A bio-friendly and economical technique for chronic implantation of multiple microelectrode arrays

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Many neurophysiological experiments on rodents and non-human primates involve the implantation of more than one multi-electrode array to record from many regions of the brain. The so-called 'floating' microelectrode arrays are implanted in cortical regions of interest and are coupled via a flexible cable to their connectors which are fixed to the skull by a cement cap or a titanium pedestal, such as the Cereport system, which has been approved for human use. The use of bone cement has several disadvantages including the creation of infection prone areas at the interface with the skull and surrounding skin. Alternatively, the more biocompatible Cereport has a limited carrying capacity and is far more expensive.

In this paper, we describe a new implantation technique, which combines the biocompatibility of titanium, a high carrying capacity with a minimal skull footprint, and a decreased chance of infection, all in a relatively inexpensive package. This technique utilizes an in-house fabricated 'Nesting Platform' (NP), mounted on a titanium headpost to hold multiple connectors above the skin, making the headpost the only transcutaneous object. The use of delrin, a durable, lightweight and easily machinable material, allows easy customization of the NP for a wide variety of floating electrodes and their connectors. The ultimate result is a longer survival time with superior neural recordings that can potentially last longer than with traditional implantation techniques.

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1. Introduction

For the past several decades, researchers have made great strides in our understanding of the brain by utilizing chronic neural recordings taken from awake behaving animals (Dolbakykan et al., 1977; Nicolelis et al., 2003). Until recently most of these chronic implants had been conducted on rodents while primate neurophysiology had primarily been implemented using acute electrode insertion through chronically implanted recording chambers (Baker et al., 1999), allowing recording sessions of only several hours at a time on restrained animals. Today laboratories around the world are chronically implanting arrays of microelectrodes into non-human primates and even humans with good results (Donoghue et al., 2007; Hochberg et al., 2006). However, the risk of infection is still high when employing the commonly used cement 'cap' method of electrode/connector attachment in which quick drying acrylic is used in combination with skull screws.

The three major issues with chronic electrode implantations are (1) the long-term stability of the implant attachment to the skull, (2) prevention of infections and (3) the long-term stability of electrodes within the neural tissue. We will address the first two of these difficulties. Both the acrylic/cement cap (Carmena et al., 2003; Nicolelis et al., 1998; Dolbakykan et al., 1977) and the titanium pedestal (Fellows and Suner, 2006; Normann et al., 1999; Donoghue et al., 2007; Hochberg et al., 2006) techniques are widely used for chronic electrode implantation, but each has their own limitations. Acrylic is exothermic and also believed to be toxic during the settling period (Albrektsson and Linder, 1984) and the newer antibiotic (Gentamycin) impregnated bone cements like Palacose (Heraeus Medical GmbH, Wehrheim, Germany) have only limited anti-bacterial effectiveness, due to low surficial contact with surrounding bacteria. The alternative, a titanium pedestal like the Cereport (Blackrock Microsystems, Salt Lake City, UT), is biocompatible and interfaces well with surrounding bone (Adams et al., 2007). However the limited carrying capacity of each Cereport (up to 96 channels per device) requires multiple Cereport pedestal implantations for a larger number of electrode implants.
Therefore, the Cereport option ultimately provides little decrease in skin margins or required skull real estate as the number of implanted electrodes increases.

To avoid the disadvantages of these previously mentioned methods we have designed and manufactured a novel apparatus in house. This apparatus, which consists of a ‘nesting platform’ (NP) mounted on a single titanium post, maintains the advantages of a traditional titanium Cereport while simultaneously increasing the carrying capacity by four times (4 × 96 electrode connectors) and reducing the transcutaneous cross section and cost. This design concept is very adaptable, and with appropriate changes in scale, could be used on animals of any size for any electrode coupled to its connector by a flexible wire; for instance floating cortical microelectrode arrays (as described here) or depth electrodes for thalamic/hippocampal implants (Behrens et al., 1994; Simuni et al., 2002). Finally, this attachment method is also very durable and does not require or impose any specific restrictions on animal movement. During the recording sessions, head movement restrictions can be imposed in order to prevent detachment of headstages from the connectors and prevent movement artifacts in the recordings, but is not required.

