Reconfigurable Neural Probes for Chronic Electrical Recording

by

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ABSTRACT

Electrical interfaces with the nervous system are critical for enabling neurally-controlled prostheses and for creating better therapies for neurological disorders. Microelectrodes that penetrate into brain tissue to record extracellular electrical activity are one such neural interface technology known for their unparalleled spatial and temporal signal resolution. However, a major challenge with reliable chronic recording with such interfaces is tissue reaction, wherein a sheath of glial cells encapsulates the neural probe, electrically insulating the probe from the surrounding neurons.

This work presents the design, fabrication, and characterization of a thin polymer probe whose body can be deflected and locked prior to insertion via a glue, storing mechanical energy in the device. After inserting into the brain and waiting for the initial glial sheath to form, the device can be deployed by melting the glue, causing the recording tip of the device to penetrate into fresh tissue. It is hypothesized that small tip dimensions (10-20 μm) should prevent the formation of an additional glial sheath post-deployment and thus enable chronic recording.

Four successive generations of devices were fabricated, characterized in benchtop tests, and subsequently tested in the rat model and in the optogenetic Thy1-ChR2-YFP mouse model. The electrical and mechanical functionality of the probe was confirmed under acute in vivo conditions in the medial prefrontal cortex of optogenetic Thy1-ChR2-YFP mice. Neural activity was successfully recorded from the probe immediately after insertion using laser stimulation of the mouse brain. Applying saline to the water-soluble glue caused the probe to deploy and neural activity was successfully recorded post-deployment. These results demonstrate the first in vivo deployment and electrical recordings from a reconfigurable neural probe with small dimensions (< 20 μm) and hold promise for the creation of chronic recording neural interfaces.

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Chapter 1: Introduction

1 INTRODUCTION

This thesis describes the design, fabrication, and testing of a new penetrating neural interface for chronic recording of extracellular electrical activity from brain cells. This chapter provides a motivation for this work and introduces the device concept, whereas subsequent chapters describe successive iterations of the device.

Section 1.1 introduces neural interfaces and their applications. Section 1.2 elaborates on recording neural interfaces with section 1.3 focusing on penetrating neural electrodes. Section 1.4 explains the phenomenon of glial reaction to penetrating neural electrodes, while section 1.5 discusses some strategies that have been attempted to date to tackle glial reaction. Lastly, section 1.6 covers the proposed design and hypothesis of this work.

1.1 Neural interfaces

A neural interface is any device that can communicate with the nervous system, either unidirectionally or bidirectionally [1], [2]. Engineering tools that can provide insights into or manipulate the nervous system are, therefore, critical to creating rehabilitative and augmentative technologies.

The nervous system consists of two different kinds of cells: electrically active neurons and electrically inactive glial cells, including oligodendrocytes, astrocytes and microglia (Figure 1-1). The main function of neurons is to transfer and process information, whereas glial cells serve to support neurons (oligodendrocytes provide electrical insulation for neurons for faster signal propagation), modulate neural activity (astrocytes can modulate synaptic activity), or act as the brain’s immune cells (microglia and astrocytes are both implicated in immune responses in the brain) [3].
Chapter 1: Introduction

Neurons can transmit information across very large distances at extremely fast speeds (over 100 m/s in humans) by means of an action potential (Figure 1-2) – a digital signal that is transmitted across the cell by a potential difference across the membrane of the cell. Once this electrical signal reaches the end of a neuron, it is translated to a chemical signal that is used to stimulate the adjacent neuron.

Many of the properties of electrically active cells, such as neurons, have been known since the 19th and early 20th century. In 1791, Luigi Galvani conducted a famous experiment where muscle contractions were evoked by electrical stimulation of an exposed frog hind limb muscle-nerve preparation [6].
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Galvani dubbed this phenomenon “animal electricity” and was the first evidence for the electrical basis of nerve cell function and muscle contractions. Since this historical study (subsequently improved upon by Alessandro Volta), over two centuries of research has served to enhance our understanding of the nervous system. A landmark study in the 20th century that laid the foundation of our current understanding of nerve cell functioning was done in the late 1940s and early 1950s by Alan Hodgkin and Andrew Huxley [7]. In this study, extensive measurements were performed on the squid giant axon and a theoretical model was developed to fit experimental observations. Hodgkin and Huxley were eventually awarded the Nobel Prize for this work, which was instrumental in providing a mathematical and physiological basis for the electrical excitability of the nerve cell [8]. The second half of the 20th century saw a whole host of technologies to probe the nervous system further. For example, in 1958, Felix Strumwasser demonstrated the ability to chronically record neural activity using microwires in hibernating squirrels [9]. The groundbreaking work of Eb Fetz in 1969 [10] showed that non-human primates can learn to control the firing rates of single neurons in the cortex. Since these early technological advances, the field of neural interfaces has expanded enormously, with technologies such as cochlear implants [11], pacemakers [12], retinal implants [13], and deep brain stimulation devices [14] now being standard treatment options for various health conditions.

Neural interfaces can be divided into various categories depending on the direction of communication with the brain (stimulation vs. recording), the modality of communication (electrical, chemical, etc.), or the target biological system (central nervous system vs. peripheral nervous system) [2].

1.1.1 Stimulation interfaces

Neural interfaces can also be used to modulate the state of the nervous system by electrical, chemical, magnetic, or optical stimulation.

1.1.1.1 Electrical stimulation

One of the most common methods of modulating neural activity is via electrical stimulation. Since neurons are electrically active, stimulating them electrically can cause firing or inhibition of action potentials, allowing for manipulation of the nervous system. This is used in clinical applications for treating various neurological disorders. For example, deep brain stimulation is an FDA-approved treatment for treating motor symptoms of Parkinson’s Disease and has also been used in treating dystonia, epilepsy, obsessive-compulsive disorder, Tourette syndrome, and depression [14]. Cochlear implants [11], first introduced in the 1970s and 1980s, are now commonplace with over 200,000 patients worldwide with such implants. Retinal implants [13] can be used to restore some visual functionality to patients with blindness. For example, Second Sight’s Argus system was approved by the FDA in 2013 for patients with retinitis pigmentosa, an inherited disease that causes severe blindness in over 1.5 million people worldwide. This device consists of an eyeglass-mounted camera that records real-time images, processes them, and wirelessly transmits them to electrodes implanted in the retina that electrically stimulate the retina [15].
Figure 1-3 Stimulating neural interface success stories. (a) Deep brain stimulation [16]. (b) Cochlear implant [17]. (c) Retinal implant [18].

Electrical stimulation may also be useful for restoring somatosensory functionality. Sensory percepts can be elicited with intracortical microstimulation of the primary somatosensory cortex (S1) region of the brain in non-human primates [19]–[22], as well as in humans [23]. Such evocation of sensory percepts can be combined with prosthetic devices to engineer prosthetics that feel similar to natural limbs [24], [25].
Chapter 1: Introduction

Although electrical stimulation is often done using intracortical penetrating electrodes, the brain can also be electrically stimulated using electrocorticography (ECoG) electrodes [26] (placed under the skull and on the brain surface) or using transcranial electrical stimulation (tES), a technique where alternating electric currents are applied through electrodes placed outside the skull [27], [28].

Like the central nervous system, peripheral nerves can also be stimulated electrically. For example, a technique called functional electrical stimulation (FES) is used to restore motor functionality in paralyzed limbs by electrically stimulating specific peripheral nerves [29].

1.1.1.2 Chemical stimulation

The easiest way to chemically stimulate the brain is through the ingestion of substances that are absorbed into the bloodstream and cross the blood-brain-barrier to modulate neural activity. The entire field of neuropharmacology is based on this concept and has led to the invention and discovery of new substances that can modulate the brain, among other organs, in some way. Because this non-invasive way to chemically stimulate the brain has been shown to be effective, invasive neural interfaces are seldom used solely for chemical stimulation. Rather, chemical stimulation via neural interfaces is typically used in combination with other functionalities, such as recording, in order to enable multi-modal neural interfaces [30]-[33].

1.1.1.3 Magnetic stimulation

Since electricity and magnetism are inherently linked, the brain can also be stimulated magnetically. Transcranial magnetic stimulation [34] is a noninvasive technique where a magnetic field can be applied through the skull to induce a controlled current pulse in a specific cortical target. While this technique is appealing because of its non-invasive nature, it has a lower spatiotemporal resolution compared to electrical stimulation using intracortical neural devices (as will be described in section 1.3).

1.1.1.4 Optical stimulation

While neuromodulation techniques have largely relied on electrical stimulation thus far, recent studies have explored the use of optogenetics [35], a technique for expressing genes in cells that render them sensitive to light. In recent years, this approach has been used in neuroscience [36] to manipulate neuronal function, whereby light-sensitive channel proteins can be expressed in certain neurons to activate or silence these neurons on demand. Figure 1-4 shows a commonly used channelrhodopsin (ChR2) isolated from the green algae Chlamydomonas reinhardtii [37], that can be engineered to be expressed in neuron cell membranes. Upon illumination with blue light, ChR2 opens and selectively allows cations through the neuronal cell membrane, leading to a depolarization of the neuron and the firing of an action potential.
Optogenetics has multiple uses. For example, it can be used to stimulate the somatosensory cortex of transgenic animals in order to elicit sensory percepts [38]. Additionally, it can serve as a very useful engineering tool for the testing of neural electrodes [36] by providing a method to elicit neural activity on demand.

1.1.2 Recording interfaces

Neural interfaces can be used to sense the state of the nervous system, either by measuring chemical activity or electrical activity.

1.1.2.1 Chemical recording

Neuronal cells, like all cells, are generally quite sensitive to their chemical environment. Therefore, information about the chemical environment in the nervous system can provide valuable information about the state of the nervous system. The most common technique utilized for probing the chemistry of the nervous system is known as fast scan cyclic voltammetry (FSCV) [39], wherein microelectrodes (typically carbon-fiber electrodes, chosen for their electrochemical properties) are used to sweep voltages between two points while the associated currents are measured. The voltage-current curves obtained using this technique are characteristic of the redox reactions taking place at the electrode site and can, therefore, be used to identify the chemicals surrounding the electrodes. For example, FSCV has been used in vivo to detect sub-second dopamine release in rodent brains [40], [41].

1.1.2.2 Electrical recording

Since neurons carry information through changes in electric potential in their cell membranes, electrical recordings from neurons can provide extremely valuable information about the nervous system [42]. Electrical recordings from neurons can be made by directly measuring the potential across a neuronal cell membrane using a technique called patch-clamp, where a micropipette of ~1 μm diameter is placed in contact with a cell membrane and a slight suction is applied [43], [44]. This technique can be used to record from whole cells or tiny patches of the cell membrane. Because of the invasive nature of the patch-clamp technique, it is typically only used in vitro. A slightly less invasive technique measures extracellular potential disturbances in the immediate vicinity of the neuron by placing microwires or
other electrodes near neurons without physically piercing through the neuronal cell membrane [3].
Electrical recordings can be done in an *in vitro* setting (where recordings are done on explanted slices of tissue [45] or on cultured cells [46]) or in an *in vivo* setting (where recordings are done on live tissue that has not been explanted). This work focuses on extracellular *in vivo* electrical recordings in the central nervous system, which are described further in section 1.2.

1.1.3 Combined recording and stimulation for closed-loop neural interfaces

Neural interfaces can have more than one of the above functionalities. In fact, the idea of a closed-loop interface remains an active area of research, wherein a single neural interface can both record and stimulate nerve cells [47]. Such closed-loop neural interfaces could enable new technologies, such as smarter prosthetics.

1.2 Recording neural interfaces for the central nervous system (CNS)

While neural interfaces can be used to record from either the peripheral nervous system (PNS) or the central nervous system (CNS), this work focuses on CNS recording. There are three broad kinds of neural interfaces that are used for extracellular *in vivo* recording in the central nervous system. Categorized from least to most invasive, they are:

1. **Electroencephalography (EEG) electrodes [48]**
   These are the least invasive electrodes and are placed on the scalp. Since a thick layer of bone (the skull) separates these electrodes from neurons in the brain from which these electrodes are trying to record, EEG electrodes have low spatial and temporal resolution, limiting their application in neuroprosthetic use.

2. **Electrocorticography (ECoG) [49]**
   These electrodes are placed on the surface of the brain, underneath the skull. Since a craniotomy needs to be performed to get these electrodes below the skull, ECoG electrodes are more invasive than EEG electrodes and require surgery to implant. However, since they do not actually penetrate brain tissue, they are considered less invasive than penetrating electrodes. Being underneath the skull (and often below the dura as well) allows ECoG electrodes to have a higher signal resolution compared to EEG electrodes.

3. **Penetrating intracortical microelectrodes [2]**
   These electrodes have shanks that penetrate the brain with electrode sites that aim to get in very close proximity to neurons in order to record extracellular action potentials from single neurons or very small groups of neurons. These electrodes offer the highest signal resolution, but are also the most invasive.
Electrical recordings from nerve cells have two important applications. First, it gives us a better understanding of the physiology of the nervous system and neural circuits, which can be useful in understanding the brain and the mechanisms behind various neurological disorders. This information can then be used to design treatments for these neurological disorders. Second, the ability to record electrical activity from neurons can enable neurally-controlled prosthetic devices that can then be used by amputees or patients with paralyzed limbs. For example, Figure 1-6 shows a recent study [53] where tetraplegic human patients used neural implants in their motor cortex (M1 region) to control a robotic arm to perform three-dimensional reach and grasp movements. While such neurally-controlled prostheses have shown promise in non-human primate studies [54], [55], as well as in human clinical trials [56], there are some significant challenges for approval of such devices to be used in patients as standard care options. These challenges will be discussed in more detail in section 1.4 and are the focus of this thesis work.
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Figure 1-6 Four sequential images of a successful trial of a patient using an implanted neural electrode to control a robotic arm to (a) grasp a bottle, (b) bring it towards her mouth, (c) drink coffee from the bottle through a straw, and (d) place the bottle back on the table [53].

In addition to intracortical recordings, EEG or ECoG signals can be used to restore movement of paralyzed arms [57]. However, the resolution of these signals is not as high as that of signals recorded with intracortical electrodes. Table 1-1 below, adapted from [2], shows the spatial and temporal resolutions of the three methods listed above. Because penetrating microelectrodes offer the highest spatiotemporal resolution, they also offer the highest functionality, making this technology an important candidate for clinical applications [56], [58]. Therefore, this work focuses on penetrating microelectrodes for recording from the central nervous system.

Table 1-1 Commonly used methods for electrical recording from the central nervous system [2].

<table>
<thead>
<tr>
<th>Method</th>
<th>Temporal resolution (seconds)</th>
<th>Spatial resolution (mm)</th>
<th>Invasiveness of procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEG</td>
<td>0.05</td>
<td>10</td>
<td>Electrodes on skull</td>
</tr>
<tr>
<td>ECoG</td>
<td>0.003</td>
<td>1</td>
<td>Electrodes under skull</td>
</tr>
<tr>
<td>Intracortical</td>
<td>0.003</td>
<td>0.05</td>
<td>Electrodes penetrating brain tissue</td>
</tr>
</tbody>
</table>

1.3 Penetrating intracortical microelectrodes for CNS recording

There are three commercially available microelectrode technologies that are commonly in use today. In addition to these three technologies mentioned above, various homemade technologies are also used by researchers in animal models.
1. Microwire arrays, commercially manufactured by companies such as Tucker Davis Technologies® and Plexon®, and also custom-made by various researchers [59], consist of an array of insulated metal wires with electrode sites at the very tip. Such arrays have been in use for decades and provide an effective way to record from neurons within the same brain layer.

2. The Utah Array, now commercially manufactured by Blackrock Microsystems®, consists of an array of micromachined silicon pillars with electrode sites at the tip of each pillar [60], [61]. Similar to microwire arrays, the Utah Array can be an effective tool for recording from neurons within the same brain layer and has been used extensively in animal studies [62], as well as in human clinical trials [56].

3. The Michigan Probe, now commercially manufactured by Neuronexus Technologies®, was first introduced in the 1990s by University of Michigan researchers and consists of a silicon shank with electrode sites along the length of the shank [63]–[65]. This technology can be used to probe neurons in different brain layers, since the electrode sites are located in the direction of probe insertion.

While these penetrating microelectrodes are very effective at recording neural activity in an acute setting (days to weeks), reliable chronic (months to years) has proven to be challenging, with a loss of 50% or more of the recording sites [53], [66], [67] during long-term (months–yrs) recording.

1.4 Glial reaction – a challenge to reliable chronic recording in the CNS

One of the most important reasons for electrode failure in chronic conditions is thought to be the body’s foreign body reaction to these electrodes [68]. Typical timelines for chronic electrode performance are described in section 1.4.1, while the glial reaction is described in more detail in section 1.4.2.

1.4.1 Timeline for electrode performance in chronic microwire implants

The typical performance of microwire electrodes is shown in Figure 1-8 below, adapted from the work of Williams et al. [59]. In this study, 8 guinea pigs were implanted with microwire arrays, each array consisting of 33 tungsten microwires (35 μm bare diameter with an additional 7 μm thick polyimide insulation, inter-column spacing of 250 μm, inter-row spacing of 400 μm). The average of the short-term implants (the dashed line in Figure 1-8) indicates that the fraction of active electrodes is ~60% one week.
post-surgery. Then, starting 1-2 weeks after surgery, the performance of the electrodes increases, peaks at \(~80\%\) around 2-3 weeks post-implantation, and then follows a gradual decline.

![Graph showing electrode performance over weeks](image1.png)

Figure 1-8 Typical performance of microwire arrays implanted in the cortex of guinea pigs [59]. Gp10, Gp13, Gp15 and Gp19 are the short-term (< 9 weeks) guinea pig implants (the average indicated by the dashed line). The solid dashed line is the reported average from the 4 animals that survived beyond 9 weeks.

This general performance timeline seems to be characteristic for neural probes in other animal models as well. Figure 1-9 below, adapted from the work of Sanchez et al. [69], shows the typical performance timeline of a 2 X 8 tungsten microwire array (50 \(\mu\)m diameter with an additional 10 \(\mu\)m polyimide insulation, inter-column spacing of 250 \(\mu\)m, inter-row spacing of 375 \(\mu\)m) implanted in the rat motor cortex. Again, the electrode performance drops right after the surgery, improves 1-2 weeks post-implantation, and then follows a gradual decline.

