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A novel simplistic fabrication technique for cranial epidural electrodes for chronic recording and stimulation in rats

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Abbreviations: CDP; cord dorsum potential, GS; gastrocnemius, L; lumbar, PDMS; polydimethysiloxane, S; sacral, SH; shoulder, T; thoracic.

Highlights:

- An implantable electrode was developed using simple wet bench fabrication
- The robust and versatile implant could be used for chronic stimulation and recording
- The electrode design had scientific, economical and ethical impact

Abstract

Background: The demand for neuromodulatory and recording tools has resulted in a surge of publications describing techniques for fabricating devices and accessories in-house suitable for neurological recordings. However, many of these fabrication protocols use equipment which are not common to biological laboratories, thus limiting researchers to the use of commercial alternatives.

New Method:

We have developed a simple yet robust implantable stimulating surface electrode which can be fabricated in all wet-bench laboratories.

Results:

Female Sprague-Dawley rats received epidural implantation of the electrodes over the fore and hind limb areas of their motor cortex. Stimulation of the motor cortex successfully evoked fore- and hind limb motor outputs. The device was also able to record surface potentials of the motor cortex following epidural stimulation of the spinal cord.

Comparisons with Existing Methods:

For stimulation of the motor cortex, often stiff stainless or copper wires are roughly tucked underneath the skull, with little accuracy of localization. While, commercially available devices utilize burr holes and screw electrodes. Our new electrode design provides us stereotaxic accuracy that was not previously available.

Conclusion:

We developed a chronic implantable electrode capable of being fabricated in all wet-labs, are robust, versatile and electrically sensitive enough for long-term chronic use. The simple and versatile electrode design provides scientific, economical and ethical benefits.

1 Introduction

Chronic implantable electrodes are of fundamental interest for both exploratory and clinical applications resulting in a well-established commercial market. These implants tend to be engineered using silicon focused microelectromechanical system (MEMS) fabrication approaches, demanding an expensive infrastructure cost. Increased functionality and reduced feature size of the implant furthers the infrastructure cost. This makes custom designs a costly option unaffordable for most neuroscience groups.

Consequently, this has driven the introduction of open-sourced technological approaches to neuroscience applications such as 3D printing for producing easily modified, print-on-demand options for implant housing (Chen et al., 2017) and chronic mechanical micromanipulators (Rogers et al., 2017). However, to our knowledge, there has yet to be a demonstration of producing cheap and reliable brain–computer interface. The testing of neuroscience hypotheses still rely on the commercial options or the simplistic penetrative microwires and tetrodes for the machine-tissue interface (Sindhurakar et al., 2017).

Unlike the above options, here we offer a quick and simple method for producing cortical stimulation and recording electrodes, encapsulated in polydimethylsiloxane (PDMS), fabricated using tools readily accessible in neuroscience labs (hotplate and desiccator).