2. Methods

2.1. Nesting platform (NP) design

The goals of this project were to create an implantation technique that would allow the implantation of up to four 96-channel microelectrode arrays while maintaining a minimal skull footprint, minimal transcutaneous cross section, and would not require the use of acrylic cement. Fig. 1 shows our final design of the NP complex, a titanium headpost (model: 6-FHP-2XF, Crist Instrument Co., Inc., Hagerstown, MD) mated with an in-house fabricated NP that holds the electrode connectors above the surface of the scalp. All designs were created using Rhinoceros v3.0 and milled out of white delrin (density 1.42 g/cm³) using an EGX-300 Desktop Engraver (Roland DG Corporation). A .3dm file format model of the NP design can be found in the online Supplementary Information.

The NP is 5.2 cm long, 4.05 cm wide, .68 cm thick, and 18.07 g in weight. This NP is mounted on a titanium headpost 6-FHP-2XF (weight 13.3 g) using the screw supplied with the headpost. Four ICS-96 connectors are then mounted onto the NP by screwing directly into appropriately positioned holes. Each of these connectors weighs ∼9 g, making the total weight of the NP with four connectors to be ∼67.4 g. This low weight caused no problems for our usage, but should a lower weight be desired, the NP could be made thinner and nonessential material (for instance the regions below the mounted connectors) could be removed with little effect on structural integrity. In addition, should one need to glue anything additional to the NP, we recommend CyPox glue (Gowest2 International, Arlington, OH) which, unlike most glues, bonds very effectively to delrin.

The effective grip between the NP and the ICS-96 connectors is provided by the self-tapping Ø .082 in. ICS-96 connector screws into the .075 in. diameter holes of the NP. If desired, one can modify the NP design to have slots for small nuts to keep the screws in place. However we have found over the course of a 6-month experiment that the structural integrity of the entire connector/NP complex was very stable.

2.2. Implantation technique

All animal procedures were approved by SUNY Downstate Medical Center IACUC and conformed to National Institutes of Health guidelines. Female Bonnet Macaques (Macaca radiata), weighing between 4 and 7 kg, were used for the experiments. Prior to attachment of the headpost to the skull, its four titanium foot processes were trimmed to adjust their length in accordance with the space...
2.3. Connector-NP assembly method

To fasten the connectors to the NP we first arranged the electrode-to-connector wire bundles into their dedicated channels on the bottom of the NP and then attached the connectors to the NP using (Ø .082 in.) screws (Fig. 2). To prevent any future intrusion of biological matter between the connectors and the NP we then applied a thin layer of cyanoacrylate along the junctions. Because cyanoacrylate does not bond very strongly to delrin this process does not interfere with future unscrewing of the connectors should it be necessary. We found it useful to label the wire bundles of each array to easily identify the connector to array pairing during surgery. All the contact edges of the NP have been smoothened to minimize the inadvertent injury to the wire bundles. Materials like silastic (Kwik-Sil™ Silicon Elastomer, World Precision Instruments, Sarasota, FL) that are inert to the wirebundles and that can give cushion can also be used to give additional insulation and protection to the wirebundles.

Recommended chronology in surgical procedure: Although many variations can be made in the implantation technique, we found that the following chronology makes the surgery more efficient and less confusing. The steps we followed are broadly as follows:

1. Create a midline incision, with coronal plane extensions at the end, to make a wide window with a blunt dissection laterally up to the temporalis ridge.

2. Blunt dissection through the temporalis ridge in order to separate temporalis muscle inside its sheath from peristeum.

3. Open a skull window exposing cortical regions of interest, in our case primary motor cortex (M1), dorsal premotor cortex (PMd) in the left frontal lobe and primary somatosensory cortex (S1) Brodmann's areas 3b, 1 and 2 of left parietal lobe (see Fig. 3(c)). We used the structural MRI of the same monkey to locate the exact coordinates of the window. Thin out the removed bone using an electric rotary tool and set it aside in ringer's solution. Note here that multiple cortical regions that are far apart anatomically can also be chosen and multiple skull windows can be drilled out. The wirebundles can then be channeled to the NP mounting site and taken out from the base of the headpost, as described in the steps that follow.

4. Choose the orientation of implants and paths of the wire bundles (all our arrays had 10 cm wire bundle length). See Fig. 3 to get a clear idea of the array orientation, the wire bundle path that we followed and the location of the second headpost. When choosing the mounting site for the headpost it is important to take into consideration clearance space for all of the attached wires between the connectors and the amplifier bank. We oriented the NP-headpost assembly so that the wire bundles passed through the posterior margin of the headpost, making it least accessible to the animal’s fingers.

5. Mount the headpost using skull-screws at its predetermined location and connect the NP to make sure that the wire bundles can sit on the skull as planned. We did not dismount the NP until step 10 as it did not interfere with the surgical field.