![Graph showing electrode performance over weeks](image2.png)

Figure 1-9 Typical performance of tungsten microwire arrays implanted in the motor cortex of Sprague-Dawley rats [69]. A total of 22 rats (designated R1 through R22) were implanted in this study, with some animals euthanized at the end of the surgery, some euthanized after 2 weeks and some euthanized between 2 and 24 weeks.
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It has been hypothesized by Prasad et al. [69] that the initial decline in performance from an intraoperative setting to immediately following surgery is likely due to swelling of the brain caused by the surgical trauma. Such brain swelling can cause neurons around the recording sites to be pushed away, leading to the low electrode yield. Swelling is reported to decrease between 3-6 days following probe insertion [70]; the increase in array yield approximately a week after implantation (in both Figure 1-8 and Figure 1-9) is consistent with this hypothesis. The gradual decline in performance seen after this time point is likely because the chronic tissue reaction sets in, leading to neuronal cell loss adjacent to the electrode sites [71].

1.4.2 Glial reaction to implanted microelectrodes
The typical glial response to electrodes implanted in the brain is shown in Figure 1-10 below. The early response is characterized by the inserted devices being surrounded by a large region containing reactive astrocytes and microglia, whereas the sustained response is characterized by the formation of a compact sheath of glial cells around the insertion site [58], [72].

![Figure 1-10 Schematic showing (A) acute and (B) chronic tissue responses following device insertion into the brain [58]. The acute response is characterized by vasculature damage, neuronal injury, plasma protein adsorption, recruitment of activated microglia, and a broad region of reactive astrocyte around inserted devices. The chronic response is characterized by a condensed sheath of cells primarily composed of activated microglia and reactive astrocytes around the insertion sites. Degeneration of neuronal processes and additional neuronal loss may also be seen.](image-url)

Figure 1-11 below, adapted from [73], shows histology images of brain slices of rat cortices implanted with 2-mm-long silicon shanks (trapezoid with base 200 μm, top 60 μm, height 130 μm) and explanted at different time points. Explanting the shank at 2 or 4 weeks disrupts any sheath structure. However, at 6 and 12 weeks, the glial sheath around the implant has solidified enough to withstand probe explantation. This serves to illustrate the difference between acute and chronic reaction timelines.
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Figure 1-11 Time course of glial scar formation at four time points as imaged by glial fibrillary acidic protein (GFAP) staining [68], [73]. At 2- and 4-week time points, the astrocytic processes fall back into the void left by the probe extraction before tissue processing. By 6 weeks, the processes have interwoven to form a stronger, denser sheath surrounding the implant. Minimal changes between the 6- and 12-week time points indicate the glial scar completion within 6 weeks.

1.5 Causes of glial reaction and strategies to tackle glial reaction

Because mitigating the glial reaction is a key factor in enabling the reliable functionality of penetrating microelectrodes in a chronic setting, there is a very large body of research investigating the causes of the glial reaction and various strategies aimed at tackling some of these causes. To date, however, these approaches have not completely eliminated the chronic tissue response, or enabled reliable chronic electrical recording.

1.5.1 Biocompatibility - material choice, surface coatings, and delivery of chemicals

Studies with different materials have shown that some materials are inherently more biocompatible than others [70]. If the brain can chemically detect the presence of a foreign neural implant, then clever material choices, bioactive material coatings, and secretion of immunosuppressants around the implants should reduce tissue reaction.

The traditional structural material for neural probes has been silicon (used by Utah-Array- and Michigan-Probe-style devices) or metal wires (used by microwire arrays), since silicon and metals such as tungsten, platinum and platinum/iridium alloys, have been shown to be generally inert in the brain. However, there is an important difference between materials that are not actively toxic to the body versus materials that are truly biocompatible and undetectable by the body. For example, while silicon and tungsten might not be toxic to the brain in the same way as certain heavy metals, devices made with these materials still get encapsulated by glial tissue, suggesting that these materials are still detected by the body. Therefore, the search continues for materials that would render neural probes truly undetectable to the body.

Researchers have attempted various material coatings and material injections in the ongoing effort to increase material biocompatibility. In 2005, Spataro et al. [74] showed that dexamethasone, an anti-inflammatory agent, administered subcutaneously post-surgery greatly attenuated the astroglial response to implanted silicon shanks. However, no electrical recordings were performed in this study. In
2011, Azemi et al. [75] showed that silicon-based neural probes coated with L1, a cell adhesion molecule, elicited significantly lower activation of microglia and astrocytes compared to uncoated control probes. However, no electrical recordings were performed in this study as well. Thus, while there is evidence to support that surface coatings or injection of certain kinds of chemicals might help reduce tissue response, it is unclear how this directly translates to better electrical recordings in a chronic setting.

Extensive research has also been done in the use of conductive polymers for neural recordings. For example, polypyrrole (PPy), seeded with various biomolecule combinations, has been shown to improve the interface between tissue and the recording sites [76], [77]. However, PPy is ill-suited for chronic, long-term implantation due to lack of stability of its electrochemical properties [78], [79]. Another conductive polymer that has recently been used in neural probe design is poly(3,4-ethylenedioxythiophene) (PEDOT). In 2006, Ludwig et al. showed that Michigan probes coated with PEDOT were marginally better than uncoated control probes in terms of recording quality, with the PEDOT electrode sites registering 17% more quality units than control sites. More recently, Kozai et al. [80] showed that electrodes coated with PEDOT can significantly improve chronic electrical recording quality. This was one of the first demonstrations of surface coatings directly improving chronic electrical recording. However, the probes used in this study had other attributes, such as small size and flexibility, which might have also contributed to the improved electrical performance in a chronic setting. The above studies suggest that PEDOT coatings might help improve chronic recordings, but only when combined with other approaches.

Thus, while current literature points to certain materials being more biocompatible than others, no materials have yet been shown to be the ultimate solution to enabling chronic electrical functionality.

1.5.2 Flexible materials to match the elastic modulus of brain tissue

Typical materials used in intracortical electrodes have Young’s moduli much higher than that of brain tissue. For example, the Utah Array and the Michigan Probe both use silicon as the structural material, which has a Young’s modulus of over 100 GPa [81]. Microwires typically use metals with Young’s moduli on the order of hundreds of GPa. Even polymers used in neural probes are quite stiff – polyimide and parylene both have Young’s moduli on the order of 1 GPa. In comparison to all of these materials, the elastic modulus of the brain is on the order of a few kPa [82]. Some have hypothesized that this elastic modulus mismatch could contribute to the brain’s tissue reaction to neural probes. If this hypothesis is true, then flexible probes should mitigate this reaction. Therefore, many researchers have started developing probes that are more flexible than the traditional silicon- or metal-wire-based probes.

For example, in 2005, Takeuchi et al. [83] described parylene flexible probes with integrated microfluidic channels. These probes were shown to pierce brain tissue and record intraoperative signals in a rat brain; however, no chronic studies were performed nor was any comparison made with stiffer probes to isolate the effect of flexibility on any improvement in electrical recordings or tissue reaction. Similar parylene or polyimide probes by other groups [84], [85] have demonstrated viable designs and preliminary in vivo tests, but no comparison of chronic recording quality or chronic tissue reaction with stiffer probes.
Perhaps the most convincing case for flexible probes was made recently by Nguyen et-al. [86], where mechanically-compliant probes implanted in the rat cortex for up to 16 weeks showed a significantly lower neuroinflammatory response compared to stiffer control probes. While lower neuroinflammation suggests that such mechanically-compliant probes would be better at chronic electrical recording, no in vivo electrical recordings were done in this study. In fact, some studies with microelectrode arrays have shown poor correlation between neuroinflammation and electrical recording performance [87].

Recently, Kozai et al. [80] demonstrated an improvement in chronic electrical recording quality by implanting flexible ultrasmall carbon-fibre-polymer composite probes in the rat brain. However, recording quality was not improved to acute levels and it was hard to attribute the improvement in recording quality to flexibility of the probes alone, since the probes were also very small (< 10 μm) and had bioactive surface coatings.

Thus, flexibility in neural probes might help reduce tissue reaction, but current literature does not support the hypothesis that flexibility alone can enable chronic neural recording. This might be because elastic modulus mismatch is not the root cause of chronic electrode failure, or it might be because even the most flexible of neural probes to-date still have elastic moduli a few orders of magnitude higher than that of the brain (~kPa). One reason why neural probes with elastic moduli as low as a few kPa have not been built is likely because neural probes need to be stiff enough to insert into brain tissue with current implantation techniques. Probes with elastic moduli similar to that of the brain would be impossible to insert into brain tissue without buckling and would be akin to pushing on a rope. Thus, there is a lower limit on how flexible neural probes can be made based on the requirement that the probes need to be stiff enough to implant into brain tissue without buckling.

To summarize, probes that are more flexible than traditional silicon probes or microwire arrays have shown to reduce chronic neuroinflammatory response. In cases where flexible probes have shown improvement in chronic recording quality, other probe attributes such as small probe size or bioactive surface coatings confound the results. Additionally, there is a lower limit on the flexibility of any probe that is to be inserted into the brain. Flexibility by itself, then, is likely not the path to chronic neural recording.

1.5.3 Tissue micromotion and untethered devices
The brain is not attached to the skull; instead, it floats inside the skull surrounded by cerebrospinal fluid. However, neural implants are typically tethered to the skull for structural support. This can result in relative micromotion between the brain tissue and the tethered neural implants [88]. It has been hypothesized that such micromotion is an important cause of the tissue reaction to neural probes.

Biran et al. [89] showed that tethered silicon microelectrode arrays implanted in the rat brain for up to 4 weeks elicited a significantly higher tissue response than untethered arrays. Other studies also point to intracortical implants without tethering cables leading to significantly diminished inflammatory responses compared to tethered electrodes [90], [91]. This is consistent with the brain micromotion hypothesis. However, there might be other factors leading to increased tissue reaction to tethered probes as well. For example, potential colonization of the electrode interface by fibroblasts migrating from the overlying meninges might be contributing factor to the increased tissue reaction [92].
Nonetheless, there seems to be strong evidence in the current literature to support the effectiveness of untethered probes in reducing tissue reaction.

It is unclear how reduced tissue reaction to untethered probes translates to improved electrical recordings, however, since studies with untethered probes so far have mostly focused on the tissue reaction alone. Electrical recording with untethered probes is more challenging since it involves implementing mechanisms to wirelessly transmit data and power across the skull, which have their own associated challenges. A compromise between conventional tethered and completely unattached wireless implants are floating microwire arrays (FMAs, manufactured by companies such as Plexon* and MicroProbes*), where microwire arrays are connected to flexible wires, which then connect to another solid part that is attached to the skull. Thus, FMAs are more free-floating in the brain than probes that are conventionally tethered directly to the skull, and have recently shown to have a reduced chronic tissue response compared to tethered probes [93]. However, chronic electrical studies with such FMAs are only recently being performed. For example, Prasad et al. [87] implanted Pt/Ir FMAs in 12 rats for up to 6 months and extensively characterized various abiotic and biotic factors leading to electrode failure. However, no tethered control probes were used in this study to compare the effects of tethering on electrical recording performance.

Thus, while there is evidence to suggest that untethered probes result in a reduced chronic tissue response, the extent to which untethering neural probes will improve chronic electrical recordings remains to be seen.

1.5.4 Blood-brain-barrier damage and strategies to minimize damage to vasculature

Insertion of neural probes typically causes significant damage to brain vasculature. Such blood-brain-barrier (BBB) damage is unavoidable during neural implant surgeries and can also persist chronically after the implantation. There is increasing evidence recently that demonstrates the importance of blood-brain-barrier breach in the failure of neural implants.

For example, Saxena et al. [94] showed a direct correlation between blood-brain-barrier damage and electrode failure for common types of microelectrodes implanted in the rat brain for up to 16 weeks. Chronically functional electrodes were found to elicit an enhanced wound healing response, whereas in poorly functioning electrodes, chronic BBB breach was shown to result in local accumulation of neurotoxic factors and an influx of pro-inflammatory myeloid cells, which negatively affect neuronal health. Such a correlation between poor electrode performance and BBB damage was corroborated by Prasad et al. [87]. In this study, electrodes (ranging in implant duration from 1 week to 10 weeks) with poor recording performance showed increased ferritin expression in histopathology studies. This is consistent with the hypothesis that electrode failure is a direct consequence of iron-mediated oxidative stress caused by BBB damage.

Strategies that minimize BBB damage, therefore, are likely to improve electrode performance.

1.5.5 Small Size

If the tissue reaction to neural probes is because the nervous system can detect the physical presence of a foreign body, then making the neural probes smaller should reduce tissue reaction. Researchers have,
therefore, attempted to reduce the size of neural implants [80], [95]–[97]. There is evidence to suggest that smaller probes elicit a reduced immune reaction, especially if they are under a specific threshold (~15-20 μm) [80], [90], [91], [96], [98]. For example, recent work [80] has described the development of new microthread electrodes (MTEs) consisting of 7-μm-diameter carbon fiber microwires (Figure 1-12a) that are coated with ~1 μm of polymer coatings (which serve as dielectric barriers and make the electrodes protein-resistant). The probe tips were coated with PEDOT to further increase biocompatibility. In vivo recordings of up to 5 weeks in the rodent brain model demonstrated that these MTEs performed significantly better than silicon electrodes (Figure 1-12b). Note that the signal-to-noise ratio (SNR) in Figure 1-12b shows that the shape of the electrode performance timeline closely resembles that of larger microwires (Figure 1-8 and Figure 1-9), with great performance acutely, followed by a dip in performance during the first week or so, a peak in performance around 1-2 weeks post-implantation, and followed by a gradual decline. The major difference is that the MTE performance timeline seems to have a vertical offset, with SNR being better overall at every time point. At 5 weeks post-implantation, Kipke’s MTEs had a significantly better performance than larger sized silicon probes, and yet their performance was not as high as acute levels. This suggests that small size might help improve chronic SNR, but, by itself, might not be sufficient to bring chronic SNR to intraoperative levels.

Figure 1-12 Ultrasmall microthread electrodes (MTE) developed by Kipke’s group [80]. (a) SEM images of the electrodes. (b) Mean SNR of the largest single unit detected on each electrode for MTE (black, N = 7) and silicon control probes (red, N = 80).

1.5.6 Repositioning Probes
Repositioning electrodes post-implantation to recover lost signals is a commonly used technique, typically done with the help of microdrives [99]. However, repositioning usually only temporarily recovers signals. For example, Figure 1-13 below shows the results from continually repositioning microelectrodes in a rodent cortex [100]. The signal-to-noise ratio (SNR) increases each time the microelectrode is pushed forward but reduces again in 2-4 days, requiring continual repositioning. Such continuous repositioning is not practical for chronic applications since the device will eventually go past the brain layer from which it is trying to record.
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Figure 1-13 Typical performance of a movable microelectrode [100]. The stars indicate a statistically significant improvement in the SNR each time the electrode was repositioned.

There is evidence to suggest that electrode repositioning after the initial glial response subsides (a few weeks) will induce a reduced secondary reaction. In a study performed by Muthuswamy’s group [101], microwires were repositioned at different time points post-implantation, as show in Figure 1-14 below. When the electrode is repositioned at day 2 post-implantation, the final tissue response is no different from the stationary control electrode. However, when the electrode is moved 14 days or 28 days post-implantation, the tissue response is significantly reduced compared to the stationary control probe.

Figure 1-14 Tissue response of electrodes repositioned 2, 14, and 28 days respectively post-implantation [101].

To enable moving of the electrode at a later time point, researchers have started developing reconfigurable probes, using either a Sandia surface-micromachined process [100], [102], [103], a triggerable variant of the Michigan probes [104], or other methods [105]. However, these existing probes either involve a very complicated fabrication process [102], have large electrode tips [102], or have not demonstrated in vivo studies [104], [105].
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1.6 Proposed Approach and Hypothesis

1.6.1 Combining small feature sizes with post-implantation repositioning

This thesis work proposes to combine the small feature size approach with post-implantation repositioning. We hypothesize that this has the potential to enable chronic neural recording for two reasons:

1. As was seen in section 1.5.5, small-sized electrodes tend to perform better than larger-sized electrodes. However, their performance timeline (Figure 1-12b) indicates that even small-sized electrodes follow a similar-shaped timeline as their larger-sized counterparts (Figure 1-8 and Figure 1-9). This might be because the surgical trauma that is the potential cause of the initial dip in post-implantation performance is unavoidable, no matter how small the electrode features. Small-sized electrodes do, however, tend to perform better in the long-term, with SNRs significantly better than larger electrodes (but still lower than their own SNRs intraoperatively).

2. As was seen in section 1.5.6, repositioning electrodes tends to temporarily recover signals up to acute levels. It is hypothesized that the temporary nature of the signal recovery is due to the large size of the electrodes (typically larger than 50 µm) used in repositionable electrode studies.

The above two effects appear to be complementary. If the hypothesis is correct that the state of the brain is different immediately after implantation versus a few weeks post-implantation (because the initial surgical trauma is unavoidable irrespective of electrode size), then it would imply that waiting a few weeks post-implantation for the brain to reach steady state and then repositioning with electrodes with very small feature sizes should recover brain signals. The small feature sizes of the probes should prevent any decay in performance that is typically seen with larger repositionable neural probes.

To our knowledge, design and in vivo testing of such small (<15-20 µm) repositionable neural probes has not been attempted before, primarily because probes with small features are more flexible and difficult to push through brain tissue without buckling. To tackle this issue, we have designed a new device with very small-sized tips supported by a larger repositionable shuttle structure, as described in the following section.

1.6.2 Proposed approach

Our approach is summarized in Figure 1-15 below. The device envisioned consists of a thin probe whose body can be deflected and locked with a glue prior to insertion, thereby storing mechanical energy in the legs (Figure 1-15a). After inserting into the brain and waiting for the initial glial sheath to form (Figure 1-15b), the device can be deployed by dissolving the glue (Figure 1-15c). This causes the recording tip of the device to penetrate into fresh tissue. It is hypothesized that designing the tip dimensions to be small (7-20 µm) should mitigate formation of an additional glial sheath post-deployment and thus improve chronic recording.
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Figure 1-15 Device operation schematic. (a) The probe bodies are deflected and glued prior to insertion into the brain. (b) A glial sheath forms over time (~weeks) due to initial surgical trauma. (c) Dissolving the glue triggers the probe body to deflect the tips carrying recording electrodes into fresh tissue. Small tip dimensions prevent subsequent glial sheath formation.

