between these sites ($P = 0.64$; one-way ANOVA) indicating that neither chronic implantation nor stimulation resulted in neural loss, compared with regions of the nerve proximal (rostral) or distal (caudal) to the electrode array. The proportion of total fascicle area compared with the total cross sectional nerve area (i.e. the fascicle packing density) was assessed between stimulated (E1 and E2) and unstimulated (E5 and E6) tissue and showed no statistically significant difference (n=4; paired two-tailed T-Test; $P = 0.86$). This fascicle packing density data from tissue adjacent to E1, E2, E5, E6 was therefore combined. There was a significant increase in the fascicle packing density between implanted tissue (23.9 ± 1.2%) versus the two non-implanted regions of the vagus nerve (17.0 ± 2.5%; two-tailed paired T-test; $P = 0.001$; **Figure 7B, G**).

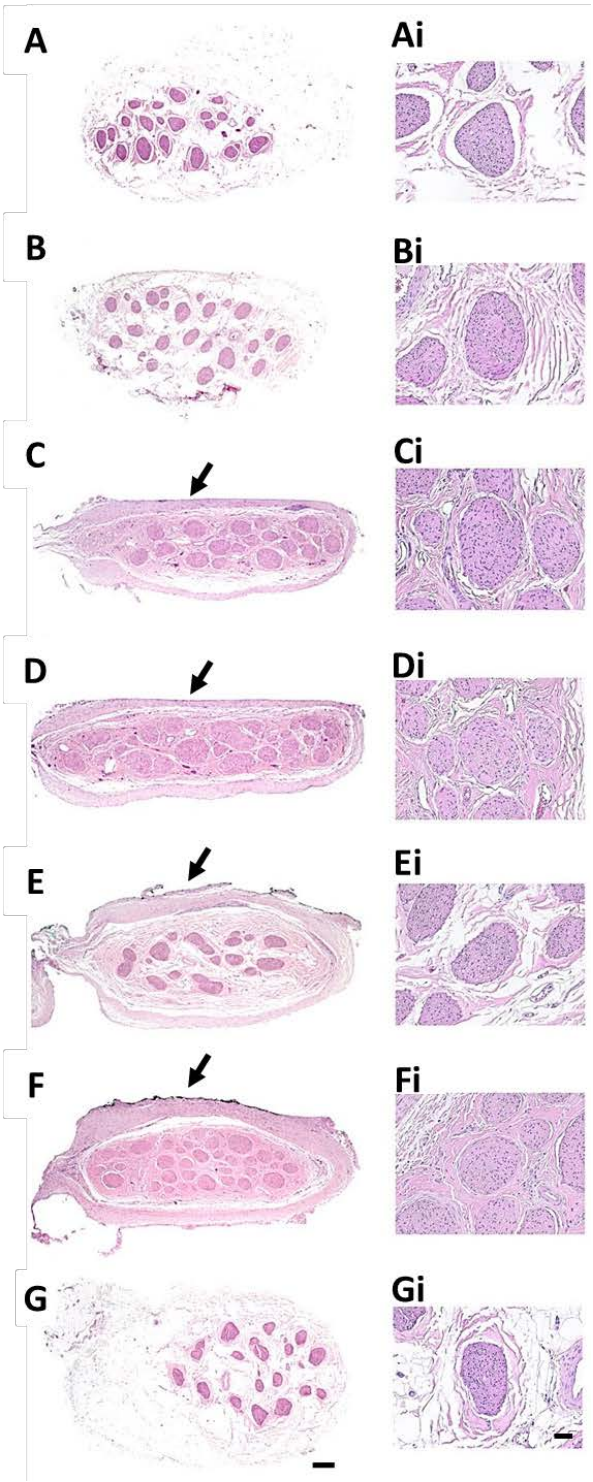

Figure 7. Histopathology of the chronically implanted anterior vagus nerve. **A-G**: Sections of vagus nerve tissue were sectioned and stained with haematoxylin and eosin (H&E) for histopathological assessment by a blinded observer. Images show non-implanted nerve (**A**), and tissue taken 20 mm rostral (**B**, VNS4) and caudal (**G**, VNS3) to the electrode array. Tissue adjacent to electrodes E6 (**C,** VN_c5), E5 (**D**, VN_c5), E2 (**E**, VNS3) and E1 (**F**, VNS1) were assessed for damage and signs of inflammation. The tissue in contact with the Pt electrode (**C-F**) is indicated by arrows. Higher power

images of each section are illustrated in **Ai-Gi**. Scale bar for images **A-G:** 200 µm; **Ai-Gi**: 50 µm.