6. Open the dura and perform somatotopic mapping of sensory cortex using a single sharp microelectrode to get a precise location of implantation site.

7. If implanting into multiple neighboring cortical regions, crowding of electrode arrays and wire bundles is inevitable. To facilitate the alignment and positioning of multiple arrays, we used multiple small padded alligator clips attached to malleable wires mounted on the stereotax (see Fig. 3C(e)). Once in position insert the arrays into the cortex following the procedures...
Fig. 3. (A) Second headpost implanted on the anterior side of the skull—note the orientation of the headpost is such that the NP fits on it from the posterior side, so that the wire bundles pass posterior to the headpost stem, making them less reachable to the animal. White arrows show medial margin of the skull window; (B) NP-connector assembly mounted on the headpost using the headpost screw (in the middle) using the top screw hole on the NP. The microelectrode arrays are yet to get implanted (see wire bundle going behind (white arrow), passing through the slits created in the sponge); (C) all 3 microelectrode arrays implanted in PMd(a), M1(b) and S1(c) and their wire bundles partially fixed on the skull using titanium straps and silastic(d) and padded alligator clips(e) were used to keep the wire bundles in place until then; (D) closure is accomplished by first placing a layer of GORE-TEX(R) membrane (artificial pericardium) between the array and the dura (b) followed by closing the dura (a) and placing a second layer of GORE-TEX(R) membrane between the dura and the skull (shown in next figure) as instructed in the Blackrock surgical manual).

Fig. 4. (A) Closure of the implantation window created on the skull using thinned out bone flap using titanium straps(a). Note another layer of GORE-TEX(R) membrane(b) sitting between the dura mater and the bone flap; (B) anterior view and (C) posterior view of the headpost-NP-connector assembly after closure of galea and skin. Note the wire bundles (black arrow) going under scalp posterior to the headpost stem; (D) post-operative lateral view of monkey with the NP-connector assembly sitting on the anterior headpost. Note that posterior headpost (white arrow) was implanted slightly above the occipital ridge a few months ago.
11. After suturing and topical antibiotic application, mount the NP method.

10. To allow ease of scalp closure around the headpost, temporarily dismount the NP taking care not to pull on the electrode wire bundles fixed on the skull. Maneuver the NP to get the closest possible scalp closure around the headpost.

9. Use GORE PRECLUDE(R) membrane (GORE PRECLUDE(R) Pericardial membrane, W.L. Gore & Associates, Inc., Flagstaff, AZ) as described in the Blackrock Microsystems’ surgical manual (Fellows and Suner, 2006). Then close the window with the thinned out bone attached (from step 3) and titanium straps. Finally, seal the open edge with a thin layer of silastic (Fig. 4A).

8. Fix the wire bundles on the skull using titanium straps and screws (P4ST-08-48 and SCR4-04-05, Bioplate, Inc., Los Angeles, CA) and silastic (Kwik-Sil™ Silicon Elastomer, World Precision Instruments, Sarasota, FL). We recommend the use of minimum amounts of silastic, making sure there are no pockets between silastic and skull, as these can be potential sites for pathogens to thrive.

7. Use systemic antibiotics daily up to day-10 post-implantation with a standard recording chamber; lost recordings at 6 months after implantation.

6. Her recordings have been consistent and in fact better than our animal becoming infected. In the above statements we are merely pointing out situations we have experienced and know others have also. Due to the unyielding nature of the cement cap, unlike scalp, the diagnosis of infection may be delayed until behavioral changes are observed, or the infection is sensed via olfaction, visual evidence or presumed via fever, in which case it is generally too late for any effective intervention, because by then a biofilm has already been formed between the scalp and the cap. This makes it impossible to replace the cement cap as the skull underneath is not healthy enough to support the new bone screws needed to attach a new cap. In addition, the implant site would be compromised once the original cement cap is removed along with the microelectrode connectors impregnated within. There are several labs that have been successfully using cement caps in their preparations for the chronic microelectrode array implants with good results, and without the animal becoming infected. In the above statements we are merely pointing out situations we have experienced and know others have as well (personal communications).