1.7 Thesis organization

The design and fabrication of the probes were performed iteratively, with each subsequent generation of devices improving upon the previous one. A set of larger-scale prototypes (hereafter referred to as “Gen 0 devices”) was first machined (chapter 2) as a proof-of-concept to validate the idea that enough mechanical energy can be stored in deflected polyimide beams to elastically move a shuttle structure back and forth, and that such a shuttle structure can be held back in place with a dissolvable/meltable glue of some kind. The first generation of true-to-scale devices (hereafter referred to as “Gen 1 devices”) were then microfabricated (chapter 3), to demonstrate acute in vivo functionality. Based on what was learned from benchtop and in vivo studies with these devices, a second generation of devices (hereafter referred to as “Gen 2 devices”) were then designed and microfabricated (chapter 4) to test integrated triggering+recording in an in vivo setting. These Gen 2 devices included numerous fabrication and packaging improvements over the previous generation. Finally, a third generation of devices (hereafter referred by “Gen 3 devices”) were designed and fabricated (chapter 5), for use in chronic in vivo studies. These devices had smaller electrode tips (10 μm vs. 20 μm for Gen 1 and Gen 2), integrated resistive heaters for on-demand device deployment (Gen 1 and Gen 2 used passive deployment), integrated capacitive sensors for sensing device deployment, and a new packaging that allowed for visually confirming deployment as well as multiplexing devices to increase the number of electrode tips per packaged device.
This chapter describes the Gen 0 devices, which were millimeter-scale lasercut prototypes designed to test the basic working principles of the device concept described in section 1.6. Two kinds of Gen 0 devices were machined:

1. Test structures to mimic the device tip (Figure 2-1a), to verify the ability of the device tips to pierce through brain tissue without buckling.
2. Prototypes of the device shuttle and legs (Figure 2-1b), as proof-of-concept that the device legs can elastically store and release energy to move the central shuttle and tip back and forth.

This chapter will discuss the relevant models (section 2.1) that were used to guide Gen 0 device design, the proposed device dimensions and model predictions (section 2.2), the process used to manufacture these devices (section 2.3) and benchtop characterization of the devices (section 2.4). Section 2.5 summarizes the lessons learned from the Gen 0 devices and how that informs the design of the microfabricated devices that will be described in chapters 3, 4 and 5.

### 2.1 Modeling

Structural mechanics theory was used to model the behavior of the device tip and legs. Note that these structural models are applicable to the prototypes (Gen 0), as well as to the microfabricated devices (Gen 1, Gen 2, and Gen 3). Predictions from these models were first used to determine tentative dimensions for the device legs and tips in the final microfabricated devices. Gen 0 prototypes were then designed to test the model predictions with easier-to-manufacture millimeter-scale prototypes. Based on results from the Gen 0 device tests (section 2.4), dimensions of the first microfabricated Gen 1 devices were finalized. Gen 2 and Gen 3 devices were similarly designed considering the structural modeling theory in conjunction with test results from each previous device generation.

There are three important structural considerations for the device:
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1. The device tip needs to be stiff enough to pierce through the brain tissue and through the resulting glial sheath without buckling.
2. The device legs need to be stiff enough to store enough energy that, upon deployment, the device delivers enough force to pierce through glial sheath.
3. The device legs need to be flexible enough to allow for a tip travel distance greater than the expected thickness of the glial sheath.

While the mechanical properties of a glial sheath have not been extensively studied, previous in vivo studies with repositionable probes can be used as a benchmark for the order of magnitude of force required to pierce through the glial sheath. For example, Muthuswamy’s group has fabricated devices that push through brain tissue 2 days post-implantation by delivering 100s of μN of force [102]. Given that these devices pierce through glial sheaths that are 2 days old, whereas the devices in this work are designed to pierce a glial sheath that forms over a few weeks, we need to add in a reasonable factor of safety. For the analytical models used here, it is assumed that a force of a few mN should be sufficient to pierce through the resulting glial sheath.

Therefore, the three constraints above can be rewritten as:

1. Predicted buckling force for device tip > few mN (force needed to pierce glial sheath)
2. Force delivered by the deflected device legs > few mN (force needed to pierce glial sheath)
3. Maximum stress induced in device < failure stress of material
4. Device deflection > 50 μm (assumed thickness of glial sheath)

A beam buckling model (section 2.1.1) was used to inform a feasible design space that would satisfy the first constraint above; whereas, elastic beam theory (section 2.1.2) was used to inform a feasible design space that would satisfy the second, third, and fourth constraints above.

2.1.1 Tip Buckling

Mechanical buckling theory was used to ensure a tip geometry that can pierce through the brain tissue and the glial sheath without buckling. The theoretical buckling force $F_{\text{buckling}}$ for a slender column is given by [106]

$$F_{\text{buckling}} = \frac{\pi^2 EI}{(KL_{\text{tip}})^2},$$

Equation 2.1

where $E$ is the Young’s modulus, $I$ is the moment of inertia, $L_{\text{tip}}$ is the length of the device tip, and $K$ is the boundary condition factor. This formula assumes a uniform moment of inertia through the entire length of the beam. The moment of inertia for a beam is given by

$$I = \int \int y^2 dx dy,$$

Equation 2.2

where $y$ is the distance from the neutral axis (in the direction of bending). For a beam with a uniform rectangular cross-section, where $dx$ goes from $-b_{\text{tip}}/2$ to $b_{\text{tip}}/2$ and $dy$ goes from $-h_{\text{tip}}/2$ to $h_{\text{tip}}/2$, equation 2.2 becomes
where $b_{\text{tip}}$ is the breadth of the device tip and $h_{\text{tip}}$ is the height of the device tip. Note that this assumes buckling in the direction of $h_{\text{tip}}$, which is the case when $h_{\text{tip}} < b_{\text{tip}}$. In the case that $b_{\text{tip}} < h_{\text{tip}}$, the tip will buckle first in the direction of $b_{\text{tip}}$ and the smaller moment of inertia will be given by

$$I = \frac{h_{\text{tip}} b_{\text{tip}}^3}{12}.$$  

Equation 2-3

Equation 2-4

For simplifying the analysis, the device tip is modeled as a fixed-free beam, for which the value of $K$ is 2. The assumption of a fixed end is a simplifying approximation that underestimates the force needed to buckle the tip, since the boundary conditions are closer to that of a pinned-free beam (for which the value of $K$ is 1). Hence, the actual device will likely be harder to buckle than estimated by this model, adding in an extra factor of safety in the buckling model predictions.

There are two points where the device tip is most likely to buckle. The device tip can either buckle at the point where the narrow region of the tip merges with the larger conical region, as shown in Figure 2-2, or it can buckle at the point where the device tip region intersects the device legs, as shown in Figure 2-3. Buckling force predictions are made for each of these two cases, in order to estimate the potential range of forces for which the device might buckle. For the first case, the area of the beam is constant over the length $l_{\text{tip,1}}$, but in the second case, the beam tapers down to a smaller cross-sectional area over its length $l_{\text{tip,2}}$. To simplify the analysis, the tip in case 2 is also assumed to have a uniform rectangular cross-section (equal to the smallest cross-section at the very tip), throughout the length $l_{\text{tip,2}}$. This approximation underestimates the buckling force by underestimating the effective moment of inertia; the actual device tips will therefore be harder to buckle than predicted by the analysis in case 2 in Figure 2-3. This adds in an extra factor of safety to the buckling predictions of case 2.

Figure 2-2 Case 1 – Upper limit for the tip buckling force.
2.1.2 Induced stress and force delivered by the deflected legs

Beam theory was used to inform optimal device leg materials and dimensions, in order to balance the force and stress design constraints discussed at the beginning of this chapter. Each device leg was modeled as a rectangular linear elastic beam with fixed-fixed boundary conditions, as shown in Figure 2-4 below.

From beam theory for linear elastic materials [106], the force $F$ required to deflect such a beam by an amount $\delta$ is given by

$$F = \frac{Ehb^3}{l^3} \delta,$$

Equation 2-5
where \( b, h \) and \( l \) are the breadth, height and length respectively of a device leg. Note that this assumes pure bending; this assumption will be examined later in this section.

The maximum stress \( \sigma_{\text{max}} \) induced in the device legs due to the deflection \( \delta \) is then given by

\[
\sigma_{\text{max}} = \frac{3Eb}{l^2} \delta. \tag{2.6}
\]

### Optimizing leg dimensions (using equations 2.5 and 2.6)

The device leg dimensions need to be optimized such that they can deliver a high-enough force (greater than a few mN) to pierce through the glial sheath, yet incur a stress less than the failure stress of the material being used. If we divide equation 2.5 by equation 2.6, we get

\[
\frac{F}{\sigma_{\text{max}}} = \frac{hb^2}{3l}. \tag{2.7}
\]

Thus, maximizing the force delivered by the device while minimizing the stress induced in the device legs requires a thick and wide device (maximize \( h, b \)) with short legs (minimize \( l \)). Moreover, increasing \( b \) is much more effective than increasing \( h \) or decreasing \( l \) (since the quantity scales with the square of \( b \) vs. linearly with the other two variables).

#### Maximizing \( b \)

If the leg width \( b \) becomes too much larger than the leg height \( h \), then it becomes much easier for the device to bend out-of-plane in the direction of \( h \), instead of in-plane in the direction of \( b \), as is being assumed. For a device where \( b \gg h \), any small out-of-plane perturbation would cause the device to deflect in the \( z \)-plane (instead of in the \( xy \)-plane) during the device priming step. This would make priming the device challenging. Therefore, \( b \) is assumed to be on the same order of magnitude as \( h \); maximizing \( h \), therefore, also indirectly maximizes the practical value that can be achieved for \( b \).

#### Maximizing \( h \)

While a larger device leg height \( h \) would help optimize over the force delivered and induced stress constraints (equation 2.7), there is an upper limit to the height \( h \). The device leg height is the same as the device tip height (since the devices are linearly uniform). Since the device is intended to test the hypothesis that electrodes with small features (7-20 \( \mu \)m) will elicit a smaller glial reaction (section 1.6), the height of the device tip cannot be more than 20 \( \mu \)m. Therefore, the dimension \( h \) cannot be greater than 20 \( \mu \)m.

Coincidentally, for the chosen fabrication process (section 3.3.1) and device material (section 2.1.2.1), the upper limit on the thickness of the devices is also approximately 20 \( \mu \)m.

#### Minimizing \( l \)

While minimizing the device leg length \( l \) would help optimize over the design constraints (equation 2.7), there is a lower limit to how small this dimension can become. For the linearly elastic pure bending
approximation to hold, the length of the beam should be greater than approximately 10 times either of the cross-sectional dimensions. Therefore, if the device legs have a 10 μm X 10 μm cross-section, then the device legs need to be at least 100 μm long. Similarly, if the device legs have a 20 μm X 20 μm cross-section, then the device legs need to be at least 200 μm long. The pure bending approximation will be examined in a later section.

2.1.2.1 Structural material choice – polyimide
In addition to satisfying the structural considerations described in the preceding sections, the device material needs to be biocompatible, as well as compatible with the chosen method of manufacturing. Since the device is designed to be a multi-layered device with microscale dimensions (10-20 μm tips, with possibly even smaller dimensions for some of the electrode sites and other features), it was decided to manufacture the device using microfabrication facilities. Thus, all device materials needed to be compatible with standard microfabrication process as well.

Some MEMS materials, such as silicon, silicon nitride, or silicon oxide, offer a high Young’s modulus $E$, allowing for higher storage of energy in the device legs for any given deflection distance; however, these materials can be brittle and easy to fracture. Others, such as polyimide or parylene, are more flexible, but can store less energy. Ultimately, each material will have trade-offs regarding biocompatibility, ease-of-fabrication, ability to store energy in deflected structure, and the resistance to failure at the required deflections. Figure 2-5 and Figure 2-6 show example tradeoffs between the force stored in the deflected device legs and the induced stress in these structures, for three different values of Young’s modulus – 100 GPa (range of $E$ for metals and silicon), 1 GPa (range of $E$ for stiff polymers like polyimide and parylene), and 100 MPa (range of $E$ for very flexible polymers). While materials with a higher Young’s modulus can deliver a higher force for a given leg length and deployment, they also experience higher induced stresses.
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Figure 2-5 Force delivered per device leg as a function of leg length. The device leg dimensions were assumed to be $h = 20 \, \mu m$, $b = 20 \, \mu m$, and the device deflection $\delta$ was assumed to be $80 \, \mu m$.

Figure 2-6 Maximum induced stress in the device legs as function of leg length. The device leg dimensions were assumed to be $h = 20 \, \mu m$, $b = 20 \, \mu m$, and the device deflection $\delta$ was assumed to be $80 \, \mu m$. 
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The structural material for the device was ultimately chosen to be polyimide for the following reasons:

1. The Young’s modulus and yield strength are such that the desired force and stress considerations require shorter minimum leg lengths (100 μm vs. 850 μm for silicon, for example). This allows for smaller device dimensions, which translates to smaller and more compact implant packages.

2. The failure mode for a polymer, such as polyimide, is yielding by plastic deformation. This is a more tolerable mode of failure than brittle fracture, which is common for some MEMS materials, such as silicon.

3. Polyimide is fairly biocompatible and a commonly used material for neural probes.

4. Polyimide is compatible with the microfabrication process.

2.1.2.2 Choosing the optimal leg length

Figure 2-7 shows example model results for polyimide informing the appropriate length of the device legs, given the tradeoff between the mechanical stress induced in the deflected device legs and the total force that can be delivered by the device. Table 2-1 lists the feasible design space for the leg length when the device thickness is varied between 10 μm and 20 μm, the device width is varied between 10 μm and 20 μm, and the leg deflection is varied between 50 μm and 80 μm, to simulate the range of possible leg geometry variations in the three microfabricated device generations in chapters 3, 4, 5.

![Figure 2-7 Plot of analytically calculated force delivered by the device (left) and the maximum stress in the device legs (right) as a function of leg length for a leg width of 20 μm, a leg thickness of 20 μm, and a deflection distance of 50 μm. The requirement that the force delivered be ≥ 1 mN (design criteria) and the maximum stress be ≤ 70 MPa (yield stress of polyimide) informs a design space for the leg length shown by the red and blue arrows on the x axis.](image-url)
Table 2-1 Feasible design space for the device leg length satisfying the criteria that maximum induced stress in the legs < 70 MPa and the force delivered by each leg > 10 μN.

<table>
<thead>
<tr>
<th>Leg width (μm)</th>
<th>Leg thickness (μm)</th>
<th>Deflection (μm)</th>
<th>Minimum leg length (μm)</th>
<th>Maximum leg length (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>20</td>
<td>50</td>
<td>330</td>
<td>1260</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>80</td>
<td>420</td>
<td>1470</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>50</td>
<td>240</td>
<td>500</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>80</td>
<td>300</td>
<td>580</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>50</td>
<td>330</td>
<td>1000</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>80</td>
<td>420</td>
<td>1160</td>
</tr>
</tbody>
</table>

2.1.2.3 Model assumptions and other considerations

Below is a discussion of the key assumptions used in equations 2.5 and 2.6, and the implications of these assumptions on the model predictions.

Assumption 1: Small deflections and the pure-bending assumption

In general, a deformed beam can have two sources of strain energy – bending and stretching. When deflections are small (usually less than the beam thickness), the strain energy in the beam is dominated by bending, and stretching can be neglected. Note that equations 2.5 and 2.6 above assume “small deflection” and pure bending (neglecting stretching). As will be seen in later sections, the device designed here encounters deflections that can be up to 4-5 times the beam thickness. Therefore, the small-deflection beam theory analysis and the pure bending approximation will not strictly hold. Nonetheless, it is a useful way to gain insights into the analytical relationships between the various design parameters in order to optimize over the various design constraints.

In reality, the force stored in the legs will be higher than predicted by equation 2.5 because the pure-bending beam equation neglects forces that arise due to beam stretching. If we wanted to account for stretching as well, an energy methods analysis would need to be performed iteratively [81], where a trial function is assumed to model the shape of the beam deflection. Note that this method is very sensitive to the particular trial function used – a poor trial function can yield an inaccurate result. This method is, therefore, used below only to compare the effects of stretching vs. bending, rather than to predict the actual beam load-deflection behavior.

The beam deflection was first assumed to have a certain trial function that models the shape of the device leg deflection (Figure 2-8). The trial function w(x) was assumed to be as sinusoidal function as follows:

\[ w = \delta/2 \times \left( 1 - \cos \frac{\pi x}{l} \right). \]  

Equation 2-8
Figure 2-8 Trial function to model the beam deflection behavior.

The total axial strain $\varepsilon_T$ in the beam is given by

$$\varepsilon_T = \varepsilon_{bending} + \varepsilon_{stretching}.$$  \hspace{1cm} \text{Equation 2-9}

where $\varepsilon_{bending}$ is the axial strain due to pure bending, and $\varepsilon_{stretching}$ is the axial strain due to stretching. These components are further defined as follows:

$$\varepsilon_{bending} = -z \frac{d^2 w}{dx^2}, \text{ and}$$  \hspace{1cm} \text{Equation 2-10}

$$\varepsilon_{stretching} = \frac{1}{l} \int_0^l \left( \frac{d w}{dx} \right)^2 dx,$$  \hspace{1cm} \text{Equation 2-11}

where $z$ is the distance from the neutral axis of the beam.

Substituting the trial function from equation 2.8 into equations 2.9, 2.10 and 2.11,

$$\varepsilon_T = -\frac{\pi^2}{2} \cos \left( \frac{\pi x}{l} \right) \left( \frac{E}{l^2} \right) \delta + \frac{\pi^2}{16} \left( \frac{E}{l^2} \right) \delta^2.$$  \hspace{1cm} \text{Equation 2-12}

The total strain energy for a beam is given by

$$W = \frac{EW}{2} \iint \varepsilon_T^2 dz dx.$$  \hspace{1cm} \text{Equation 2-13}

where $z$ goes from $-b/2$ to $b/2$ and $x$ goes from 0 to $l$.