Electrode neural interface examination

By embedding and sectioning electrodes E3 and E4 together with the vagus nerve contained within these electrodes, we were able to examine the electrode neural interface in some detail. As illustrated in **Figure 8A**, the vagus nerve and its fibrous tissue capsule can be identified within the cavity (C) formed around the nerve by the cuff electrode. In all animals examined (n = 5), the nerve and its associated tissue capsule did not always occupy the entire cavity. While we cannot dismiss the possibility of tissue shrinkage, these results indicate that the nerve may not form a tight interface with the electrodes. However, the cross-sectional profile of the nerve reflects the longitudinal distortion observed in the histological sections (**Figure 7C-F**). Inspection of the region of the cavity in each animal showed no evidence of tissue adhesions, cellular debris or an accumulation of inflammatory cells. As illustrated in **Figure 8A**, the Pt electrode (indicated by arrows) is evident at the interface between the silicone carrier (S) and the cavity. The vagus nerve is positioned ~100-200 µm from the electrode and this was typical for all animals examined using this technique.

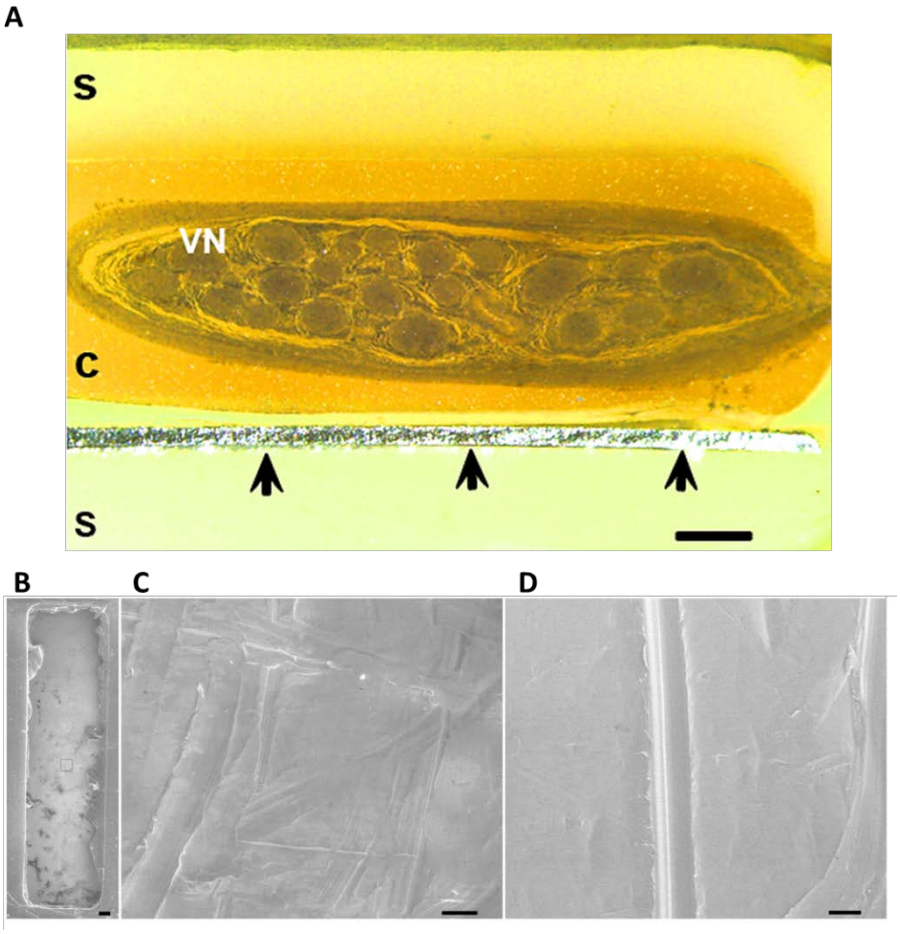

Figure 8. Assessment of electrode–neural interface and Pt electrode surface. **A**: Representative image of epoxy resin embedded electrode array (unstimulated, E3, VN_c5), silicone cuffs (S), nerve cavity (C) and implanted vagus nerve (VN). Arrows indicate the Pt electrode surface. **B-C**: Low (B) and high (C) magnification micrographs of an electrode used for chronic stimulation (E2; VNS1). Despite score marks associated with manufacturing, there was no sign of corrosion. **D**: High magnification micrograph from an electrode not used for chronic stimulation (E5, VNS2), again illustrated manufacturing score marks without evidence of corrosion. Scale bar for **A**: 200 μm; **B**: 100 μm; **C-D**: 10 μm.

No corrosion seen in chronically stimulated electrodes

Figure 8 illustrates the typical surface features associated with a chronically stimulated (**Figure 8C**) and an un-stimulated recording electrode (**Figure 8D**). Typical surface features included manufacturing marks; however there was no evidence of Pt corrosion observed on any electrode in the present study. Stimulated electrodes did show significantly more protein and fibrous tissue on the electrode surface compared with the unstimulated electrodes ($P < 0.002$; Mann-Whitney rank sum test).

4. Discussion

The present study evaluated the long-term safety of a novel multi-cuff peripheral nerve electrode array in a pre-clinical ovine model, and trialled a new target location on the anterior vagus nerve in the lower thorax, below the branches to the larynx, heart and lungs in order to reduce off-target effects during stimulation. The results show that chronic implantation and stimulation of this peripheral nerve array is safe and provides an effective means of monitoring electrically-evoked vagus nerve activity. The