5. When an infection was suspected we would clean the skin margin with betadine and nolvasan and apply topical antibiotic ointment daily. In addition, systemic antibiotics were given. If an animal was healthy the skin margin was left undisturbed. Animals were individually housed in very large baboon cages conveniently situated to allow grooming between neighboring animals. In addition, the animals were given free time to run around and explore the housing space. As the cement cap is not a biological tissue, antibiotics given systemically barely reach the infection site. Also, due to the unyielding nature of the cement cap, unlike scalp, the diagnosis of infection may be delayed until behavioral changes are observed, or the infection is sensed via olfaction, visual evidence or presumed via fever, in which case it is generally too late for any effective intervention, because by then a biofilm has already been formed between the scalp and the cap. This makes it impossible to replace the cement cap as the skull underneath is not healthy enough to support the new bone screws needed to attach a new cap. In addition, the implant site would be compromised once the original cement cap is removed along with the microelectrode connectors impregnated within. There are several labs that have been successfully using cement caps in their preparations for the chronic microelectrode array implants with good results, and without the animal becoming infected. In the above statements we are merely pointing out situations we have experienced and know others have as well (personal communications).

4. Cap created with palacos cement with impregnated antibiotics; 1.0 mm Utah array, microwires and multi-site ceramic arrays implantation; infection occurred ∼6-months after implantation.

3. Cap created with palacos cement with impregnated antibiotics; 1.0 mm Utah array, microwires and multi-site ceramic arrays implantation and a standard recording chamber; lost recordings at 6 months after implantation, but the animal and the cap are healthy after 11 months.

2. Cap created with dental acrylic; microwire array implantation; infection occurred ∼6-months after implantation.

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3.1. Comparison with other techniques

3.1.1. Cement cap

Our lab has experience with the chronic microelectrode implantation of five non-human primates (M. radiata), and over 50 rats. Below is a summary of the non-human primate implants and prognosis.

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1. Cap created with dental acrylic; microwire array implantation; infection occurred after implantation.

Fig. 5. Comparison of NP method of chronic MEA implantation with other methods. (A) The skin margin stays the same with NP method irrespective of number of arrays used, and that it is less than other methods. (B) The surface area occupied by the connector assembly impregnated into it. (C) Expenses incurred by both NP and cement cap method are the same and are almost half the expenses by the Cereport method.

3. Results

We have implanted this single nesting platform with three 1.5 mm length microelectrode arrays in cortical areas PMd, M1, and S1 on a female Bonnet Macaque (M. radiata) weighing 4.2 kg on February 19, 2009 and she has been completely healthy to date (August 08, 2009); see recordings in Fig. 7. She was given systemic antibiotics daily up to day-10 post-implantation with no later antibiotic applications needed. This animal is our third animal with the Utah array implant, but the first one utilizing the nesting platform (see the section below for more details). Her recordings have been consistent and in fact better than our previously implanted two animals with the same type arrays (1.0 mm microelectrode length) implanted with the traditional cement cap covering (Palacos).

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5. See the next section for the NP implantation.
meninges had grown under the microelectrode array and pushed it up and out of the cortex. Since the Utah arrays are floating and not fixed to the cap, as many microwire implants have been, this problem can occur when there is no structure to keep the array pressed against the cortex. However, this is not a problem with the newer techniques such as those in the Blackrock surgical instruction manual that we have expanded on. It appears that this problem can be solved by closing the dura up properly; however, this can only be done currently with floating electrodes. This last point clearly has nothing to do with the type of connector housing one is using.

3.1.2. Titanium pedestal (Cereport)

Titanium’s bio-friendly nature and bone-cell attracting quality (Adams et al., 2007) makes the Titanium Pedestal, such as the Cereport, the implantation method of choice when compared to the cement cap. However, when implanting multiple arrays as stated previously, one quickly ends up with a transcutaneous area comparable to that of cement caps when using multiple pedestals. It should also be noted that the manufacturing of titanium pedestal is expensive and thus the overall cost of the Cereport is almost double when compared with the same array using an ICS-96 connector type.

3.1.3. The nesting platform (NP)

Using the NP implantation technique with scalp closure we implanted two 1.5 mm Utah arrays and one 1.5 mm IrOx coated Utah array. We removed the implants along with NP (leaving the anterior headpost in situ) 6 months post-implantation because of a local skin infection as a preventive measure to intra-cranial extension of infection, although we were still obtaining recordings from the arrays (see Figs. 6 and 7). This animal has fully recovered from the superficial infection and will be re-implanted after several months off studies. We observed that two of the three wire bundles were cut at the headpost stem level, which we believe is in part due to vigorous cleaning of the anterior post margin by rhythmic rubbing of a piece of gauze almost daily for a few weeks, although this is speculative. Dismounting the NP might have caused avulsion of the wire bundles which were tightly glued to the headpost with the help of cyanoacrylate.