From equation 2.12 and 2.13, the total strain energy $W$ becomes

$$W = \left( \frac{\pi^4}{192} \right) \left( \frac{E h b^4}{l^3} \right) \delta^2 + \left( \frac{\pi^4}{512} \right) \left( \frac{E h b}{l^3} \right) \delta^4.$$  \hspace{1cm} \text{Equation 2-14}

The work done by the external force is simply $F\delta$, hence the total potential energy is given by

$$U = W - F\delta.$$  \hspace{1cm} \text{Equation 2-15}
Taking the derivative of the total potential energy $U$ with respect to $\delta$ and setting the result to zero, gives the load-deflection characteristic of the beam to be

$$F = \frac{\pi^4}{96} \left( \frac{Ebh}{l^3} \right) \delta + \frac{\pi^4}{128} \left( \frac{Ebh}{l^3} \right) \delta^3.$$  \hspace{1cm} \text{Equation 2-16}

The first term in the above equation comes from small-deflection pure bending, while the second term comes from stretching. The numerical factors in front of each of the two terms result from the particular trial function used earlier and, therefore, might not be completely accurate. However, equation 2.16 does provide insights into the dependencies of the beam load-deflection behavior on the beam dimensions and material properties. For example, ignoring the numerical pre-factors and comparing the component of the force due to bending to the component of the force due to stretching, we get,

$$\frac{F_{\text{bending}}}{F_{\text{stretching}}} \propto \left( \frac{b}{\delta} \right)^2.$$  \hspace{1cm} \text{Equation 2-17}

Typical device legs that are 20 $\mu$m wide and undergo deflections of 80 $\mu$m can, therefore, can have forces due to stretching that are 16 times higher than forces due to bending. This is, of course, neglecting the numerical pre-factors in equation 2.16, which are highly sensitive to the particular trial function used, and can modify this result by some amount. Nonetheless, the preceding discussion serves to underscore the importance of stretching, which has been neglected in equations 2.5 and 2.6 that are used (for ease of analysis) to optimize the device dimensions. In reality, the device legs can likely deliver a larger force than predicted by our models.

The axial stress induced in the legs due to the axial strain is given by Hooke’s Law as

$$\sigma = E\epsilon_\ell.$$  \hspace{1cm} \text{Equation 2-18}

Substituting equation 2.12 in equation 2.18, the axial stress induced in the beam is given by

$$\sigma = -\frac{\pi^2}{2}\cos\left(\frac{nx}{l}\right) \left( \frac{E^2 \ell}{l^2} \right) \delta + \frac{\pi^2}{16} \left( \frac{E^2 \ell}{l^2} \right) \delta^2,$$  \hspace{1cm} \text{Equation 2-19}

where the first term is the stress induced due to pure bending, and the second term is the stress induced due to stretching. The maximum value of the bending stress occurs at $\ell = 0$ and $\ell = l$ when the cosine function in the equation has a value of 1, and at the maximum value of $z$ which is $b/2$. The ratio of the maximum bending stress, $\sigma_{\text{bending,max}}$ to the stretching stress $\sigma_{\text{stretching}}$ is, therefore, given by

$$\frac{\sigma_{\text{bending,max}}}{\sigma_{\text{stretching}}} \propto \frac{b}{\delta}.$$  \hspace{1cm} \text{Equation 2-20}

Typical legs that are 20 $\mu$m wide and undergo 80 $\mu$m of deflection could experience stresses due to stretching that are 4 times higher than stresses due to pure bending. The specific numerical prefactors, which are neglected in the equation above, and which are highly sensitive to the choice of trial function used, might affect this ratio by some amount. Nonetheless, equation 2.20 gives us a similar insight as equation 2.17 did previously – the effects of stretching are important to consider in our design. This
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indicates that our stress analysis may substantially underestimate the total stress, thereby necessitating a reasonable factor of safety to be included when using the stress predictions of our models.

Assumption 2: Neglecting plastic deformations

The discussion so far has assumed fully elastic deformations. If the device legs experience stresses higher than the yield stress of the material being used, they will deform plastically. Benchtop experiments with the devices (sections 2.4.2, 3.4.2, 4.4.2, and 5.4.2.2), however indicate minimal plastic deformation, if any, during the triggering tests. The elastic deformation assumption for our models is, therefore, valid.

Stress induced in other areas of the device

The stress predicted by equation 2.6 only predicts the stress in the deflected device legs. Other areas of the device might also experience high stresses, however. A particular area of concern is the device head structure (Figure 2-9) during the triggering process. As will be seen in later chapters, the device is “primed” by hooking a micromanipulator or small pin into the hole structure in the device head and pulling it back to deflect the device legs. During this process, the device head area can experience significant stresses. A fairly common mode of failure during the priming process was the tearing of the device in the head area, likely because of high stresses in this region.

![Figure 2-9 Schematic of the device head during device priming.](image)

COMSOL® models

Finite element modeling using COMSOL® was also used to double-check the stress analysis above. For a 25 µm wide by 25 µm thick by 1 mm long device leg, the force due to a 200 µm deflection of is predicted by equation 2.5 to be 195 µN, and the maximum stress induced in the leg is predicted by equation 2.6 to be 37.5 MPa. A sample COMSOL® simulation (Figure 2-10) with these same leg dimensions and deflection values predicts the maximum induced stress in the device legs to be 36.25 MPa, which agrees well with the bending stress of 37.5 MPa predicted by our analytical model. Note that the COMSOL model (similar to equation 2.5 and 2.6) uses small-deflection pure bending theory in its constitutive
equations; the effects of stretching, therefore, still need to be accounted for by adding in appropriate factors of safety in the design.

Figure 2-10 COMSOL model showing the stress induced when a central polyimide shuttle structure supported by 10 legs per side is deflected by 200 µm. The shuttle is 1 mm X 2.5 mm and each leg is 25 µm wide by 25 µm thick by 1 mm long. The model predicts a maximum stress induced in the legs to be 36.25 MPa.

2.2 Proposed design and model predictions
This section describes the proposed dimensions and corresponding structural model predictions for the two types of Gen 0 test structures described in Figure 2-1.

2.2.1.1 Tip test structures
Tip test structures (Figure 2-11) were designed and manufactured as an indirect method to probe the force required to pierce brain tissue – by pushing increasingly floppier tips into brain tissue (section 2.4.1), we can determine the tip that just pierces through brain tissue. The theoretical buckling force for this tip (section 2.1.1) is then the force required to pierce through the brain tissue.
As discussed in the beginning of section 2.1, the force required to pierce through brain tissue is expected to be on the order of a few mN. A device tip with a 0.9 mN predicted buckling force was first manufactured, and upon successful piercing of brain tissue (section 2.4.1), increasingly floppier tips were manufactured and tested, until the tips started buckling. Table 2-2 lists the dimensions of the test structures (widths varying from 60 μm to 450 μm, and lengths varying from 1.3 mm to 28 mm) with a range of predicted buckling forces (0.9 mN to 0.03 mN).

Table 2-2 Proposed tip dimensions for some of the 25 μm thick Gen0 tip test structures and corresponding buckling force predictions (based on equation 2.1).

<table>
<thead>
<tr>
<th>Tip length $l_{tip}$ (mm)</th>
<th>Tip width $w_{tip}$ (μm)</th>
<th>Predicted buckling force (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>450</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>0.5</td>
</tr>
<tr>
<td>1.3</td>
<td>60</td>
<td>0.29</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>0.2</td>
</tr>
<tr>
<td>10</td>
<td>250</td>
<td>0.02</td>
</tr>
<tr>
<td>17</td>
<td>300</td>
<td>0.008</td>
</tr>
<tr>
<td>28</td>
<td>300</td>
<td>0.003</td>
</tr>
</tbody>
</table>

2.2.1.2 Moveable device shuttle structures
Shuttle structures (Figure 2-12) were also machined to test if the device legs can withstand the stresses induced when deflected by ~100 μm and if they undergo mostly elastic deformation.
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Moveable shuttle     Device legs

Hole for hook to pull shuttle back

Figure 2-12 Schematic of Gen 0 device shuttle test structures.

Three such millimeter-scale prototypes of the device concept were manufactured. Since the smallest features that can be reliably made by the laser ablation method (section 2.3) used for making these is 50 μm, the leg widths for these Gen 0 prototypes were designed to be 50 μm (Table 2-3). This is slightly scaled-up from the final microfabricated devices (Gen 1, Gen 2, and Gen 3), which are designed to have legs widths of 10-20 μm. Devices A and B were designed to have legs that would have predicted induced stresses of 38 MPa when deflected by 100 μm (the yield stress for polyimide is ~70 MPa). Upon successful benchtop tests with devices A and B, a third device C was also made with smaller overall dimensions, and with a stiffer device leg geometry that would incur high stresses upon deflection, but would also be capable of delivering higher forces.

Table 2-3 Proposed dimensions for 25 μm thick shuttle test structures and corresponding force and stress predictions (based on equations 2.5 and 2.6) for a 100 μm leg deflection.

<table>
<thead>
<tr>
<th>Device</th>
<th>Leg width (μm)</th>
<th>Leg length (mm)</th>
<th>Number of legs per shuttle</th>
<th>Maximum stress induced (MPa)</th>
<th>Force per leg (mN)</th>
<th>Total force (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50</td>
<td>1</td>
<td>20</td>
<td>38</td>
<td>0.8</td>
<td>16</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>1</td>
<td>40</td>
<td>38</td>
<td>0.8</td>
<td>32</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>0.25</td>
<td>40</td>
<td>600</td>
<td>50</td>
<td>1000</td>
</tr>
</tbody>
</table>

2.3 Fabrication

All test structures were laser-cut from 25 μm thick DuPont™ Kapton® HN polyimide film (Grainger catalog # 15CS14) using a Resonetics® LPX 200 KrF laser (wavelength = 248 nm, spot size = 10-200 μm, energy per pulse = 200 mJ, firing rate = 1-100 Hz) in MIT’s Microsystems Technology Laboratory (MTL). Note that these Gen 0 test structures were polyimide-only structures, without any metal electrode layer.

A sample laser-cut tip test structure is shown in Figure 2-13 below.
The three devices to test the moveable shuttle concept are shown in Figure 2-14, Figure 2-15, and Figure 2-16 below. Device A in Figure 2-14 has a central shuttle supported by 10 legs on each side, with each leg designed to have a length of 1 mm, a width of 50 μm, and an inter-leg distance of 500 μm. Device B in Figure 2-15 is identical to device A, with the addition of a tip at the end of the shuttle, a reduced inter-leg distance of 300 μm, and 20 legs per side instead of 10. Both devices have a triangular hole on the shuttle, which is intended to serve as a space for a micromanipulator tip or any other hook-like structure to hook into during the device priming step. Device C in Figure 2-16 was designed to have smaller dimensions compared to devices A and B. Note that the picture of device C was taken after a benchtop test that resulted in tearing of the device head during the priming process.
Figure 2-15 Image of Gen 0 device B. The shuttle is supported by 20 legs per side that are designed to be 1 mm long and 50 μm wide with an inter-leg distance of 300 μm.

Head structure ruptured during benchtop trigger test

Figure 2-16 Image of Gen 0 device C. The shuttle is supported by 20 legs per side, with each leg designed to be 250 μm long and 50 μm wide.

An artefact of the laser ablation process was undesired waviness on some straight edges, or corners having additional undesired etching (Figure 2-17).
Figure 2-17 Microscope picture of the leg area of Device A, showing undesirable artefacts of the laser ablation manufacturing process.

2.4 Benchtop characterization

2.4.1 Tip buckling tests

Benchtop tests with the tip test structures were aimed at getting insights into two areas:

1. What is the force required to pierce brain tissue?
2. Can microscale polyimide test structures be designed such that they can deliver this force without buckling?

Both the questions above were simultaneously investigated with the tests described in this section. The benchtop setup for these tests is shown in Figure 2-18 below. Freshly sacrificed rat pup brains (Figure 2-18a), were placed in front of a metal support taped down to an underlying substrate (Figure 2-18b). The majority of the tests were performed with whole brains (without nicking the brain membranes or cutting the brain tissue); however, for some of the longer and floppier test structures, the brains were either sliced in a coronal plane (as shown in Figure 2-18b), or the dura and pia were nicked. Tip test structures were taped onto a needle attached to a syringe taped down onto a moveable platform that
could be moved back and forth using a micromanipulator (Figure 2-18c). The tip structures were then pushed into the brain tissue to test if the structures could pierce brain tissue without buckling.

Figure 2-18 Test setup for testing the Gen 0 tip test structures. (a) Freshly sacrificed rat pup brains in saline. (b) Portion of the rat pup brain sliced in a coronal plane, and placed in front of a metal support structure. (c) Tip test structure taped onto a needle and syringe setup, and attached to a base moveable with a micromanipulator.

The dimensions for the various structures tested are listed in Table 2-4 below, along with their predicted buckling force and the result of the piercing tests. The structures are listed in the table in decreasing order of their predicted buckling force. Buckling is observed for structures that have a predicted buckling force of approximately 8E-3 mN. Unless otherwise noted, the piercing results indicate piercing the brain tissue with all brain membranes intact.

Table 2-4 Dimensions and buckling force predictions (based on equation 2.1) for Gen0 tip test structures.

<table>
<thead>
<tr>
<th>Tip length (mm)</th>
<th>Tip width (μm)</th>
<th>Predicted buckling force (mN)</th>
<th>Did the device pierce the rat pup brain?</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>450</td>
<td>0.90</td>
<td>Pierce</td>
</tr>
<tr>
<td>2</td>
<td>350</td>
<td>0.70</td>
<td>Pierce</td>
</tr>
<tr>
<td>2</td>
<td>320</td>
<td>0.64</td>
<td>Pierce</td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>0.60</td>
<td>Pierce</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>0.50</td>
<td>Pierce</td>
</tr>
<tr>
<td>1.5</td>
<td>140</td>
<td>0.50</td>
<td>Pierce</td>
</tr>
<tr>
<td>2</td>
<td>180</td>
<td>0.36</td>
<td>Pierce</td>
</tr>
<tr>
<td>2.16</td>
<td>200</td>
<td>0.34</td>
<td>Pierce</td>
</tr>
<tr>
<td>1.3</td>
<td>60</td>
<td>0.29</td>
<td>Pierce</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>0.18</td>
<td>Pierce</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>0.18</td>
<td>Pierce</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>0.18</td>
<td>Pierce</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>0.18</td>
<td>Pierce</td>
</tr>
</tbody>
</table>
2.4.2 Testing of millimeter-scale laser-cut polyimide shuttle prototype

Benchtop tests with the shuttle structures described in section 2.3, were aimed at investigating three questions:

1. Can a device shuttle supported by polyimide leg structures be pulled back without breaking the legs?
2. Is there a biocompatible meltable glue that would be strong enough to hold the device in this primed position?
3. Upon melting or dissolution of this glue, can the device legs elastically deform back to their original shape in order to move the shuttle forward?

The benchtop test for Device A is shown in Figure 2-19. First, the device edges were taped onto a piece of paper to hold the device in place. The central shuttle was then manually pulled back by using a pin to hook into the triangular hole in the shuttle (Figure 2-19a). The device was held in this position by pushing the pin into the underlying paper. Some polyethylene glycol (PEG) (Sigma Aldrich®, molecular weight 1000) was then manually deposited around the shuttle, melted using a heatgun and then allowed to resolidify. The pin holding the shuttle in place was then removed, leaving the solidified PEG to now hold the shuttle in the primed position (Figure 2-19b). PEG, being water soluble, dissolves upon application of water (Figure 2-19c), and the shuttle moves forward as the device legs regain their original shape. Note from the image in Figure 2-19c that the device legs do not entirely straighten out to their original position, indicating some plastic deformation. This is not unreasonable given that the device legs were deformed by 150-200 μm (visual approximation based on Figure 2-19a), and the induced stress for a 200 μm deflection is 76 MPa (based on equation 2.6), which is higher than the polyimide yield stress of 70 MPa. Nonetheless, it is encouraging that a majority of the leg deflection was elastic and that the shuttle moved forward by at least approximately 100 μm upon triggering.
Figure 2-19 Device A triggering pictures. (a) Device being pulled back by pin, by approximately 150 to 200 μm. (b) Device held back in place with polyethylene glycol. (c) PEG dissolved upon application of water, releasing the elastic energy stored in the deflected legs and causing the shuttle to move forward.

The benchtop test for device B is shown in Figure 2-20. The device was primed in a similar fashion to device A above, but was triggered by heating the PEG instead of dissolving it with water. Figure 2-20a shows the device being pulled back by approximately 200 μm (based on visual estimation). Upon heating with a heat gun, the PEG melts and the shuttle moves forward by 100-150 μm (Figure 2-20b). Note the slight plastic deformation of the device legs in this case as well.

Figure 2-20 Device B triggering. (a) Device in the primed position with PEG holding the device back. (b) Device in the deployed position with PEG melting upon application of heat.

The head structure in device C tore off during the priming process (likely due to the induced stresses exceeding the failure stress of polyimide), as can been seen in Figure 2-16. Hence, no benchtop triggering tests could be performed on this device. The device failure was expected given the very high predicted induced stresses for this device (Table 2-3).
Chapter 2: Gen 0

2.5 Conclusions

The main goal of Gen 0 devices was to de-risk the critical parts of the device concept, namely the ability of polyimide tips to pierce brain tissue without buckling and the viability of the moveable shuttle concept.

The conclusions drawn from the tip buckling tests (section 2.4.1) are that the force required to pierce freshly sacrificed rat pup brains must be approximately $8 \times 10^{-3}$ mN for the tip geometries that were used in these tests. While adult live rat brains and glial sheaths might be stiffer than this, these tests give us a good sense of the orders of magnitude of force required to pierce brain tissue for tips that have geometries and dimensions similar to those of the tips used here. All our microfabricated devices are designed to deliver forces greater than a few mN, thereby incorporating an extremely large factor of safety that should offset the uncertainty in the actual force required to pierce through a live rat brain or through a glial sheath. Moreover, if device tips are designed to have predicted buckling forces much higher than $8 \times 10^{-3}$ mN (with a reasonable factor of safety), they should be able to pierce brain tissue without buckling. As will be seen later (Table 3-1, Table 4-1, Table 5-1), it is possible to design polyimide tips with microscale dimensions such that the predicted buckling forces are much higher than $8 \times 10^{-3}$ mN.