multi-cuff array remained functional during the implantation period and the electrode properties were unaffected by chronic electrical stimulation. Following implantation of the array, the vagus nerve showed no change in ECAP threshold over time, and no adverse inflammatory response or neural loss was seen in the histology. There were no measurable changes to eating, defection and few sustained changes to heart rate during stimulation. While respiratory rate exhibited small changes at the onset of stimulation in three animals, they diminished with implant duration. Taken together, these findings support the use of this novel peripheral nerve array for VNS at a site located below the branches to the larynx, heart and lungs, as a treatment of IBD.

Device design evaluation

The cuff array developed in the present study was manufactured using biocompatible materials (platinum and medical grade silicone), which have long histories of successful

application in medical devices [33]. The cuff design aimed to reshape the nerve slightly for optimal contact onto the active Pt electrode surface. This design is known as the flat-interface nerve electrode (FINE) and has been used extensively by Durand and colleagues to improve recording of somatic (peripheral) nerves in animal and intraoperative human studies [20, 34-36]. Furthermore, FINE electrode arrays have been successfully implanted onto the peripheral nerves of amputees for over a year, in order to evoke touch sensations via electrical stimulation [37]. The electrode array used in the present study was safely implanted chronically onto the peripheral nerve of a large animal model, and all electrodes remained functional and impedances stable over the implantation period. There was more variability in the impedance of the stimulated electrodes between 5 – 12 weeks due to one electrode exhibiting a rise in impedance (**Figure 2**). However, this electrode remained functional, as indicated by the electrode's ability to evoke neural responses. The absence of tissue adhesions between the nerve and electrode array suggests that the cuff-array can be removed with minimal trauma to the underlying nerve. The ability to safely remove an electrode array is an important design consideration for the development of a safe device for clinical application [21].

A feature of the present electrode array was the ability to record multiple neural populations. The electrode array has two recording sites, 10 mm and 20 mm from the stimulating electrode pair. A subpopulation of thoracic vagal fibers exhibit fast conduction so there is a need to have some separation (i.e. 20 mm) between the stimulating and recording electrodes in order to record these responses outside the latency of the stimulus artefact. In contrast, a majority of thoracic vagal fibers exhibit slow conduction, are poorly synchronized and recordings are diminished with increasing distance between the stimulating and recording electrodes. Therefore, using a more proximal recording site (10 mm) is optimal for a slower conduction fiber type.