2 Materials and Methods

2.1 Electrode design

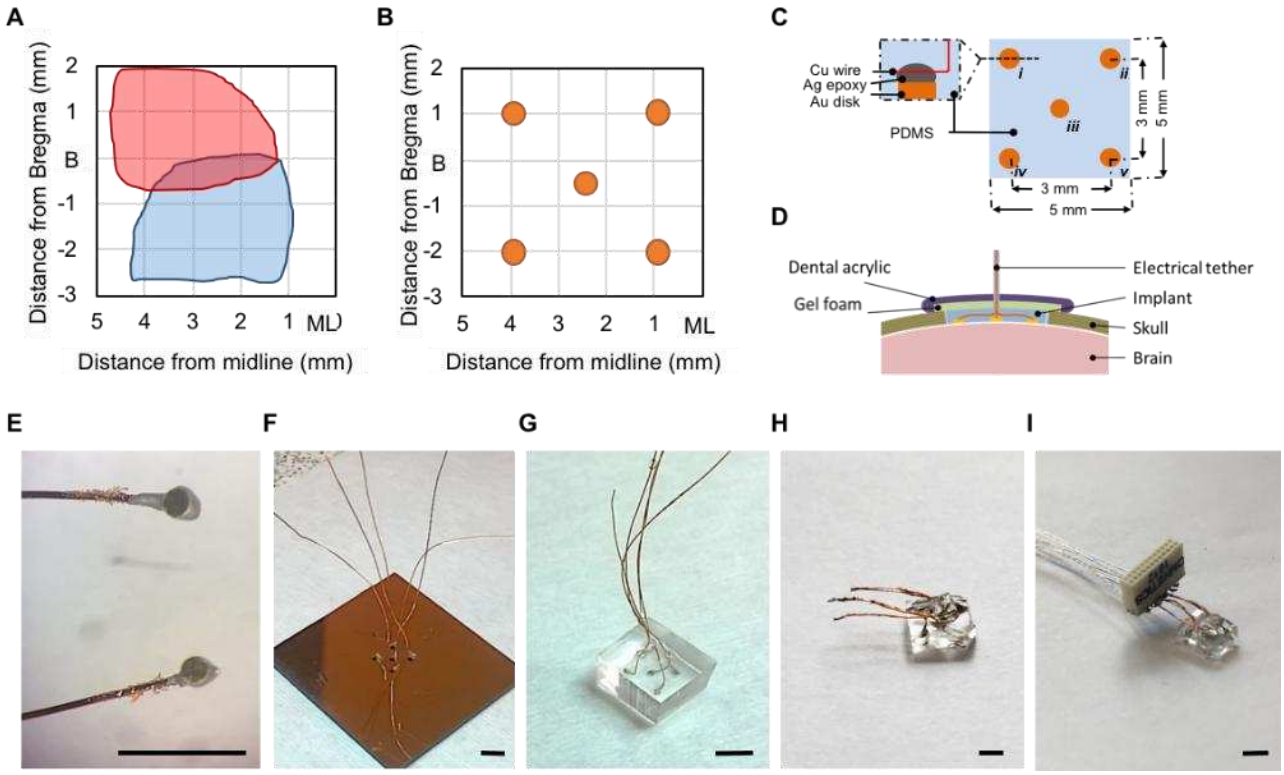

Figure 1. Rational and fabrication of electrode. Cortical mapping of forelimb (red) and hind limb (blue) regions within the motor cortex of intact rats (A) based on coordinates from (Neafsey et al., 1986). Electrode placement on motor cortex for selective activation of fore/hind limb (B). Schematic of the electrode (C) showing the array of five (*i* - *v*) 500-µm-diameter gold contacts encapsulated in 2-mm-thick cast PDMS. Schematic of fixing implant into position, secured by the gel foam and the dental acrylic (D). Fabrication of device (E-I). Photographs of the gold disks epoxied onto the copper wire (E) glued into position on a glass slide (F) and set in PDMS (G). Wires are cut to size and stimulation was either delivered differentially, using the pairs rostral (*i* - *ii*) to caudal (*iv* - *v*), or unipolar against the ground (iii) (H). Electrode attached to transdermal interconnect (Omnetics) used for stimulation and recording (I). PDMS; polydimethysiloxane. Scale bars 2 mm.

Copper wire (40 AWG; 79 µm diameter) insulated with polyurethane were mechanically stripped of insulation, 1 mm from the tips. Using silver loaded epoxy (RS components), gold disks (500 µm diameter, 150 µm thick; LEW Techniques) were bonded to the copper wire and heat cured at 100 ℃ for 2 hours (Fig. 1E). An optically transparent, temporary host substrate (microscope slide) was placed over a pre-defined alignment grid. Using the alignment grid as a guide (a

square array with an orthogonal separation of 3 mm, and a fifth contact at the center), the gold disks are glued (UHU ultrafast superglue) into position, before the remaining exposed host substrate is covered using an acetone-soluble coating (Shipley S1805 photoresist; MicroChem) (Fig. 1F). Biocompatible polydimethysiloxane (PDMS; Slygard 184 Silicone Elastomer; Dow Corning) was mixed in a 1:10 ratio-by-volume, hardener to resin and degassed to remove any air bubbles. The PDMS is then pour cast over the gold disks to the required thickness (ensuring all silver epoxy is encapsulated; thickness of 2 mm used in this work) and cured at 120 ℃ for 2 hours. Dissolving the glue and S1805 in acetone, the head-plugs are released from the host substrate and rinsed in de-ionised water to remove any remaining solvents. The PDMS is cut to size and shape (Fig. 1G-H) with the copper wires electrically connected to the electrical transdermal interconnector (Fig. 1I).