The conclusions that can be drawn from the shuttle tests (section 2.4.2) are that the polyimide shuttle can be pulled back without breaking the device legs, that a biocompatible glue such as PEG is strong enough to hold the device back in the primed position, and that the device deforms (mostly) elastically up to 100-200 μm of deflection. This de-risks the moveable shuttle concept idea.

Gen 0 devices, thus, paved the way for the microfabricated devices that will be described in chapters 3, 4, and 5.
Chapter 3: Gen 1

3 GEN 1

This chapter describes the Gen 1 devices, which were the first microfabricated batch of devices. Microfabrication was chosen as the manufacturing method for Gen 1, Gen 2, and Gen 3 devices because:

1. Microfabrication allows for easy integration of multi-layer geometry. Thus, the microfabricated devices can be made to incorporate a metal electrode layer sandwiched between two polyimide structural layers, with the ability to easily adjust the thicknesses and properties of each layer. A metal layer is critical, since these devices are intended to serve as electrodes.
2. Microfabrication allows for incorporation of finer microscale features, whereas features in the laser ablated devices were limited by the resolution (~50 μm) of the laser ablation system used, and were subject to undesirable structural artefacts as seen in Figure 2-17. Incorporation of small features (as small as 5-10 μm) is critical to testing the hypothesis of the thesis described in section 1.6.
3. Microfabrication allows for easier production of large quantities and variations of devices. For example, while each laser ablated test structure (such as the one shown in Figure 2-14) takes approximately 10-12 hours to manufacture, a batch of microfabricated devices could yield on the order of thousands of devices in a period of a couple weeks.

This chapter will discuss the relevant models (section 3.1) that were used to guide the Gen 1 device design, the proposed device dimensions and model predictions (section 3.2), the microfabrication process used to manufacture these devices (section 3.3), benchtop characterization of the device tips, the device shuttle structure and the electrode sites (section 3.4), and in vivo tests (section 3.5) in the rodent model.

3.1 Modeling

The theory used to model Gen 1 device legs and tips was the same as described in section 2.1. The only additional component in Gen 1 devices was the set of electrode sites.

3.1.1 Electrode site areas and impedance

A key factor that governs the spatial recording resolution of neural electrodes is the projected area of the electrode sites. However, there is a tradeoff between spatial recording resolution and the noise levels for neural electrodes. Larger-sized electrodes typically have a lower noise floor, but also a lower spatial resolution, and tend to be better at recording activity from populations of neurons instead of single neurons. Smaller-sized electrodes have higher noise floors, but also a higher spatial resolution. When in close proximity to a neuron, these smaller electrodes are very effective at recording isolated extra-cellular action potentials; however, they tend to be noisier if no neurons are present in very close proximity to the electrode sites. One way to ameliorate this issue is by surface treatments that increase the effective surface area of the electrodes sites, while maintaining a given projected area. Because electrode site impedance is dependent on the effective surface area and the spatial resolution is
dependent on the projected surface area, surface treatments can serve to reduce noise while maintaining the localized nature of small electrodes.

Existing neural electrode technologies were used as a benchmark to estimate the optimal electrode site areas for the Gen 1 probes. Electrode site areas in Gen 2 and Gen 3 were then fine-tuned based on the impedance testing results of the fabricated Gen 1 probes.

3.2 Proposed design and model predictions

Three styles of Gen 1 devices were fabricated (Figure 3-1) – larger-sized A-type devices, medium-sized B-type devices, and smaller-sized C-type devices. A-type devices have a larger hole (1 mm diameter) in the device head, making them easier to pull back during the priming step. They also have the lowest predicted induced stress and force delivered out of the three styles of devices (Table 3-3), thus trading a higher factor of safety in stresses induced for lower values of the force delivered. The B-type devices are smaller overall, and can deliver more force when deployed than the A-type devices. But this comes at the expense of higher induced stress. Moreover, they have a smaller device head, thereby requiring smaller micromanipulator tips to pull the device back during the priming step, making the priming step more challenging. C-type devices are the smallest of the three types, capable of delivering the most force and were fabricated as an extreme case in the very unlikely event that both the A-type and the B-type devices survived the priming step. The C-type devices did not end up being used for any tests.
3.2.1 Gen 1 – proposed tip dimensions and buckling model predictions

For each Gen 1 device, the tip region tapers to the final tip width, as shown below in Figure 3-2. Buckling models (section 2.1.1) are then used to predict the lower and upper bounds of the force that would be required to buckle this tip structure (Table 3-1). Benchtop tests with Gen 0 devices (section 2.4.1) indicated that forces required to pierce freshly sacrificed rat pup brains were on the order of a few μN. Gen 1 devices were therefore made so that the predicted buckling forces (Table 3-1) would be much higher (0.08 mN to 1.65 mN at the lower limit) than a few μN.
Figure 3-2 Typical tip region of the Gen 1 devices. Green lines indicate the edge of the polyimide structure, blue lines indicate the metal trace, and magenta lines indicate the exposed areas of the metal.

Table 3-1 Proposed tip dimensions for Gen1 devices and corresponding buckling force predictions for 20 μm thick devices, calculated using equation 2.1. The upper limit is calculated using \( l_{\text{tip},1} \) as the tip length, and the lower limit is calculated using \( l_{\text{tip},2} \) as the tip length. Actual dimensions may differ from these designed dimensions by a few μm due to microfabrication processing limitations.

<table>
<thead>
<tr>
<th>Device type</th>
<th>Tip length ( l_{\text{tip},1} ) (μm)</th>
<th>Tip length ( l_{\text{tip},2} ) (μm)</th>
<th>Tip width ( b_{\text{tip}} ) (μm)</th>
<th>Predicted buckling force - upper limit (mN)</th>
<th>Predicted buckling force - lower limit (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A20-E1</td>
<td>200</td>
<td>985</td>
<td>20</td>
<td>2.1</td>
<td>0.08</td>
</tr>
<tr>
<td>A20-E2A</td>
<td>200</td>
<td>985</td>
<td>20</td>
<td>2.1</td>
<td>0.08</td>
</tr>
<tr>
<td>A20-E2B</td>
<td>200</td>
<td>985</td>
<td>15</td>
<td>0.9</td>
<td>0.04</td>
</tr>
<tr>
<td>A20-E2C</td>
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<td>885</td>
<td>15</td>
<td>3.5</td>
<td>0.04</td>
</tr>
<tr>
<td>A40-E1</td>
<td>200</td>
<td>985</td>
<td>40</td>
<td>4.1</td>
<td>0.17</td>
</tr>
<tr>
<td>A40-E2A</td>
<td>200</td>
<td>985</td>
<td>30</td>
<td>3.1</td>
<td>0.13</td>
</tr>
<tr>
<td>A40-E2B</td>
<td>200</td>
<td>985</td>
<td>15</td>
<td>0.9</td>
<td>0.04</td>
</tr>
<tr>
<td>B20-E1</td>
<td>200</td>
<td>445</td>
<td>20</td>
<td>2.1</td>
<td>0.42</td>
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<tr>
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<td>445</td>
<td>20</td>
<td>2.1</td>
<td>0.42</td>
</tr>
<tr>
<td>B20-E2B</td>
<td>200</td>
<td>445</td>
<td>15</td>
<td>0.9</td>
<td>0.18</td>
</tr>
<tr>
<td>B20-E2C</td>
<td>100</td>
<td>345</td>
<td>15</td>
<td>3.5</td>
<td>0.29</td>
</tr>
<tr>
<td>B40-E1</td>
<td>200</td>
<td>445</td>
<td>40</td>
<td>4.1</td>
<td>0.83</td>
</tr>
</tbody>
</table>
3.2.2 Gen 1 – proposed electrode site dimensions

Two types of electrode site designs were proposed for Gen 1 device tips, as shown in Figure 3-3. E1-type tips had two small sites with approximately similar areas, whereas E2-type tips had a smaller site at the end of the device tip and a larger site farther up the device tip.

![Figure 3-3 Gen 1 electrode site variations. (a) E1-type tips. (b) E2-type tips. Green lines indicate the edge of the polyimide structure, blue lines indicate the metal trace, and magenta lines indicate the exposed areas of the metal.](image-url)

As mentioned in section 3.1.1, electrode site areas for Gen 1 are modeled on existing neural electrode technologies. Typical electrode areas for the Utah array are 750 µm² [60], for the Michigan probe are 170 to 700 µm², and for 50-µm-diameter microwires are ~2000 µm². Based on this, the smaller Gen 1 electrode sites were designed to be approximately 50 to 170 µm², whereas the larger electrode sites were designed to be approximately 650 to 750 µm² (Table 3-2).

Table 3-2 Proposed electrode site area variations for Gen 1 devices. Actual dimensions may differ from these designed dimensions by a few µm due to microfabrication processing limitations.

<table>
<thead>
<tr>
<th>Device type</th>
<th>Electrode site 1 area (µm²)</th>
<th>Electrode site 2 area (µm²)</th>
<th>Distance between electrode sites (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A20-E1</td>
<td>166</td>
<td>138</td>
<td>65</td>
</tr>
<tr>
<td>A20-E2A</td>
<td>166</td>
<td>637</td>
<td>261</td>
</tr>
<tr>
<td>A20-E2B</td>
<td>52</td>
<td>637</td>
<td>263</td>
</tr>
<tr>
<td>A20-E2C</td>
<td>52</td>
<td>637</td>
<td>163</td>
</tr>
<tr>
<td>A40-E1</td>
<td>102</td>
<td>138</td>
<td>65</td>
</tr>
</tbody>
</table>
3.2.3 Gen 1 – proposed leg dimensions and structural model predictions

Benchtop tests with Gen 0 devices (section 2.4.2) had indicated that devices with induced stresses of 38 MPa (Gen 0 devices A and B) were easy to prime, whereas the device with an induced stress of 600 MPa (Gen 0 device C) could not be primed without breaking. Therefore, there were three types of Gen 1 devices (Table 3-3) that were designed: A-type devices with induced stresses in the legs predicted to be 7.5-15 MPa, B-type devices with induced stresses in the legs predicted to be 188-375 MPa, and C-type devices with induced stresses predicted to be over 1 GPa. There was a high degree of confidence that the A-type devices would be easy to trigger, since their predicted induced stress (7.5-15 MPa) was less than the predicted induced stress (38 MPa) of the Gen 0 devices that were primed in Figure 2-19 and Figure 2-20. However, these A-type devices would also deliver the least amount of force during triggering and would be the largest of the three Gen 1 variations. There was less certainty regarding triggering ease for the B-type devices, since their predicted induced stress (188 MPa) was higher than that of the Gen 0 devices that were easily primed, but lower than that of the Gen 0 devices that could not be primed. However, these devices were smaller than the A-type devices, and would therefore have smaller implant packages, making them better candidates for in vivo testing. C-type devices had a very high value of predicted stress induced (> 1 GPa for a 50 μm device leg deflection), and were almost certain to fail during the priming step. However, they were fabricated to potentially implant in the unprimed configuration, since they would have the smallest implant package of the three Gen 1 device variations and the stiffest device tip.

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A40-E2A</td>
<td>166</td>
<td>716</td>
<td>318</td>
</tr>
<tr>
<td>A40-E2B</td>
<td>67</td>
<td>716</td>
<td>325</td>
</tr>
<tr>
<td>B20-E1</td>
<td>166</td>
<td>138</td>
<td>65</td>
</tr>
<tr>
<td>B20-E2A</td>
<td>166</td>
<td>751</td>
<td>235</td>
</tr>
<tr>
<td>B20-E2B</td>
<td>47</td>
<td>751</td>
<td>247</td>
</tr>
<tr>
<td>B20-E2C</td>
<td>47</td>
<td>751</td>
<td>147</td>
</tr>
<tr>
<td>B40-E1</td>
<td>166</td>
<td>138</td>
<td>65</td>
</tr>
<tr>
<td>B40-E2A</td>
<td>166</td>
<td>659</td>
<td>247</td>
</tr>
<tr>
<td>B40-E2B</td>
<td>32</td>
<td>659</td>
<td>264</td>
</tr>
<tr>
<td>C</td>
<td>28</td>
<td>38</td>
<td>53</td>
</tr>
</tbody>
</table>
Table 3-3 Proposed leg dimensions for Gen 1 devices and corresponding predictions of force delivered (calculated using equation 2.5) and maximum induced stress (calculated using equation 2.6), for 20 μm thick devices with a 50 μm leg deflection. Actual device dimensions may differ from these designed dimensions by a few μm due to microfabrication processing limitations.

<table>
<thead>
<tr>
<th>Device type</th>
<th>Leg width b (μm)</th>
<th>Leg length l (μm)</th>
<th>Number of legs per shuttle</th>
<th>Maximum induced stress (MPa)</th>
<th>Force per leg (mN)</th>
<th>Total force (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A20-E1</td>
<td>20</td>
<td>1000</td>
<td>40</td>
<td>7.5</td>
<td>0.02</td>
<td>0.8</td>
</tr>
<tr>
<td>A20-E2A</td>
<td>20</td>
<td>1000</td>
<td>40</td>
<td>7.5</td>
<td>0.02</td>
<td>0.8</td>
</tr>
<tr>
<td>A20-E2B</td>
<td>20</td>
<td>1000</td>
<td>40</td>
<td>7.5</td>
<td>0.02</td>
<td>0.8</td>
</tr>
<tr>
<td>A20-E2C</td>
<td>20</td>
<td>1000</td>
<td>40</td>
<td>7.5</td>
<td>0.02</td>
<td>0.8</td>
</tr>
<tr>
<td>A40-E1</td>
<td>40</td>
<td>1000</td>
<td>40</td>
<td>15</td>
<td>0.16</td>
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<td>0.16</td>
<td>6.4</td>
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<td>A40-E2B</td>
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<td>1000</td>
<td>40</td>
<td>15</td>
<td>0.16</td>
<td>6.4</td>
</tr>
<tr>
<td>B20-E1</td>
<td>20</td>
<td>200</td>
<td>40</td>
<td>188</td>
<td>2.5</td>
<td>100</td>
</tr>
<tr>
<td>B20-E2A</td>
<td>20</td>
<td>200</td>
<td>40</td>
<td>188</td>
<td>2.5</td>
<td>100</td>
</tr>
<tr>
<td>B20-E2B</td>
<td>20</td>
<td>200</td>
<td>40</td>
<td>188</td>
<td>2.5</td>
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</tr>
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<td>B20-E2C</td>
<td>20</td>
<td>200</td>
<td>40</td>
<td>188</td>
<td>2.5</td>
<td>100</td>
</tr>
<tr>
<td>B40-E1</td>
<td>40</td>
<td>200</td>
<td>40</td>
<td>375</td>
<td>20</td>
<td>800</td>
</tr>
<tr>
<td>B40-E2A</td>
<td>40</td>
<td>200</td>
<td>40</td>
<td>375</td>
<td>20</td>
<td>800</td>
</tr>
<tr>
<td>B40-E2B</td>
<td>40</td>
<td>200</td>
<td>40</td>
<td>375</td>
<td>20</td>
<td>800</td>
</tr>
<tr>
<td>C1</td>
<td>20</td>
<td>75</td>
<td>10</td>
<td>1333</td>
<td>47</td>
<td>474</td>
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<tr>
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<td>20</td>
<td>75</td>
<td>10</td>
<td>1333</td>
<td>47</td>
<td>474</td>
</tr>
</tbody>
</table>

3.3 Fabrication and packaging

The fabrication process flow for Gen 1 devices is described in section 3.3.1 below. The subsequent microfabricated devices (Gen 2 and Gen 3) also used a similar process with minor modifications as described in sections 4.3.1 and 5.3.1. Since Gen 1 was the first microfabricated generation, the detailed recipes for each step described in section 3.3.1 had to be developed from scratch. While some processes, such as etching alignment marks on silicon (step 1) are standard, some other processes, such as spinning, patterning, and curing the polyimide layers, needed significant recipe development.

3.3.1 Fabrication process flow

There are six major steps to the Gen 1 device microfabrication process flow (Figure 3-4).
Step 1: Etch alignment marks

The process starts with a clean single-side polished 6-inch diameter silicon wafer. A thin positive photoresist layer is then patterned onto the wafer and serves as a mask to etch alignment marks onto the underlying silicon wafer using a magnetically-enhanced reactive ion etcher (Applied Materials® Model P5000; Cl₂ flow rate: 20 sccm, HBr flow rate: 20 sccm; pressure: 20 mT, power: 350 W). The photoresist is then removed by running the wafer through a piranha etch (3:1 concentrated sulfuric acid to 30% hydrogen peroxide).
Chapter 3: Gen 1

Step 2: Deposit aluminum layer

A 1-µm-thick aluminum layer is then sputter-deposited onto the wafer using an Applied Materials® Endura® physical vapor deposition system. This layer acts as the eventual sacrificial layer for device release in step 6.

Step 3: Spin-on and pattern first polyimide layer

A 10-µm-thick base layer of photo-patternable polyimide (HD Microsystems® 4100) is spun onto the wafer and patterned using photolithographic masks. The polyimide is then partially cured (320°C for 30 min) to create a stable surface for subsequent processing, while leaving some unterminated bonds for attaching to the top polyimide layer.

Step 4: Electrode metal layer

A 10-nm-thick titanium + 200-nm-thick gold layer is deposited using electron beam evaporation (Temescal® Model VES2550), and patterned using an acetone liftoff process. This gold layer forms the electrode sites and traces for the probe.

Step 5: Spin-on and pattern second polyimide layer

The 10-µm-thick top layer of polyimide (HD Microsystems® 4100) is spun onto the wafer and patterned. The wafer is then fully cured (360°C for 60 min under a nitrogen environment) to complete the full cure of both polyimide layers. For Gen 3 devices, an oxygen plasma clean step (Matrix® stripper system with a pressure of 0.5 Torr and RF forward power of 250W) is then performed to clean any polyimide residue from the electrode sites.