These electrically-evoked slow conduction fibers had an average conduction of 1.4 m/s. This is in the same range as C-fiber conduction [15], suggesting that the slow conduction fibers evoked by the vagus nerve array are C-fibers. Furthermore, stimulation levels were supra-threshold throughout the duration of the study. This is an important property of the electrode array, as it is likely that abdominal vagal C-fibers are involved in driving the anti-inflammatory mechanisms of VNS [16, 17].

Biocompatibility and physical safety of the multi-cuff array

Peripheral nerves are highly vascularised and consist of fibers that transfer sensory and motor information, *via* action potentials to and from the brain [38], in addition to the transport of cellular components via retrograde and anterograde transport. Therefore, compression from the electrode cuff may cause occlusion, disruptions to the nerve physiology, disruption of axonal transport and even neural degeneration [39, 40]. Depending on the severity of the compression, injury-induced neural degeneration is likely to be observable by 3 months [41]. In the present study, histopathological

assessment of the implanted vagus nerve showed no evidence of neural loss. Furthermore, total fascicle area (containing vagal axon fibers) was similar to rostral/caudal tissue, suggesting no significant neural degeneration had occurred as a result of the chronic implantation of these cuff electrodes. Numerous blood vessels were observed throughout intra- and extra-fascicular areas of the implanted tissue. In addition, ECAPs were recorded throughout the implantation period, suggesting that vagus nerve function was not disrupted.

Electrical safety of stimulation parameters

The stimulation parameters used in the present study (0.4 μC/phase developing a charge density of 27 $\mu C/cm^2$/phase), were well within the safety limit defined by Shannon (1992) or Pt corrosion limits defined electrochemically [24, 42]. Therefore, it is not surprising that no electrically-induced neural damage or Pt corrosion was seen.

Previous clinical studies that investiagted VNS using a Cyberonics device as a therapy for Crohn's disease applied continuous stimulation of 10 pps, 500 μs pulse width, 1.25 mA current and duty cycle of 30 s ON, 300 s OFF [12]. Here, we continually using a higher rate (30 pps) and current (2 mA), while the duty cycle was nearly doubled (30 s ON, 150 s OFF). A shorter pulse width (200 μs/phase) was selected to reduce the duration of the electrical artefact in order to improve the quality of the ECAP recordings. The sem analysis of stimulated Pt electrodes showed no corrosion, compared with unstimulated electrodes. Taken together, this result, combined with the histopathological observations of the implanted vagus nerve, suggests that our chronic VNS regime did not result in irreversible electrochemical reactions and Pt dissolution.

Minimal off-target effects of VNS

Stimulation of the vagus nerve in the lower thorax activates afferent and efferent fibers which connect the brain to the gastrointestinal tract, pancreas, gallbladder and liver [43]. Therefore, stimulation of the vagus nerve in the lower thorax may have effects on food intake. Bilateral stimulation of the gastric vagus nerve has been shown to be effective in limiting weight gain, food consumption and intake of sweet food in obese mini-pigs [44]. Here, we observed no evidence of a change in eating behaviour following the onset of suprathreshold VNS (note that no conclusion can be drawn on the effect of VNS on increasing food intake, as sheep usually ate all the food they were given). Furthermore, no significant detectable change to faecal output was seen following the onset of stimulation. There were minimal effects to heart rate and respiration during the onset of stimulation.

Any changes in heart rate and respiration patterns following the initial onset of stimulation were typically not evident at later time points. The decrease in cardiac and

respiratory side-effects over time is probably related to the development of tissue encapsulation around the electrode array reducing current spread to other structures. Furthermore, since changes were small and recorded in awake animals, it is possible that they indicated awareness of the stimulus rather than off-target stimulation of vagal fibers controlling heart rate or respiration. In summary, chronic stimulation at levels well above those required to evoke C-fiber activity produced few sustained off-target effects and no measurable effects on the animal's behaviour.