2.2 Experimental design

Five female Sprague-Dawley rats aged 6-8 weeks and weighing 229 ± 5 g were used in this study. Animals were housed individually on a 12-hour light-dark cycle at 21 ℃ and had *ad-libitum* access to food and water. One animal was used to test the functionality of the electrode under terminal anaesthesia (Fig 2A). Following successfully isolated responses in the terminal animal, the remaining four animals received chronic implantation of the stimulating electrodes over the dura of the motor cortex, along with EMG electrodes (multi-stranded stainless-steel wires, AS632, Cooner Wire, Chatsworth, CA, USA) into the mid-belly of the *Latissimus Dorsi* and *Gastrocnemius* muscle. Epidural wires were also sutured to the dura of the spinal cord at the 2^{nd} lumbar (L2) and 1^{st} sacral (S1) segments for either recording of cord dorsum potentials (CDP) or epidural stimulation of the spinal cord. All animal experimentation was approved by the University of Leeds Animal Welfare and Ethics Committee and was conducted in accordance with UK Animals (Scientific Procedures) Act 1986.

2.2.1 Surgical procedure

Animals were anaesthetised with Isoflurane: induced with 5 % IsoFlo® in O_2 (0.4 L min^{-1} kg^{-1}) and maintained at 0.28 L min^{-1} kg^{-1} during surgery. After confirmation of induction into the surgical plane an incision is made on the midline of the skull, and the mid-thoracic region of the

back. EMG wires are run from the head down through the incision on the back, and to the implant sites (shoulder; deltoid muscle, spinal cord, L2 and S1, and hind limb ankle extensor, gastrocnemius). EMG wires were implanted in the muscle using a 23 G needle, running the wires through the mid-belly of the muscle, and sutured into place. Laminectomies were performed on vertebral thoracic (T) levels 12/13 and L2 exposing spinal L2 and S1. Epidural wires were passed between the two laminectomies and sutured to the dura along the midline with non-absorbable suture (Ethilon 9-0, W2871, Ethicon). Finally, a 5x5 mm craniotomy was performed, exposing the motor cortex (coordinates in Fig. 1A-B). Three screws were implanted around the craniotomy site to secure the implantable electrode, placed on the dura by securing the connector. Dental acrylic (Simplex Rapid Powder, Kemdent) was then applied to cover the screws and implant, anchoring the transdermal interconnector to the skull (Fig. 1D). The incisions of the neck, back and hind limbs were closed with non-absorbable sutures (Ethilon 5-0, 1865G, Ethicon). All surgeries were completed under aseptic conditions, on a heat pad, regulated to maintain temperature at 37 ℃. Upon completion of surgery, animals were administered saline (10 ml), analgesic (buprenorphine; 0.015 $mg.kg^{-1}$) and antimicrobial (Baytril; 2.5 $mg.kg^{-1}$) subcutaneously and repeated at 24 and 48 hours post-surgery.

2.2.2 Motor evoked potentials, cortical and EMG recordings

Animals moved freely in a custom animal cage, with stimulating/recording cables attached to the externalized electronic hardware. A single square wave pulse (200 µs duration, 333 Hz biphasic stimulus pulse) was delivered across the implanted electrode for cortical stimulation and the epidural wires during cortical recording. Recordings were filtered (10 – 1000 Hz) and captured in Signal (CED).

3 Results

Five implants were fabricated (for less than 5 USD). One was used in a terminal anaesthetised animal to test the functionality of the implant, while the remaining four were successfully implanted into the epidural space of the motor cortex of the rats. In the anesthetised animal we were able to selectively activate a forelimb and hindlimb response (Fig. 2A), using differential pairings of electrodes. In an awake chronically implanted animal, stimulation of the whole motor

cortex successfully evoked a motor response in the shoulder and hind limb of the rat (Fig. 2B). The device was also used to record evoked motor cortex surface potentials (Fig. 2C) following epidural stimulation of the spinal cord. The implanted electrode was used successfully to evoke limb muscle responses for the whole duration of 70 days without any change in the required current strength of 10µA (Fig. 2D).