Step 6: Release etch to separate polyimide from silicon wafer

The polyimide sheet is removed from the silicon wafer by dissolving the sacrificial aluminum in aluminum etchant (Transene® – type A). All die geometries were designed so that the final dies could be easily separated by hand after peeling the polyimide film from the silicon wafer, thereby avoiding a final diesaw step.

A microscope picture of a B-type Gen 1 device is shown in Figure 3-5 below. Examples of the leg variations described in section 3.2.3 are shown in Figure 3-6, and examples of the tip variations (described further in section 3.2.1 and 3.2.2) are shown in Figure 3-7.
3.3.2 Optimizing the fabrication process flow

The particular steps in the process flow chosen above involve design choices that result in tradeoffs between the ease of fabrication and the various properties of the fabricated devices. These fabrication design choices are discussed below.
3.3.2.1 Choice of structural material – photopatternable polyimide

Polyimide vs. parylene

An important microfabrication design decision made was to use polyimide for the device structural material. Modeling results (section 2.1.2.1) indicated that any polymer with a Young’s modulus on the order of 1 GPa would satisfy the structural requirements of the device. Therefore, a polymer such as parylene (Young’s modulus ~2.5 GPa) could have as well been used for the devices here, since parylene is both biocompatible [85] and our microfabrication facilities have the capability to deposit parylene. Polyimide was chosen over parylene as the structural material for the device due to prior expertise in our research group fabricating microscale devices using polyimide [107].

Choice of polyimide

Another important fabrication design choice made was to use photodefinable polyimide instead of non-photodefinable polyimide. If a non-photodefinable polyimide would have been chosen, the process flow would have had to have been modified to etch both polyimide layers using an oxygen plasma hard mask towards the end of the fabrication process flow (similar to what is described in [107]). However, the tool in our microfabrication facility that would be used to generate the oxygen plasma to pattern any non-photodefinable polyimide is only compatible with 4-inch silicon wafers. It was desirable to keep our process compatible with 6-inch wafers for increasing the device throughput per fabrication batch, and because some of the other fabrication tools that we wished to use were only compatible with 6-inch wafers.

The drawback to using a photodefinable polyimide is that the gold liftoff process (step 4 in Figure 3-4) is more challenging to perform over a non-uniform topography (step 3 in Figure 3-4 results in features that can be as thick as 10 μm). A non-photodefinable polyimide would result in a flat topography before the liftoff step – this would have made the liftoff process significantly easier.

The particular polyimide used in this work (HD 4100) was chosen because of its photodefinable nature, and its ease of availability. Additionally, this polyimide results in cured film thicknesses in the range of 4-13 μm per layer. This allows us to use the same polyimide in the 20 μm thick Gen 1 and 2 devices (with 10 μm per layer), as well as the 10 μm thick Gen 3 devices (with 5 μm per layer).

3.3.2.2 Choice of electrode material

Any metal that can satisfy the failure current density criteria for the given device dimensions (section 5.1.2), has good recording characteristics, and is biocompatible, could be used for the electrode material. Many metals, such as gold, platinum, etc., satisfy these criteria. From a microfabrication point of view [81], gold is easy to deposit via electron beam evaporation (the preferred deposition method for metal liftoff), is adhesive to many commonly used underlying substrates, and is compatible with the microfabrication facilities used. Therefore, it was chosen as the electrode material.

Titanium is a commonly used adhesion layer used with gold to promote adhesion of the gold to the underlying substrate [81]. Titanium is also easy to evaporate and is compatible with the microfabrication facilities used. Therefore, it was chosen as the adhesion layer for the gold electrode layer.
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3.3.2.3 Choice of final release step using a sacrificial etch

The final device release was done by etching a sacrificial metal layer and manually separating the various devices in a post-fabrication step. This method avoids any diesaw step, a common final step with microfabricated devices where the wafer is physically cut with a saw into the individual dies.

The metal-etch combination chosen was aluminum and aluminum etchant (nitric and phosphoric acid mixture). An alternative combination that could have been used is aluminum and hydrofluoric acid [107].

A major drawback of using a chemical wet etch to dissolve the sacrificial aluminum is the amount of time taken for etch completion (typical etches take 3-5 days). An alternative method to dissolve the sacrificial aluminum layer is using anodic aluminum dissolution in a sodium chloride solution [108]. This method is faster than the chemical wet etch and is claimed to result in lower residual stresses in the fabricated structures [84]. While this method was not used in any of the device generations described in this thesis, it might be worth considering for future generations or other similar devices.

3.3.2.4 Recipe development for the polyimide layer

An important accomplishment with Gen 1 device fabrication was developing the recipes for fabricating the polyimide layers. Processing of polymers such as polyimide can be very dependent on the particular tools in use. Moreover, polyimide can be very sensitive to temperature and humidity, and therefore, can be very sensitive to the particular microfabrication facility being used. As a result, recipes for the polyimide layers had to be developed and carefully tuned in order to achieve the desired characteristics in the fabricated devices.

A very common issue initially encountered while processing the polyimide was the presence of bubbles in the polyimide. There were two hypothesized causes for such bubbles. Air bubbles might be getting trapped during the manual deposition of the polyimide precursor onto the wafer before the spin step. Alternatively, moisture on the device wafer might be evaporating during the bake steps after spinning on the polyimide and getting caught in the overlying polyimide, leading to water vapor bubbles. The manual deposition was performed very slowly with considerable wait times to allow any dissolved bubbles to escape. Bake steps were also added at various points in the process to remove any moisture from the wafer. These process modifications resolved the issue of bubbles.

The detailed steps involved with fabricating the polyimide layers (step 3 and 5 in Figure 3-4) are described below. The only difference between the recipes for the two polyimide layers is the final cure step; the first polyimide layer is only partially cured, whereas a full cure is performed after depositing and patterning the second polyimide layer.

1. Piranha clean
   The wafers were cleaned with piranha solution (concentrated sulfuric acid and hydrogen peroxide mixture). Starting with clean wafers helps avoid contaminants on the surface of the wafer than can lead to bubbles in the polyimide.
2. Pre-spin bake
   The wafers were placed on a 150°C hot plate for 3 minutes. This helps remove any moisture that can lead to bubbles in the polyimide.

3. Spin on polyimide
   a. The wafer was attached to the vacuum chuck of the spinner.
   b. HD-4100® polyimide was manually poured onto the wafer. Care was taken to pour very slowly to avoid getting any bubbles trapped. The wafer was then left on the chuck for approximately 10 minutes while the polyimide slowly spread out. This helped ensure that any small bubbles that might been introduced during pouring have time to evaporate.
   c. The polyimide was spun out in two steps: (1) a 1000 rpm spin for 25 seconds (with a 200 rpm/s ramp) to get the polyimide to spread out on the wafer; and (2) a final spin speed for 60 seconds (with a 500 rpm/s ramp). Typical final spin speeds ranged from xxx to xxx depending on the final device thickness desired.
   d. The wafer was then left on the chuck for 1-2 minutes.

4. Post-spin bake
   A short post-spin bake (90°C hot plate for 3 minutes followed by a 120°C hot plate for 3 minutes) was performed.

5. Photolithography exposure
   a. An interval exposure was performed through a quartz mask, with soft contact. Typical exposure settings were 6 cycles of 5 second exposure interspersed with a 8 second delay.
   b. The wafer was rested for 5 minutes, which was then followed by a post-exposure bake on an 80°C hot plate for 60 seconds.

6. Develop
   To develop the polyimide, the wafer was first put in the beaker containing the PA-400D® developer solution, and agitated for approximately 4 minutes. The wafer was then moved to a second beaker containing a 50:50 solution of PA-400D® and PA-400R®, and agitated for approximately 10-15 seconds; this intermediate step is important to remove any developer residues. Finally, the wafer was moved to a third beaker containing PA-400R® rinse solution, agitated for approximately 10-15 seconds, and dried with an air-gun.

7. Post develop bake
   The wafer was placed on a 150°C hot plate for 2 minutes, followed by a 200°C hot plate for an additional 2 minutes. This removes any undesired volatiles from the developing step.

8. Cure
   An optimal polyimide cure is important because undercured films tend to not be very robust in terms of structural strength or resistance to chemical etching, whereas overcured films tend to be brittle. Optimal parameters for the cure step were found to vary significantly depending on the oven used. Two different ovens were used for curing the devices described in this work, since the oven in the microfabrication facility being used was replaced in a routine equipment upgrade over the course of this work. Therefore, Gen 1 and Gen 2 devices used one oven, whereas Gen 3 devices used a different oven.
For the partial cure of the first polyimide, the oven temperature was ramped to 300° C and held for 30 minutes before ramping back down. The parameters for this partial cure step were the same for both ovens used.

For the full cure step at the end the second polyimide patterning step, the parameters with the first oven (used in Gen 1 and Gen 2 devices) were as follows: the temperatures was first ramped up to 200° C and held for 30 minutes, then ramped up to 375° C and held for 1 hour, before ramping back down. For the second oven (used in Gen 3 devices), the temperature was only ramped up to 340° C in the final step, as higher temperatures resulted in burning of the polyimide.

All cure steps were performed in a nitrogen environment.

### 3.4 Benchtop characterization

Benchtop tests were used to characterize Gen 1 device features (device tips, trigger mechanism, and electrode sites) before testing the devices in vivo (section 3.5). Tip buckling tests (section 3.4.1) were used to verify that the device tips can pierce brain tissue without buckling, triggering tests (section 3.4.2) were used to verify that the device trigger mechanism worked as designed, and electrode impedance tests (section 3.4.3) were used to characterize the impedance of the electrode sites.

#### 3.4.1 Tip buckling tests

Gen 1 device tips were pierced into freshly sacrificed rat pup brains to test tip buckling, similar to tests with Gen 0 device tips in section 2.4.1. Approximately 30 piercing tests were performed with three B40-2A devices, and all tests resulted in the tips piercing the brain tissue. Since the predicted buckling forces (Table 3-1) for all Gen 1 devices are similar (in the 0.08 to 4.1 mN range) and are all at least 1-2 orders of magnitude higher than the forces (~8E-3 mN) at which Gen 0 device tips buckled (section 2.4.1), it was assumed that Gen 1 device tips would be strong enough to pierce live rat brain tissue. As will be seen in section 3.5, Gen 1 device tips do indeed pierce live rat brain tissue without buckling.

Figure 3-8 shows an example B40-E2A Gen 1 device tip piercing into freshly sacrificed rat pup brain tissue.

![Figure 3-8 Example of a B40-E2A Gen 1 device piercing a freshly sacrificed rat pup brain. (a) Device tip attached to a coverslip. (b) Brain tissue dimpling as the tip is about to pierce tissue. (c) Tip piercing into the tissue. Scale bars = 500 μm.](image-url)
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3.4.2 Trigger mechanism
Gen 1 devices were primed using a similar process as Gen 0 devices (section 2.4.2). Figure 3-9 below shows an A20 device with a leg width of 20 μm, a leg length of 1 mm, and 40 legs per device. A pin is first hooked into the hole in the shuttle head and used to pull the device back (Figure 3-9a). Some PEG is manually applied to the device head region, melted with a heat gun, and allowed to resolidify. The pin is then removed from the device, leaving the PEG to hold the device back in the primed position (Figure 3-9b). Upon application of water, the PEG dissolves and the device legs deflect back to their original shape, causing the shuttle to move forward (Figure 3-9c). Tests with other A-type devices indicated similar ease of priming.

![Figure 3-9 Gen1 triggering demonstration. (a) The device is pulled back using a thin metal pin and polyethylene glycol (PEG) is melted onto the device head. (b) Once the PEG solidifies, the pin is taken out (the PEG holds the device in place). (c) Upon application of water, the PEG dissolves, thereby releasing the mechanical energy stored in the deflected legs and pushing the device forward. Scale bars = 2 mm.](image)

B-type devices were designed to be able to deliver a higher force upon device triggering than A-type devices. Gen 0 device triggering tests (section 2.4.2) had indicated that, while devices with predicted induced stresses of 38 MPa were easy to prime, devices with predicted induced stresses of 600 MPa were quite challenging to prime and often resulted in failure due to tearing of the device head during the priming step. Gen 1 B-type devices were designed to have induced stresses on the order of 188 MPa (well below 600 MPa). However, triggering tests with B-type devices also resulted in device head failure, indicating that a stress of 188 MPa is still too high. Therefore, most Gen 2 devices were designed to incur stresses lower than this value (section 4.2.3).

3.4.3 Electrode impedance testing
A third important functional component of the Gen 1 devices, in addition to the device tip and legs, is the set of electrode sites. Proper electrical functionality of these sites is critical to the success of the device. Because Gen 0 devices had no metal layer and no electrode sites, Gen 1 devices were the first set of devices for which electrode impedance characterization was performed.

Figure 3-10 below shows the benchtop setup to characterize the impedance of the electrode sites. Stainless steel wires (50 μm diameter) were attached onto the gold contact pads of the device using silver epoxy (MG Chemicals #8331). Epoxy (DevCon® 5-minute) was applied over the silver epoxy to electrically insulate the contact pads and to act as an additional adhesive for the stainless steel wires. The device was then attached onto a glass coverslip for support and the device tip was dipped into a beaker filled with saline solution. Wires from the device were attached to a VersaSTAT 3® potentiostat.
A platinum electrode was used as a counter electrode and the two device electrode sites were used as working and reference electrodes, respectively. The impedance between the working and reference electrodes was measured, while a 10 mV amplitude AC voltage was swept between 1 Hz and 100 kHz.

The impedance measurement for a sample B40-E2A device is plotted as function of frequency (Figure 3-11 and Figure 3-12). The magnitude of the impedance measured at 1 kHz is the most physiologically relevant impedance measurement, since the time period of nerve cell action potentials is approximately 1 millisecond. As can be seen from Figure 3-11, the measured impedance at 1 kHz is ~ 10 MΩ. This is the combined impedance from two sites, one with an area of 166 μm² and the other with an area of 751 μm². Assuming that the impedance of these sites is proportional to their area, the larger site would have an impedance of 1.7 MΩ and the smaller site would have an impedance of 8.3 MΩ. While the smaller site impedance is likely too high for the recording system being used in the *in vivo* tests, the larger site impedance is in a borderline acceptable range (impedances on the order of a few hundred kΩ to a few MΩ are ideal for the recording system in use).
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Figure 3-11 Impedance magnitude as a function of frequency for a B40-2A Gen 1 device, when the electrode sites were submerged in saline solution.

As was discussed in section 3.2.2, commercially available neural probe technologies with comparable electrode site areas have impedances on the order of a few hundred kΩ to a few MΩ. A hypothesized reason for the higher impedance of Gen 1 device electrode sites is the incomplete etching of the polyimide from the electrode site area and polyimide residue on the sites (Figure 3-13). It is also possible that the impedance is higher because the gold on the electrode sites is untreated (most commercial neural probe technologies use various surface modifications to roughen the electrode site surface to increase the effective surface area and, thereby, reduce the impedance).
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Figure 3-13 Gen 1 electrode sites showing polyimide residue and incomplete etching. (a) E2A-type site. (b) E1-type site. (c) E2A-type site. Scale bars = 100 μm.

3.5 In vivo studies

Gen 1 devices were the first generation of devices that were tested in a rodent model (adult female Long Evans rats). There were three major aims of the in vivo studies with Gen 1 devices:

1. Development of a device package that can provide a robust interface with the recording system (section 3.5.1).
2. Validation of the ability of device tips to pierce live rodent brain tissue (section 3.5.2).
3. Validation of the ability of the device electrode sites to record neural activity (section 3.5.2).

All surgeries and electrophysiology data analyses were performed by Michael Sunshine and Aiva levins in Prof. Chet Moritz’ research lab in the University of Washington.

3.5.1 Device packaging and experimental setup

Since Gen 1 devices were intended to be used in in vivo experiments, there were two functions critical to the device package:

1. The package needed to provide enough structural support to enable reliable insertion of the device into the brain and be compact enough to allow for easy integration into the animal skullcap.
2. The package needed to enable easy electrical interfacing of the device electrodes to the recording system being used.

In order to enable these functionalities, a device package was designed as shown in Figure 3-14 below. The polyimide device was first cut using a razorblade in a rectangular outline around the main device features. Device edges next to the electrode tip were then cut off, so they would not get in the way during device insertion into brain tissue. The polyimide device was then attached to a glass coverslip using epoxy (DevCon® 2-Ton or 5-minute epoxy), and the coverslip was, in turn, attached to a custom-designed PCB with acrylic. The coverslip serves as the structural support for the device during insertion into brain tissue. The gold contact pads on the device were then connected to wires using silver epoxy (MG Chemicals #8331), and these wires were then soldered onto the appropriate vias of the PCB. The DF-30 connector on the PCB can be connected to the Tucker Davis Technologies® recording system cable.
3.5.2 Device insertion in live tissue and intraoperative acute recordings

The goal of the \textit{in vivo} tests with Gen 1 devices was twofold:

1. Since the insertion capabilities of these devices had previously only been tested in an \textit{ex vivo} setting (section 3.4.1), the \textit{in vivo} tests were meant to serve as demonstration of the device tip piercing live brain tissue without buckling.

2. Since the electrical functionality of the devices had previously only been tested in benchtop tests (section 3.4.3), the \textit{in vivo} tests were meant to demonstrate the electrical functionality of the electrodes in an \textit{in vivo} setting.

To demonstrate the ability of Gen 1 devices to pierce live brain tissue, terminal surgeries were performed on two adult female Long Evans rats (a common species of rat used in many research applications including testing of neural probes). The first animal was implanted with a B40-E1 device (predicted buckling force 0.83 mN at the lower limit; Table 3-1). Upon successful insertion of the device into the brain (Figure 3-15), a second animal was then implanted with a more flexible B20-E2C device (predicted buckling force 0.29 mN at the lower limit; Table 3-1). These tests demonstrated the ability of the Gen 1 devices to pierce live brain tissue without buckling, and also validated the benchtop piercing tests in section 3.4.1.
To demonstrate electrical functionality of the electrodes in an *in vivo* setting, untriggered probes were inserted in the motor cortex of adult female Long Evans rats. Three rats were implanted – two with a B20-E2C device each, and one with a B40-E1 device. Neural data were recorded intra-operatively under ketamine/xylazine anesthesia upon initial insertion of these devices using a commercially available recording system (Tucker Davis Technologies) and single unit waveforms were discriminated using time-amplitude windows.