Clinical relevance

For clinical application, it is anticipated that this electrode array would be placed sub-diaphragmatically, onto the anterior abdominal vagus nerve above the junction of the celiac and hepatic branches. As noted in the Introduction, the abdominal vagus nerve is composed mainly of C-fibers that innervate visceral organs and is below branches to the larynx, heart and lungs, thereby reducing any potential off-target effects experienced with cervical VNS. This is supported by previous studies that found no changes to heart rate during abdominal vagal nerve stimulation in pigs [45, 46]. Although abdominal surgery is more invasive than cervical VN implantation surgery, laparoscopic implantation of a neural stimulator around vagal gastric branches of the stomach already has FDA approval for the treatment of obesity [47, 48]. We anticipate that the ability to record evoked neural activity, which is a feature of this electrode array, will allow for the titration of stimulus parameters so that precise stimulation levels can be selected for optimal therapeutic benefit and for stimulation to be tailored to an individual's specific needs.

Study limitations

Although we have shown that chronic implantation and stimulation of the anterior vagus nerve is safe and feasible, we did not evaluate the efficacy of stimulating the vagus nerve in the lower thorax in alleviating IBD in the present study. While the anti-inflammatory mechanisms of VNS for IBD have not yet been unequivocally defined, it is likely that stimulation of the vagus nerve below the branches to the larynx, heart and lungs would result in similar therapeutic effects on inflammatory diseases to those seen when stimulation is applied to the cervical vagus nerve [11, 12].

5. Conclusion

Chronic implantation and electrical stimulation using this novel vagus nerve electrode array at a site below the branches to the larynx, heart and lungs, is safe and results in

minimal sustained off-target effects. Furthermore, the novel design of this electrode array is expected to allow the titration of stimulus parameters so that precise stimulation levels can be selected for optimal therapeutic benefit.

6. References

1. Donaldson N, Brindley GS. The historical foundations of bionics. In: *Neurobionics: The biomedical engineering of neural prostheses*, R.K S (Ed.^(Eds).John Wiley & Sons Inc. Hoboken 49-51 (2016).
2. Shepherd RK, Villalobos J, Burns O, Nayagam DA. The development of neural stimulators: A review of preclinical safety and efficacy studies. *J. Neural. Eng.* 15(4), 041004 (2018).
3. Payne SC, Furness JB, Stebbing MJ. Bioelectric neuromodulation for gastrointestinal disorders: effectiveness and mechanisms. *Nat. Rev. Gastroenterol. Hepatol.* doi:10.1038/s41575-018-0078-6 (2018).
4. Thin NN, Horrocks EJ, Hotouras A *et al*. Systematic review of the clinical effectiveness of neuromodulation in the treatment of faecal incontinence. *Br. J. Surg.* 100(11), 1430-1447 (2013).
5. Dinning PG, Hunt L, Patton V *et al*. Treatment efficacy of sacral nerve stimulation in slow transit constipation: A two-phase, double-blind randomized controlled crossover study. *Am. J. Gastroenterol.* 110(5), 733-740 (2015).
6. Sulkowski JP, Nacion KM, Deans KJ *et al*. Sacral nerve stimulation: A promising therapy for fecal and urinary incontinence and constipation in children. *J. Pediatr. Surg.* 50(10), 1644-1647 (2015).
7. Siegel SW, Catanzaro F, Dijkema HE *et al*. Long-term results of a multicenter study on sacral nerve stimulation for treatment of urinary urge incontinence, urgency-frequency, and retention. *Urology* 56(6 Suppl 1), 87-91 (2000).
8. Schachter SC. Vagus nerve stimulation therapy summary: Five years after FDA approval. *Neurology* 59(6 Suppl 4), S15-20 (2002).
9. Nemeroff CB, Mayberg HS, Krahl SE *et al*. VNS therapy in treatment-resistant depression: Clinical evidence and putative neurobiological mechanisms. *Neuropsychopharmacology* 31(7), 1345-1355 (2006).
10. Borovikova LV, Ivanova S, Zhang M *et al*. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405(6785), 458-462 (2000).
11. Koopman FA, Chavan SS, Miljko S *et al*. Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis. *Proc. Natl. Acad. Sci. U S A.* 113(29), 8284-8289 (2016).
12. Bonaz B, Sinniger V, Hoffmann D *et al*. Chronic vagus nerve stimulation in Crohn's disease: A 6-month follow-up pilot study. *Neurogastroenterol. Motil.* 28(6), 948-953 (2016).
13. Handforth A, Degiorgio CM, Schachter SC *et al*. Vagus nerve stimulation therapy for partial-onset seizures: A randomized active-control trial. *Neurology* 51(1), 48-55 (1998).
14. Degiorgio CM, Schachter SC, Handforth A *et al*. Prospective long-term study of vagus nerve stimulation for the treatment of refractory seizures. *Epilepsia* 41(9), 1195-1200 (2000).
15. Castoro MA, Yoo PB, Hincapie JG *et al*. Excitation properties of the right cervical vagus nerve in adult dogs. *Exp. Neurol.* 227(1), 62-68 (2011).
16. Payne SC, Furness JB, Stebbing M. Bioelectric neuromodulation for gastrointestinal disorders: effectiveness and mechanisms. *Nat Rev Gastroenterol Hepatol* In Press (2018).