Since the NP mounts on a headpost the number of arrays or connectors do not increase the exposed skin margin proportionately. By using the NP multiple Utah arrays with up to 4 ICS-96 connectors can be used while maintaining a minimal skin margin around a single headpost, which is almost 20% less than that of a single Cereport connector. Fig. 5 and Table 1 summarizes the comparison points between the NP we propose here with the cement cap and the titanium Cereport pedestal. We are only using the Utah array as one of many possible floating array types in this work, and do not wish to make claims as to what electrode type is best past the advantages of the floating style.

An additional feature of the NP is that one can use the headpost and NP again on another animal after removal and sterilization. We are presently developing a NP that can be mounted to the same headpost used for head restraint, thus decreasing further the surgical trauma to the animal and post surgical skin margins. Meanwhile, the cement cap design is extremely difficult if not impossible to re-use on other animals and we do not believe that the Cereport system is designed for re-use.

4. Discussion

We demonstrate in this paper a novel technique of micro-eletrode array implantation that is cost-effective, bio-friendly, reusable and easy to implement in light of growing interest in the field of chronic multi-site, multichannel electrophysiological recordings. The NP design can be modified to meet the customized
Fig. 7. Waveform recordings from all available channels shown approximately one and 6 months after implantation, waveform mean (solid line) ± standard deviation (dashed lines). Recordings were made at 40 kHz digitizing frequency with appropriate gain, threshold and sorted using PCA method on recorded waveform tracings online using SortClient software (Plexon Inc., Dallas, TX). The waveform location represents actual anatomical location of the microelectrode in the arrays and the arrays are arranged as implanted (see Fig. 3C). So, consider left side as anterior, right as posterior, top as medial and bottom as lateral side for individual microelectrode location in the array. Different waveform patterns with unique shade represent different unit activity (i.e. possible single neuron action potential activity in the vicinity of a given electrode) recorded from the same channel. All 3 arrays as depicted in Fig. 3C are shown here. Dorsal premotor (PMd), primary motor (M1) and primary somatosensory (S1) cortical array recordings are arranged in rows from top to bottom in that order.

needs for individual research labs and with the advent of sophisticated milling and 3D printing technology can be finely tuned to a specific experimental setup. Thus the NP design can be changed according to the type of headpost used (small pinhead size headpost for rats, for example or different shape and diameter of headposts as commercially available by different companies.) and the physical properties of the connectors for a given MEA (e.g., ICS-96 connectors, Omnetics connectors etc. as well as custom made connectors.) Apart from that, different hardware including wireless transmitter or antennas can also be mounted on the NP while minimally, if at all, disturbing biological tissues. This would expand its use manifold and give an experimenter some degree of freedom in engineering the novel technology without much worrying about the installation or mounting of the required hardware straight on the skull or scalp and fixing it with less biologically friendly materials. The NP described here is a simple demonstration of this idea which is specifically designed for non-human primates with headpost model 6-FHP-2XF and connector type ICS-96. Use in different species with different headpost model and connector type would need a customized size and design of the NP, which can be easily produced by use of 3D designing software and manufactured by a milling/3D printing process with a custom choice of material to use in the making of the NP.

The next generation of our NP will fit onto the same headpost needed for head restraint during awake, behaving macaque neurophysiological experiments. In our case mild head restraint is needed to keep the animal from destroying the expensive preamp headstages. Using our in house developed nesting platform we have shown that it is possible to record from hundreds of microelectrodes while greatly limiting the exposed skin margin vulnerable to infection. While the continuing development of wireless transfer technologies promise a future without the need
Table 1

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<th>Parameter</th>
<th>NP</th>
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<th>Cement cap</th>
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<td>Surface area (cm²) covered on skull</td>
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<td>2.953</td>
<td>2.953</td>
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<td>Base diameter (~2 cm) dependent</td>
<td>Neck diameter dependent</td>
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<tr>
<td>Skin margin (cm)</td>
<td>Post diameter dependent (~.94)</td>
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<tr>
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<td>3.691</td>
<td>&gt;11.2</td>
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<td>Quad array</td>
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<td>Bone cement + 10 screws</td>
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<td>10 screws</td>
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<tr>
<td></td>
<td>1200</td>
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for trans-cutaneous protrusions, realization of such implants is in its nascent stage with their capacity limited to a few channels (Song et al., 2009). In the meantime the described nesting platform allows possibly the most bio-friendly and economical means of conducting chronic neurophysiological experiments with multiple microelectrode arrays.

Conflict of interest statement

At the time of publication, no authors have any real or potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jneumeth.2010.02.006.

References