It was found that the larger electrode sites of the B20-E2C devices (site area ~750 μm²; Table 3-2) were better at successfully recording neural activity than the smaller electrode sites of the B20-E2C devices (site area ~50 μm²; Table 3-2), or any of the electrode sites of the B40-E1 device (electrode site areas ~150 μm²; Table 3-2). Figure 3-16 shows a representative pile plot of the sorted waveform (action potentials) and the distribution of the inter-spike intervals recorded from the larger electrode site of a B20-E2C device. These *in vivo* recording tests demonstrate the effectiveness of the device in recording neural activity in an acute *in vivo* setting. Furthermore, they give insight into the optimal electrode site areas for successfully recording neural activity. These insights are used in the design of the electrode sites of Gen 2 devices (section 4.2.2).
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3.6 Conclusions

Gen 1 devices were the first microfabricated version of the design and were the first set of devices that underwent *in vivo* testing of any kind.

The major accomplishments of the Gen 1 devices were:

1. The device design was scaled down from the larger dimensions in the Gen 0 prototypes to microscale dimensions suitable for animal tests.
2. A new microfabrication process flow was developed and validated. This involved significant recipe development and recipe tuning to achieve the desired device characteristics.
3. A new *in vivo* test package and interface was developed to interface with the recording system used in the animal tests.
4. The ability of the device tips to pierce live rodent brain tissue was validated.
5. The ability of the device electrode sites to record neural activity was validated.

As was seen in section 3.4.2, the B-type Gen 1 devices were not as easy to prime as the A-type devices. However, the A-type devices were larger in size and using them in *in vivo* studies would have required larger craniotomies, which increases the complexity of the surgeries as well as the risk of post-surgical complications. Therefore, it was decided to use unprimed smaller B-type devices in the Gen 1 *in vivo* studies. As a result, the trigger mechanism of Gen 1 devices was not tested in an *in vivo* setting.

Some important lessons from Gen 1 devices that were used in the development of future devices were as follows.

**Optimal device size and geometry**

1. The shape and size of the Gen 1 B-type devices was shown to be compatible with the animal test setup. However, the device head hole was too small and the device legs were too stiff for priming the device. As a result, Gen 2 devices were designed to have similar dimensions as Gen 1 B-type devices, but with larger device head holes and slightly more flexible legs (section 4.2).
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2. An unintended side-effect of the particular geometry of the Gen 1 devices was the presence of undesired device “wings” around the electrode tips (Figure 3-14), which got in the way during device insertion into brain tissue. Gen 2 devices were, therefore, designed to eliminate the presence of such undesired structures around the device tip (section 4.2).

Optimal electrode site design

3. As was seen in the benchtop tests in section 3.4.3, the impedance of the Gen 1 electrode sites was quite high. This can make reliably picking up neural activity challenging. It was, therefore, concluded that it would be important to find a way to reduce electrode impedance in future device generations. Gen 2 and Gen 3 devices both have larger electrode site areas (sections 4.2 and 5.2) and include processing steps designed to clean any undesired polyimide residue from the electrode site region (sections 4.3.1.1 and 5.3.1.1).

Fabrication process design

4. Some of the Gen 1 devices had traces that were not properly patterned due to issues with the gold liftoff process (step 4 in Figure 3-4). This issue was most commonly observed in the device leg region where the metal traces are the narrowest. An important lesson from Gen 1 devices was the need to add more redundancy in the electrode traces in the device leg region. Therefore, Gen 2 and Gen 3 devices both have multiple traces going over the device legs (section 4.3).

5. The time taken to fabricate Gen 1 devices was dominated by the sacrificial etch step (step 6 in Figure 3-4), which took approximately a week. Fabrication process improvements that would reduce the time taken for this sacrificial etch step would help to significantly reduce overall fabrication time. Therefore, Gen 2 and Gen 3 devices both used release holes to reduce the sacrificial etch time (section 4.3.1 and 5.3.1).

Thus, Gen 1 devices built on what was learned from the Gen 0 prototypes in order to enable the first microfabricated set of devices. Benchtop tests and in vivo tests with Gen 1 devices, in turn, led to valuable insights into potential improvements in the device design, fabrication, and testing setup for future device generations.
Chapter 4: Gen 2

4 GEN 2

This chapter describes the Gen 2 devices, which built on what was learned from Gen 1 devices (chapter 3) to incorporate various improvements in the device design and fabrication process:

1. Gen 2 devices were designed to have a larger head hole (for easier device priming) and a die geometry that eliminated the wings issue of Gen 1 (Figure 3-14) (for easier device implantation).
2. Gen 2 devices had three electrode-related design improvements. First, Gen 2 devices included two types of electrode sites – smaller sites with areas comparable to Gen 1 device electrode sites, and larger sites. These larger sites were designed to reduce the electrode site impedance, compared to Gen 1, with the goal of improving the ease of recording neural activity. Second, a nanostrip etch step was added to the device fabrication step to clean any undesired polyimide residue from the small sites of Gen 2 devices. Third, the number of electrode sites was also increased from 2 sites in Gen 1 to 3 sites in Gen 3.
3. More redundancy was added in the trace lines going from the electrode sites to the contact pads to minimize device failure due to improper gold liftoff in areas where the traces were narrowest (e.g. over the device leg region). While Gen 1 had a single unbranched trace throughout, Gen 2 device traces branched off into multiple, redundant traces over the device leg region.
4. Gen 2 devices included release holes to speed up the sacrificial etch step, which was the bottleneck in device fabrication. This helped reduce the time for this step from over a week to 3-4 days.
5. Gen 2 devices were the first to include a variation (AH-type devices) with an integrated resistive heater for on-demand deployment of the device. However, it was found that the resistive heaters had connectivity issues and were, therefore, not used in this generation of devices. Functional resistive heaters were developed later for Gen 3 devices (chapter 5); the analytical models for the resistive heater are, therefore, included in chapter 5.

Gen 2 devices were used to test the integrated functionality (recording + deployment + recording) of the device in an acute in vivo setting, whereas Gen 1 devices had only been used to validate the recording characteristics of the device in vivo (with no in vivo device deployment).

This chapter will discuss the relevant models (section 4.1) that were used to guide the Gen 2 device design, the proposed device dimensions and model predictions (section 4.2), the microfabrication process used to manufacture these devices (section 4.3), benchtop characterization of the device tips, the device shuttle structure and the electrode sites (section 4.4), and in vivo tests (section 4.5) in the rodent model.

4.1 Modeling

The theory used to model Gen 2 device legs and tips was the same as described in section 2.1. Gen 2 electrode sites were designed based on comparable technologies (section 3.1.1), as well as results from Gen 1 device tests (section 3.4.3).
Chapter 4: Gen 2

4.2 Proposed design and model predictions

Gen 2 devices were fabricated based on analytical model predictions (section 2.1) in conjunction with benchtop (section 3.4) and in vivo (section 3.5) test results of Gen 1 devices. A typical Gen 2 device is shown in Figure 4-1 below and the major device style variations are shown in Figure 4-2. Most of the devices were A or B type devices (Figure 4-2 b and c), but some other variations were also fabricated. AH-type devices (Figure 4-2a) are similar to A-type devices, but have an additional resistive heater; B-type devices (Figure 4-2c) are similar to A-type devices, but with additional release holes for easier release of the devices during the sacrificial etch step (step 6 of Figure 3-4); and C- and D-type devices (Figure 4-2d and e) have multiple shuttles per device or multiple tips per shuttle. E-type devices (Figure 4-2f) were made with extra exposed metal pads on different parts of the device to test/develop the concept of capacitance sensing of the shuttle deployment (section 5.1.3).

Figure 4-1 Schematic of a typical Gen2 device. Green lines indicate the edge of the polyimide structure, blue lines indicate the metal trace, and magenta lines indicate the exposed areas of the metal.
4.2.1 Gen 2 - proposed tip dimensions and buckling model predictions

All AH-, A-, and B-type devices had four variations in the tip geometry, as shown in Figure 4-3. T2-type tips are similar to T1-type tips except with smaller tip lengths $l_{tip,2}$. Similarly, T4-type tips are similar to T3-type tips except with smaller tip lengths.

A major change in Gen 2 device tips compared to Gen 1 device tips is the much longer distance, $l_{tip,2}$, between the very tip of the device and the device legs (~1.5 mm for Gen 2 vs. less than 1 mm for Gen 1). This change was motivated by the fact that Gen 1 devices could not be inserted into brain tissue at depths required (~1.5 mm) to reach the target neurons without also inserting part of the device leg structure into the brain tissue (since even the largest Gen 1 devices had values of $l_{tip,2}$ less than 1 mm). In order to avoid having to insert the device leg region into brain tissue for Gen 2 (which would minimize tissue reaction by minimizing the amount of foreign material in the brain), Gen 2 device tips were designed to be longer with values of $l_{tip,2}$ all around 1.5 mm (Table 4-1). The longer Gen 2 tips also makes them floppier than Gen 1 tips (the lower limit of the predicted buckling forces range from 0.013 to 0.018 mN for Gen 2, vs. 0.08 to 1.65 mN for Gen 1 (Table 3-1)). However, this was predicted to not be an issue because Gen 1 device tips had all successfully pierced brain tissue in benchtop and in vivo tests, and because Gen 0 benchtop tests had indicated that forces on the order of a few µN were sufficient to pierce brain tissue.

It should also be noted that T1- and T2-type tips are more tapered variations of T3- and T4-type tips. While the lower limit of the predicted buckling force (Table 4-1) is the same for T3- and T4-type tips as for T1- and T2-type tips; however, in reality, the T3- and T4-type tips will be stiffer than the T1- and T2-type tips because of the higher effective moment of inertia than assumed by the simplified buckling model - case 2 (section 2.1.1). Also, T3- and T4-type tips allow for two of the three electrode sites to be significantly larger in area, enabling testing of additional variations in the designed electrode impedances.
Chapter 4: Gen 2

Figure 4-3 Schematic of the four tip types for Gen 2 devices – (a) T1-type tip, (b) T2-type tip, (c) T3-type tip, and (d) T4-type tip. Green lines indicate the edge of the polyimide structure, blue lines indicate the metal trace, and magenta lines indicate the exposed areas of the metal.

Table 4-1 Proposed tip dimensions for 20 μm thick Gen 2 devices and corresponding buckling force predictions calculated using equation 2.1. Actual dimensions may differ from these designed dimensions by a few μm due to microfabrication processing limitations.
4.2.2 Gen 2 – proposed electrode site dimensions

In vivo tests (section 3.5.2) with Gen 1 devices had indicated a difficulty in reliably recording neural activity, possibly because the electrode site impedance was too high (section 3.4.3). It was hypothesized that this was because the electrode site area was not large enough or because there was undesired polyimide residue on the electrode sites as an artefact of the microfabrication process. Gen 1 electrode site areas ranged from 50 to 170 \( \mu m^2 \) for the smaller sites and 650 to 750 \( \mu m^2 \) for the larger sites (section 3.2.2). Therefore, Gen 2 electrodes sites (Figure 4-4) were all designed to have areas larger than Gen 1 site areas (Table 4-2) with an aim to reduce the electrode site impedance. Additionally, while T1- and T2-type Gen 2 tips had rectangular slit-like electrodes similar to Gen 1, T3- and T4-type tips had larger triangular sites, in order to minimize any underdevelopment of the polyimide during the photolithography step that might lead to undesired residues on the sites.

![Non-tip sites](a) | Tip site | (b) Non-tip sites | Tip site |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(c) Non-tip sites</td>
<td>Tip site</td>
<td>(d) Non-tip sites</td>
<td>Tip site</td>
</tr>
</tbody>
</table>

Figure 4-4 Gen 2 electrode site variations. (a) T1-type tip. (b) T2-type tip. (c) T3-type tip. (d) T4-type tip. Green lines indicate the edge of the polyimide structure, blue lines indicate the metal trace, and magenta lines indicate the exposed areas of the metal.

Table 4-2 Proposed electrode site area variations for Gen 2 devices. Actual dimensions may differ from these designed dimensions by a few \( \mu m \) due to microfabrication processing limitations.

<table>
<thead>
<tr>
<th>Tip type</th>
<th>Tip electrode site area ((\mu m^2))</th>
<th>Non-tip electrode site area ((\mu m^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>1460 (rectangular; 5 ( \mu m ) X 288 ( \mu m ))</td>
<td>1560 (rectangular; 6 ( \mu m ) X ~260 ( \mu m ))</td>
</tr>
<tr>
<td>T2</td>
<td>440 (rectangular; 5 ( \mu m ) X 88 ( \mu m ))</td>
<td>1560 (rectangular; 6 ( \mu m ) X ~260 ( \mu m ))</td>
</tr>
<tr>
<td>T3</td>
<td>1460 (rectangular; 5 ( \mu m ) X 288 ( \mu m ))</td>
<td>3150 (triangular; base 74 ( \mu m ), height 85 ( \mu m ))</td>
</tr>
</tbody>
</table>
4.2.3 Gen 2 – proposed leg dimensions and structural model predictions

All AH-, A-, and B- type devices had three variations in the leg geometries, as shown in Figure 4-5 below. All three leg variations have the same width (20 μm), but different lengths: L1-type legs are 200 μm long, L2-type legs are 300 μm long, and L3-type legs are 500 μm long. These variations are meant to test tradeoffs (Table 4-3) between the stress induced in the legs and the force delivered by these legs during device deployment. Benchtop tests of Gen 1 devices (section 3.4.2) had indicated that these devices can survive predicted stresses well above 15 MPa (for Gen 1 A-type devices). Therefore, for Gen 2 devices, the L2-type legs are designed to be standard legs with a predicted induced stress of over 80 MPa for a 50 μm device deflection. The L1-type legs are designed to be even stiffer with a predicted induced stress of 188 MPa for a 50 μm device deflection in the unlikely event that the L2-type legs are not as strong as predicted by the analytical models. L3-type legs are designed to be more flexible than the L2-type legs with a predicted induced stress of 30 MPa for a 50 μm device deflection, yet have a high enough force capability at over 6 mN.

<table>
<thead>
<tr>
<th>Device type</th>
<th>Leg width b (μm)</th>
<th>Leg length l (μm)</th>
<th>Number of legs per shuttle</th>
<th>Maximum stress induced (MPa)</th>
<th>Force per leg (mN)</th>
<th>Total force (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>x-L1-xx</td>
<td>20</td>
<td>200</td>
<td>42</td>
<td>188</td>
<td>2.5</td>
<td>105</td>
</tr>
<tr>
<td>x-L2-xx</td>
<td>20</td>
<td>300</td>
<td>42</td>
<td>83</td>
<td>0.7</td>
<td>31</td>
</tr>
</tbody>
</table>

Figure 4-5 Schematic of leg variations for AH-, A- and B-type Gen 2 devices. (a) L1-type leg, (b) L2-type leg, (c) L3-type leg. Green lines indicate the edge of the polyimide structure, and blue lines indicate the metal trace.

Table 4-3 Proposed leg dimensions for 20 μm thick Gen 2 devices and corresponding predictions of force delivered and maximum induced stress (calculated using equations 2.5 and 2.6) for a device deflection of 50 μm. Actual dimensions may differ from these designed dimensions by a few μm due to microfabrication processing limitations.
4.3 Fabrication and packaging

4.3.1 Fabrication

The fabrication process for Gen 2 devices is the same as that for Gen 1 devices as described in section 3.3.1. Four batches of devices were fabricated, each consisting of 4-5 wafers.

Optical images of sample fabricated Gen 2 devices are shown in Figure 4-6 and Figure 4-7 below.

Figure 4-6 Optical image of a typical Gen 2 fabricated device (BL2T2). The device consists of a 10 μm polyimide/ 200 nm gold/ 10 μm polyimide sandwich structure with the gold exposed at the electrode sites on the tip and at the contact pads. Scale bars = 300 μm.
4.3.1.1 Nanostrip etching to remove webbed legs

One issue that was commonly observed with Gen 2 devices was that some areas of the device, especially those with smaller feature sizes, were not properly patterned. For example, some of the devices exhibited webbed legs as shown in Figure 4-8. Smaller features, such as the inter-leg regions, are more susceptible to undesirable residue during photolithography of negative-tone resists such as the one being used here, since any light that might diffuse into the smaller areas might end up partially cross-linking the photoresist polymers.

Figure 4-7 Pictures of Gen 2 devices showcasing fabrication variations. (a) BL2T2 device. (b) AL3T3 device. (c) AL3T1 device. Scale bars = 500 μm.
The issue of webbed legs with Gen 2 devices was addressed by soaking the affected devices in a nanostrip solution (Cyantek Nano-Strip® - stabilized sulfuric acid and hydrogen peroxide solution) to dissolve away the webbing. After a few minutes of soaking, the webbing began to dissolve away (Figure 4-9). It was found that an 18 to 21 hour nanostrip soak was required to completely dissolve away the webbing between the legs and in any other small gaps (Figure 4-10, Figure 4-11, Figure 4-12).
Figure 4-10 Effects of nanostrip soaking on a AL1T1 Gen 2 device. (a) Device prior to etch. (b) Device after an 18 hour soak in nanostrip solution. Scale bars = 100 μm.

Figure 4-11 Effects of nanostrip soaking on a AL1T2 device. (a) Device prior to etch. (b)-(c) Device after an 18 hour soak in nanostrip solution. Scale bars = 100 μm.

Figure 4-12 Effects of nanostrip soaking on a BL1T4 device. (a) Device prior to etch. (b) Device after a 21 hour soak in nanostrip solution. Scale bars = 100 μm.
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An undesired side-effect of post-fabrication nanostrip soaking was the overall thinning of the devices. Estimating based on the images in the figures above, an 18-21 hour nanostrip etch resulted in approximately 5 μm of thinning of the devices in each direction. Thus, the 20 μm wide legs are observed to be approximately 10 μm wide after the nanostrip etch. Assuming isotropic etching, the nanostrip etch thins the 20 μm thick devices down to a 10 μm thickness. This thinning of the devices makes them more prone to buckling during insertion into brain tissue. Gen 2 devices that were prone to such buckling because of a nanostrip treatment were stiffened with PEG prior to insertion (section 4.5).