17. Martelli D, Mckinley MJ, Mcallen RM. The cholinergic anti-inflammatory pathway: A critical review. *Auton. Neurosci.* 182 65-69 (2014).
18. Howland RH. Vagus Nerve Stimulation. *Curr. Behav. Neurosci. Rep.* 1(2), 64-73 (2014).
19. Hoffman HH, Schnitzlein HN. The numbers of nerve fibers in the vagus nerve of man. *Anat. Rec.* 139 429-435 (1961).
20. Tyler DJ, Durand DM. Chronic response of the rat sciatic nerve to the flat interface nerve electrode. *Ann. Biomed. Eng.* 31(6), 633-642 (2003).
21. Shepherd RK, Villalobos J, Burns O, Nayagam DaX. The development of neural stimulators: a review of preclinical safety and efficacy studies. *J Neural Eng* 15(4), 041004 (2018).
22. Shannon RV. A model of safe levels for electrical stimulation. *IEEE Trans. Biomed. Eng.* 39(4), 424-426 (1992).
23. Merrill DR, Bikson M, Jefferys JG. Electrical stimulation of excitable tissue: Design of efficacious and safe protocols. *J. Neurosci. Methods* 141(2), 171-198 (2005).
24. Cogan SF, Ludwig KA, Welle CG, Takmakov P. Tissue damage thresholds during therapeutic electrical stimulation. *J. Neural. Eng.* 13(2), 021001 (021013 pp) (2016).
25. Mccreery D. Tissue reaction to electrodes: The problem of safe and effective stimulation of neural tissue. In: *Neural Prosthesis: Theory and Practise*, Horch DS,Dhillon GS (Ed.^(Eds).World Scientific Publishing River Edge, NJ (2004).
26. Langenberg C, Wan L, Egi M, May CN, Bellomo R. Renal blood flow in experimental septic acute renal failure. *Kidney. Int.* 69(11), 1996-2002 (2006).
27. Fallon JB, Senn P, Thompson AC. A highly configurable neurostimulator for chronic pre-clinical stimulation studies. *Neural Interfaces Conferences* (2018).
28. Patrick JF, Seligman PM, Money DK, J.A K. *Engineering in Cochlear Prostheses*. Churchill Livingston, Edinburgh 99-124 (1990).
29. Richardson RT, Wise AK, Thompson BC *et al*. Polypyrrole-coated electrodes for the delivery of charge and neurotrophins to cochlear neurons. *Biomaterials* 30(13), 2614-2624 (2009).
30. Villalobos J, Nayagam DA, Allen PJ *et al*. A wide-field suprachoroidal retinal prosthesis is stable and well tolerated following chronic implantation. *Invest. Ophthalmol. Vis. Sci.* 54(5), 3751-3762 (2013).
31. Shepherd RK, Wise AK, Enke YL, Carter PM, Fallon JB. Evaluation of focused multipolar stimulation for cochlear implants: A preclinical safety study. *J Neural Eng* 14(4), 046020 (2017).
32. Nayagam DA, Durmo I, Mcgowan C, Williams RA, Shepherd RK. Techniques for processing eyes implanted with a retinal prosthesis for localized histopathological analysis: Part 2 epiretinal implants with retinal tacks. *J. Vis. Exp.* 96 e52348 (52349 pp) (2015).
33. Clark GM, Shepherd RK, Patrick JF, Black RC, Tong YC. Design and fabrication of the banded electrode array. *Ann. N. Y. Acad. Sci.* 405 191-201 (1983).
34. Tyler DJ, Durand DM. Functionally selective peripheral nerve stimulation with a flat interface nerve electrode. *IEEE Trans. Neural. Syst. Rehabil. Eng.* 10(4), 294-303 (2002).
35. Schiefer MA, Polasek KH, Triolo RJ, Pinault GC, Tyler DJ. Selective stimulation of the human femoral nerve with a flat interface nerve electrode. *J. Neural. Eng.* 7(2), 26006 (2010).
36. Dweiri YM, Stone MA, Tyler DJ, Mccallum GA, Durand DM. Fabrication of high contact-density, flat-interface nerve electrodes for recording and stimulation applications. *J Vis Exp* 116 e54388 (2016).
37. Tan DW, Schiefer MA, Keith MW, Anderson JR, Tyler J, Tyler DJ. A neural interface provides long-term stable natural touch perception. *Sci. Transl. Med.* 6(257), 257ra138 (2014).
38. Fallon JB, Carter PM. Principles of recording from and electrical stimulation of neural tissue. In: *Neurobionics: The biomedical engineering of neural protheses*, Shepherd RK (Ed.^(Eds).John Wiley & Sons, Inc. New Jersey (2016).
39. He Z, Jin Y. Intrinsic control of axon regeneration. *Neuron* 90(3), 437-451 (2016).