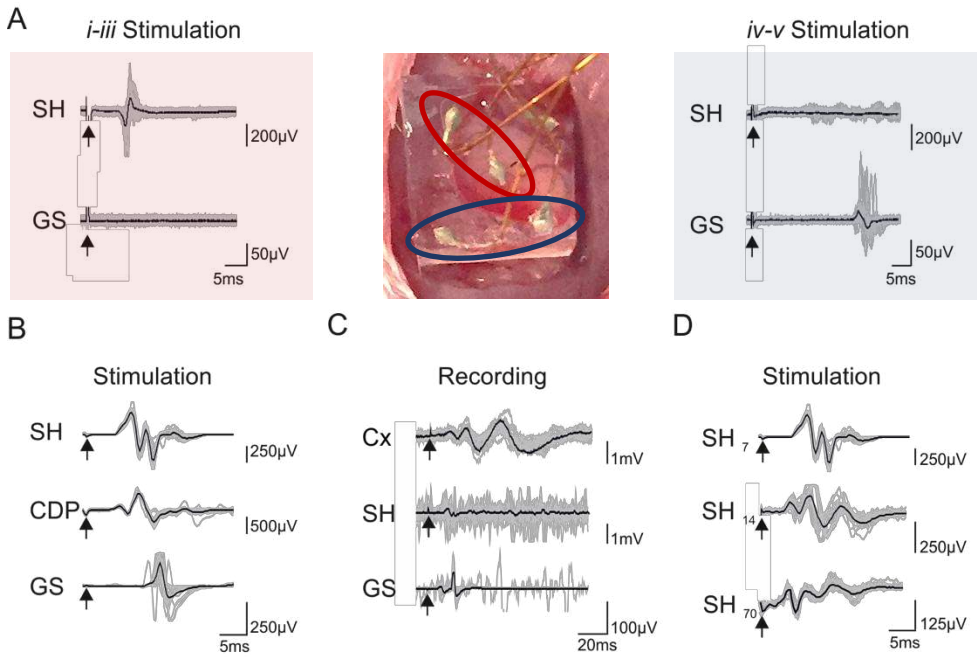

Figure 2. Motor evoked potential and cortical recording from implanted electrode. Stimulation of motor cortex forelimb electrodes i-iii evoked shoulder (SH) response and hindlimb electrodes iv-v evoked gastrocnemius (GS) response in an anaesthetised animal (A). Stimulation of whole cortex in an awake animal evoked SH, lumbar cord dorsum potential (CDP) and GS response (B). Epidural stimulation of spinal cord region L2 – S1, evoked GS and motor cortex response (Cx) (C). Longevity of implant demonstrated by the robust shoulder response seven (SH $_{7}$), 14 (SH $_{14}$) and 70 (SH $_{70}$) days after implant (D). Traces contain 20 overlapped individual sweeps (grey) with the average on top (black). Black arrow indicates stimulus artefact.

4 Discussion

PDMS was selected as the substrate material for this work as it is well established in bioengineering applications, particularly for lab-on-chip studies where small microchannels have

been patterned. This is owed to the many beneficial characteristic of the PDMS such as the optical transparency, gas permeability and adaptable stiffness (Toepke and Beebe, 2006). For the latter, the mixing ratios of the resin and hardener can be tuned to optimize the stiffness of the PDMS (Ochsner et al., 2007) to better match the stiffness of the brain tissue. Alternatively, the stiffness can be further tuned by mixing a 1:10 ratio PDMS with a dielectric gel (Slygard 527 Silicone Dielectric Gel) (Palchesko et al., 2012). Once cured, the surface chemistry of the PDMS can be further manipulated to improve the biocompatibility of the material (Almutairi et al., 2012, Zhou et al., 2010). The selection of this methodology is to reflect the end application and should be approached with caution. For example, the permeability of the PDMS will allow the tissue to breathe, facilitating an exchange of oxygen and carbon dioxide, at the same time can absorb molecules affecting drug studies (Toepke and Beebe, 2006).

This material may be useful for reducing the foreign body response to the implant when implanted with an appropriate drug to leach out post-surgery (Stone and Hollins, 2016). As this is a casting methodology, there are few restrictions on the shape of the PDMS structure providing a suitable mould is formed. Hence, this technique can be shaped to the brain topology opposed to the flat structure demonstrated here using a method similar to (Chen et al., 2017). Using this casting method, pipes too can be introduced to allow pharmacological studies. Currently, the PDMS primarily serves as a structural hold, maintaining the separation between the gold electrical, holding the connections in predefined positions.