40. Somann JP, Albors GO, Neihouser KV *et al*. Chronic cuffing of cervical vagus nerve inhibits efferent fiber integrity in rat model*. J. Neural. Eng.* 15(3), 036018 (2018).
41. Berkelaar M, Clarke DB, Wang YC, Bray GM, Aguayo AJ. Axotomy results in delayed death and apoptosis of retinal ganglion cells in adult rats*. J. Neurosci.* 14(7), 4368-4374 (1994).
42. Brummer SB, Turner MJ. Electrochemical considerations for safe electrical stimulation of the nervous system with platinum electrodes*. IEEE Trans. Biomed. Eng.* 24(1), 59-63 (1977).
43. Bonaz B, Picq C, Sinniger V, Mayol JF, Clarencon D. Vagus nerve stimulation: From epilepsy to the cholinergic anti-inflammatory pathway*. Neurogastroenterol. Motil.* 25(3), 208-221 (2013).
44. Val-Laillet D, Biraben A, Randuineau G, Malbert CH. Chronic vagus nerve stimulation decreased weight gain, food consumption and sweet craving in adult obese minipigs*. Appetite* 55(2), 245-252 (2010).
45. Stakenborg N, Wolthuis AM, Gomez-Pinilla PJ *et al*. Abdominal vagus nerve stimulation as a new therapeutic approach to prevent postoperative ileus*. Neurogastroenterol. Motil.* 29(9), e13075-13086 (2017).
46. Wolthuis AM, Stakenborg N, D'hoore A, Boeckxstaens GE. The pig as preclinical model for laparoscopic vagus nerve stimulation*. Int. J. Colorectal. Dis.* 31(2), 211-215 (2016).
47. Camilleri M, Toouli J, Herrera MF *et al*. Intra-abdominal vagal blocking (vBloc therapy): Clinical results with a new implantable medical device*. Surgery* 143(6), 723-731 (2008).
48. Apovian CM, Shah SN, Wolfe BM *et al*. Two-year outcomes of vagal nerve blocking (vBloc) for the treatment of obesity in the ReCharge trial*. Obes. Surg.* 27(1), 169-176 (2017).