Other material choices in this work can be later refined following further lifetime studies where biodegradation of materials, leeching and micro-cracking are scrutinised; one such refinement is copper being replaced in favour of stainless-steel wire due to the cytotoxicity of copper. The copper here is encased in silver loaded epoxy, the polyurethane insulation or the solder connections; these materials themselves are encapsulated in a thick (1 mm min.) PDMS.

Unlike MEMS fabrication approaches, the choice of interface materials remain restricted to commercially available options; the gold disks here are repurposed from gold wire interconnect technologies. Gold is regularly used for recordings and sufficient for stimulations though better materials have been cited for stimulators; iridium, rhodium, platinum and palladium (Geddes and Roeder, 2003). The gold disks are 150 µm thick (thicker than MEMS fabricated electrodes), suggesting longevity of stimulation experiments should the charge injection need to be taken

above the faradaic limits leading to a reduction of the gold electrodes at a risk of a time limited measure and loss of consistency of stimulations. Alternatively, adopting post-fabrication roughening methods such as electrodeposition should be considered for increasing the charge injection capacity of the electrodes (Kim et al., 2013). The repeatability of the measurements in this work, 70 days post implant implies a stable implantation design for this application.
The electrical contact areas here are restricted by commercial availability but found to be effective. Should larger contacts be necessary, an array of multiple contacts may be considered with the larger inter-electrode spacing yielding an increase in detection depth but at a loss of bandwidth and signal amplitude (Fuglevand et al., 1992).

Chronic implants for cortical stimulation are currently varied and unrefined in practice. Previously, we have used simple copper wires tucked underneath the skull, with poor accuracy of stimulation site, while other groups use crude solid electrodes (Carmel et al., 2014). Unlike the previous tools used by this group and still in use by many others, the new electrode design allows one to select relevant stereotaxic stimulation sites pre-fabrication, choose dimensions of the implanted PDMS base to suit the need, with a short turnaround of 2 days. Furthermore, the offer to be able position and fix electrical contacts geometrically with respect to one another and against a biological reference (in this case, the bregma) without being rushed by the operation. This has significant effects on efficacy of the study and the ease of carrying out the implantation. The charge injection to evoke limb muscle response here was 2 nC which is low when compared with screw electrodes (15 – 200 nC (Mishra et al., 2017, Wang et al., 2012) and surface placed wires (180 nC (Carmel et al., 2014)). When compared with MEMS fabricated electrodes, there was comparable charge injection albeit at a lower spatial resolution; surface electrodes (2.13 nC (Molina-Luna et al., 2007)) and intracortical needle electrodes (15 nC (Wang et al., 2013)). Unlike the screw and intracortical electrodes, the electrodes in this work do not mechanically damage the tissue and offer repeatability of current threshold to evoke muscle response.
The duration of implantation of these new devices has been reduced significantly from anything in the range of 1-2 hours to a meagre 45 minutes, where 10 minutes is what it takes to place the implant on the intended site on the dura, and the rest is in waiting for the acrylic to seal. The greatest hurdle that has been eliminated with this device is the uncertainty and unnecessary

loss of time in ensuring that the inflexible wires are where one had placed them prior to being sealed with dental acrylic.

The surgery now requires a measured craniotomy, and the electrode is simply laid over the desired stimulation sites, which is then cemented into place. There were no notable foreign body response to the chronic implant, but there was bone growth over the implant that further anchored the device into place. It was possible to remove the implant and reuse in subsequent terminal recordings, it has not been retested under chronic recording or stimulation conditions, but one assumes it is likely to be as effective.

The drastic reduction of time required for implanting these devices means, thus reduced exposure of the animals to anaesthesia, which has subsequent impacts on successful recovery following surgery, are a critical ethical and welfare issue. The ease of surgical times for implants and the increased efficacy also means less stressed surgeons, reduced loss of animals in these experiments and a more easily reproducible data, which in all should reduce the number of animals used and their overall welfare.

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Declaration of interest

Conflicts of interest: none.

Author contribution

RWPK and CR are equal first authors of the manuscript. RWPK, CR, DPS and SC contributed to the conception and design of the study. CR completed the fabrication of all implants, while RWPK and SC implanted all the devices. RWPK and SC were involved in the acquisition and analysis of data. RWPK, CR, DPS and SC were equally involved in the drafting and revising of the manuscript and approved the final submission.

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