4.3.1.2 Device curling and PEG stiffening

Even in some devices that were not soaked in nanostrip, device curling was sometimes observed (Figure 4-13 below). Such device curling had not generally been observed in Gen 1 devices, likely because Gen 1 device tips were shorter than Gen 2 device tips, with 0.345 to 0.985 mm of the Gen 1 device tip freestanding beyond the coverslip packaging (Table 3-1) vs. 1.445 to 1.645 mm of the Gen 2 device tip freestanding beyond the coverslip packaging (Table 4-1).

Gen 2 device curling was remedied by manually straightening the devices by using a tweezer to lightly push the device tip until it was straightened and then applying some melted PEG to the tip region and allowing the PEG to solidify. The solidified PEG is strong enough to fix the devices in the straightened position. The PEG dissolves upon implantation into the brain within a few seconds. Therefore, as long as the implantation is done within a few seconds, the PEG helps the device tip pierce the brain tissue without buckling.

Figure 4-13 Optical image of an AL2T4 Gen 2 device showing the device tip curling upward. Scale bar = 1 mm.

4.3.2 Packaging and connectorization

Custom printed circuit boards (PCBs) were designed and manufactured (Figure 4-14) by Andres Canales, from Prof. Polina Anikeeva's research group at MIT. These PCBs interface the device with the Tucker Davis Technologies recording system used in the in vivo studies.
Figure 4-14 PCBs used in Gen 2 in vivo tests. (a) Picture of front of the PCB with an attached DF-30 connector. (b) Picture of back of PCB without a DF-30 connector. (c) Schematic of the PCB showing the electrical connections.

The device was first attached onto a piece of glass coverslip by using epoxy (DevCon® 5-minute epoxy or DevCon® 2-Ton epoxy) to glue the edges of the device onto the coverslip, with the device tip hanging out from over the coverslip support (Figure 4-15). Stainless steel wires (50 µm diameter) were then attached onto the gold contact pads of the device using silver epoxy (MG Chemicals #8331). Epoxy (DevCon® 5-minute) was applied over the silver epoxy to electrically insulate the contact pads and to act as an additional adhesive for the stainless steel wires. The other ends of these wires were then soldered into the vias of custom PCBs (Figure 4-14).

To prime the device, a micromanipulator was used to hook into the hole in the device head and pull it back. With the device pulled back, a meltable glue, such as PEG (melting point 50 to 70°C, depending on molecular weight) or bonewax (melting point approximately 65°C), was manually placed onto the device head area and melted using a heat gun, and then allowed to cool down and resolidify. The micromanipulator hook was then removed, with the solidified PEG/bonewax holding the device pulled back in the primed position.
Figure 4-15 T typical Gen 2 packaging setup. (a) Image of a packaged BL2T4 device. (b) Top-view schematic of packaging setup. (c) Side-view schematic of packaging setup. Scale bar = 1 mm.

4.4 Benchtop characterization

Benchtop tests were used to characterize Gen 2 device features (device tips, trigger mechanism, and electrode sites) before testing the devices in vivo (section 4.5). Tip buckling tests (section 4.4.1) were used to verify that the device tips can pierce brain tissue without buckling, triggering tests (section 4.4.2) were used to verify that the device trigger mechanism worked as designed, and electrode impedance tests (section 4.4.3) were used to characterize the impedance of the electrode sites. Additionally, the devices were used to measure a physiological-looking but artificially generated signal in saline solution (section 4.4.4), in order to simulate in vivo conditions.

4.4.1 Tip buckling tests

Gen 2 device tips were pierced into freshly sacrificed rat pup brains to test tip buckling, similar to tests with Gen 0 (section 2.4.1) and Gen 1 device tips (section 3.4.1). Note that while predicted buckling forces of Gen 2 device tips (0.013 to 2.4 mN, Table 4-1) were lower than the predicted buckling forces of Gen 1 device tips (0.08 to 4.1 mN, Table 3-1), they were still higher than the predicted buckling forces (0.008 mN) of Gen 0 device tips that buckled in benchtop tests (section 2.4.1). Figure 4-16, Figure 4-17, Figure 4-18 below show a T1-, T2-, and T3-type tip respectively piercing brain tissue.
4.4.2 Trigger mechanism

Gen 2 devices were primed slightly differently from Gen 0 (section 2.4.2) and Gen 1 (section 3.4.2) devices – instead of a large pin, a micromanipulator was used to hook a small metal probe into the Gen 2 device head hole and pull the shuttle back. Holding the device in place, some PEG was then manually
applied to the device head region, melted with a heat gun, and allowed to resolidify. The metal probe was then removed from the device, leaving the PEG to hold the device back in the primed position (Figure 4-19a). Upon application of saline, the PEG dissolved and the device legs deflected back to their original shape, causing the shuttle to move forward (Figure 4-19b). Trigger mechanism tests performed with Gen 2 devices indicated that L1-type devices (predicted induced stress of 188 MPa for a 50 μm deflection, Table 4-3) were generally harder to prime and frequently resulted in failure due to tearing of the head structure. This was as expected given that the B-type Gen 1 devices with predicted induced stresses of 188 MPa (Table 3-3) were also challenging to prime (section 3.4.2). L2- and L3-type devices could both be easily primed up to a 50 μm shuttle deflection. Since L2-type devices can deliver a higher force upon deployment than L3-type devices (Table 4-3), they were the preferred type of devices for tests requiring device deployment.

Note that benchtop tests were successfully performed with PEG of molecular weights ranging from 1000 to 8000. Tests were also successfully performed using bonewax as the glue instead of PEG, where the bonewax was melted with a heatgun to deploy the device. This indicates that bonewax is also compatible with the device triggering approach.

![Figure 4-19 Gen 2 triggering demonstration of an L3-type device.](image)

**Figure 4-19 Gen 2 triggering demonstration of an L3-type device.** (a) The pulled-back probe body is held in place with PEG, storing energy in the deflected legs. (b) The PEG is dissolved by adding saline, thereby straightening the legs and pushing the tip forward by approximately 50 μm. Scale bars = 500 μm.

4.4.3 Electrode impedance testing

The impedance of the electrode sites of packaged Gen 2 devices (Figure 4-15) was tested with a setup (Figure 4-20) similar to Gen 1’s impedance testing setup described in section 3.4.3. However, instead of using the Versastat® potentiostat, which was capable of sweeping frequencies from 0 to 1 MHz, an Agilent 4294A precision impedance analyzer was used. This new impedance analyzer had a faster setup time and was capable of sweeping frequencies from 40 Hz to 110 MHz, which enabled an increased range of frequencies over which impedance was measured, giving additional insight into the electrochemical behavior of the device sites. Impedance was measured as a 10 mV amplitude AC voltage was swept between 40 Hz to 110 MHz.
Note that the measured impedance represents electrochemical reactions occurring at the device electrode site and also at the stainless steel wire. Approximately 1 cm of the stainless steel wire was exposed and immersed in the saline. This represents a surface area of approximately $1.5 \times 10^6 \, \mu\text{m}^2$, which is three orders of magnitude higher than the device electrode site area of $\sim 1 \times 10^3 \, \mu\text{m}^2$. Since impedance is inversely proportional to surface area, the impedance due to electrochemical reactions at the stainless steel wire interface can be neglected compared to the device electrode site impedance. Any contact resistances that might be introduced into the system due to the silver epoxy or other electrical connections can also be neglected since such contact resistances are typically on the order of a few ohms (based on product specifications for the silver epoxy and also verified by separate benchtop tests not shown here), which is negligible compared to the impedances measured with our setup. Therefore, it is reasonable to assume that measured impedances represent the impedance of the device electrode sites.

![Diagram of impedance test setup for Gen 2 devices.](image)

Representative impedance curves from two T4-type Gen 2 devices are shown in the figures below. Figure 4-21 and Figure 4-22 show the respective magnitude and phase angle of the impedance of a BL3T4 device that had not been etched in nanostrip. At the physiologically relevant frequency of 1 kHz, the impedance magnitudes were 271 kΩ for the tip site, 331 kΩ for one non-tip site, 284 kΩ for the second non-tip site, and 10 MΩ for the open configuration where the device tip was taken out of the saline solution. Note that the impedance phase plots indicate that the tip site for this device is resistive in behavior at low frequencies (a phase angle of 0° indicates a purely resistive behavior whereas a phase angle of 90° indicates a purely capacitive behavior). This is unusual (the electrode sites are expected to show some degree of capacitive behavior) and might indicate a potential short in the circuit or issues with the electrical insulation of the traces of the tip site.

Figure 4-23 and Figure 4-24 show the respective magnitude and phase angle of the impedance of an AL3T4 device that had previously been soaked in nanostrip for 10.5 hours. At 1 kHz, the impedance...
magnitudes were 2.5 MΩ for the tip site, 164 kΩ for one non-tip site, 328 kΩ for the second non-tip site, and 6 MΩ for the open configuration where the device tip was taken out of the saline solution.

Typical impedances of Gen 2 devices are summarized in Table 4-4 below. It was found that the non-tip electrode sites of the T4-type Gen 2 devices had the lowest measured impedances at 1 kHz, likely due to their large site area. Consequently, these sites were also most effective at reliably picking up neural activity in in vivo tests (section 4.5). Additionally, it was found that while nanostrip soaking was effective at removing webbing between device legs (section 4.3.1.1), there was no clear correlation between nanostrip soaking and the impedance of the tip electrode sites.
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Figure 4-21 Representative impedance (magnitude) plot of a BL3T4 Gen 2 device (no nanostrip soak). The black squares represent the impedance magnitude when the device was not immersed in saline. The red triangles represent the impedance magnitude of the tip electrode sites whereas the black circles and purple diamonds represent the impedance magnitude of the non-tip electrode sites.

Figure 4-22 Representative impedance (phase) plot of a BL3T4 Gen 2 device (no nanostrip soak). The black squares represent the impedance phase when the device was not immersed in saline. The red triangles represent the impedance phase of the tip electrode sites whereas the black circles and purple diamonds represent the impedance phase of the non-tip electrode sites.
Figure 4-23 Representative impedance (magnitude) plot of an AL3T4 Gen 2 device (previously soaked in nanostrip for 10.5 hours). The black squares represent the impedance magnitude when the device was not immersed in saline. The red triangles represent the impedance magnitude of the tip electrode sites whereas the black circles and purple diamonds represent the impedance magnitude of the non-tip electrode sites.

Figure 4-24 Representative impedance (phase) plot of an AL3T4 Gen 2 device (previously soaked in nanostrip for 10.5 hours). The black squares represent the impedance phase when the device was not immersed in saline. The red triangles represent the impedance phase of the tip electrode site whereas the black circles and purple diamonds represent the impedance phase of the non-tip electrode sites.
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Table 4-4 Measured impedances in saline at 1kHz for some Gen 2 devices. Measured values represent the range of single measurements from multiple \( n \geq 2 \) devices of each tip type.

<table>
<thead>
<tr>
<th>Tip type</th>
<th>Site type</th>
<th>Site area</th>
<th>Typical measured impedances (MO) at 1 kHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Tip site</td>
<td>1460</td>
<td>1 to 3</td>
</tr>
<tr>
<td>T2</td>
<td>Tip site</td>
<td>440</td>
<td>1 to 3</td>
</tr>
<tr>
<td>T1/T2</td>
<td>Non-tip site</td>
<td>1560</td>
<td>0.5 to 1</td>
</tr>
<tr>
<td>T3</td>
<td>Tip site</td>
<td>1460</td>
<td>0.3 to 3</td>
</tr>
<tr>
<td>T4</td>
<td>Tip site</td>
<td>440</td>
<td>0.3 to 3</td>
</tr>
<tr>
<td>T3/T4</td>
<td>Non-tip site</td>
<td>3150</td>
<td>0.1 to 0.4</td>
</tr>
<tr>
<td>Open</td>
<td></td>
<td></td>
<td>1 to 7</td>
</tr>
</tbody>
</table>

4.4.4 Measuring a known signal through the electrodes

To test the functionality of the electrode sites, the device tips were immersed in a saline solution (Figure 4-25) and a known physiologically-shaped signal (peak-to-peak voltage 1.9 V, frequency 100 Hz) was input using a Coulbourn Systems® Bio-System Calibrator®. The signal was recorded through the non-tip electrode sites of a BL3T4 device (impedances of the sites had previously been recorded to be 190 kΩ and 200 kΩ respectively at 1 kHz). Two tungsten wires were also attached to the device, with one of the wires being used as a reference, while the other being used as a control electrode (previously recorded impedance of 500 kΩ at 1 kHz).

Figure 4-25 Schematic of test setup for measuring a known signal through the electrode sites.
The signal measured through the device electrode sites and through the tungsten wire is shown in Figure 4-26 below. The peak-to-peak amplitude of the signal recorded through the device electrode sites was measured to be approximately 1.7 V, whereas the peak-to-peak amplitude of the signal recorded through the tungsten wire was measured to be approximately 1 V. The higher impedance of the tungsten wire compared to the device electrode sites (500 kΩ vs. 190-200 kΩ) explains the lower amplitude of the signal recorded by the tungsten wire.

![Figure 4-26 Measuring a 1.9 V peak-to-peak signal through a BL3T4 Gen 2 device. (a) Device non-tip electrode site 1 (impedance 190 kΩ). (b) Device non-tip electrode site 2 (impedance 200 kΩ). (c) Tungsten wire (impedance 500 kΩ).](image)

This experiment demonstrates that the device can successfully record the electrical signals through saline solution, without much attenuation. This indicates that the devices are ready for *in vivo* testing.

### 4.5 *In vivo* studies

Various studies were performed in the rat and mouse models to validate the functionality of the device in an *in vivo* setting. First, the recording-only capability of the Gen 2 devices was tested (section 4.5.1), similar to what was done with Gen 1 devices (section 3.5.2), before testing the triggering capability of the device *in vivo*. However, similar to Gen 1 devices, it was found that recording endogeneous neural activity was challenging. This might have been because the electrode sites were not functioning as desired or because the sites were not in close proximity to active neurons. To ensure the functionality of the electrode sites, two types of animal studies were designed to artificially elicit neural activity in the region surrounding the electrode sites. First, rat brain tissue was electrically stimulated in the opposing brain hemisphere to the implanted electrodes, with the goal of activating neural networks that would eventually cause neurons around the device electrode sites to fire (section 4.5.2). Second, the device electrodes were implanted in optogenetic mice and a laser waveguide attached to the device was used to optically stimulate the region surrounding the device electrode sites, with the goal of eliciting a neural response in that region (sections 4.5.3 and 4.5.4). Both these types of animal studies were successful in recording neural activity through the device in an acute setting, as well as in validating the integrated (deployment+recording) functionality of the device in an *in vivo* setting.

For the rat studies (sections 4.5.1 and 4.5.2), all surgeries and electrophysiology data analyses were performed by Michael Sunshine and Aiva levins in Prof. Chet Moritz’ research lab in University of Washington. For the optogenetic mouse studies (sections 4.5.3 and 4.5.4), all surgeries and electrophysiology data analyses were performed by Dr. Ulrich Froriep in Prof. Polina Anikeeva’s research lab.
lab at MIT. The histology studies shown in section 4.5.1 were performed in Prof. Bill Shain's research lab in the University of Washington.

4.5.1 Recording-only studies in the rat model and preliminary histology
Recording-only studies were performed similar to Gen 1 (section 3.5) to demonstrate the electrical functionality of the device in vivo.

To demonstrate the ability of Gen 2 device tips to pierce brain tissue without buckling, three different devices were implanted into the motor cortex of a ketamine/xylazine anesthesized adult female Long Evans rat: an AL3T3 device (predicted buckling force lower limit 0.013 mN, Table 4-1), a BL3T4 device (predicted buckling force lower limit 0.017 mN, Table 4-1), and an AL1T1 device (predicted buckling force lower limit 0.013 mN, Table 4-1). All devices successfully penetrated into brain tissue without buckling, although the AL3T3 device suffered from the curling issues described in section 4.3.1.2 and had to be coated with PEG for additional stiffening before successful device insertion.

To demonstrate the electrical functionality of Gen 2 devices, untriggered probes were inserted in the motor cortex of 5 adult female Long Evans rats under ketamine/xylazine anesthesia (Table 4-5). Neural data were recorded intra-operatively as well as post-implantation using a commercially available recording system (Tucker Davis Technologies) and single unit waveforms were discriminated using time-amplitude windows. One such recording (from the large electrode site of animal 4; 24 days-post-implantation) is shown in Figure 4-27 below. These preliminary tests demonstrate the ability of the device to record neural activity in an acute in vivo setting. However, in general, it was found that reliably recording endogeneous activity was challenging – no single unit waveforms were seen in animals 1 and 2, and only occasional single unit waveforms were observed from the large electrode sites of animals 3, 4, and 5.

Table 4-5 Recording-only tests performed in rats.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Device</th>
<th>Implant duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BL3T4</td>
<td>Terminal</td>
</tr>
<tr>
<td>2</td>
<td>AL3T4 (left hemisphere)</td>
<td>Terminal</td>
</tr>
<tr>
<td></td>
<td>BL3T4 (right hemisphere)</td>
<td>Terminal</td>
</tr>
<tr>
<td>3</td>
<td>BL3T3 (left hemisphere)</td>
<td>18 days</td>
</tr>
<tr>
<td></td>
<td>BL2T3 (right hemisphere)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>BL3T3 (left hemisphere)</td>
<td>45 days</td>
</tr>
<tr>
<td></td>
<td>Tungsten microwire array (right hemisphere)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>BL1T4 (left hemisphere)</td>
<td>47 days</td>
</tr>
<tr>
<td></td>
<td>AL2T3 (right hemisphere)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4-27 Time-amplitude sorted waveform recorded intraoperatively from a Gen 2 device immediately following device insertion.

Preliminary histology studies were also performed on Gen 2 devices by Prof. Bill Shain’s lab in the University of Washington. Figure 4-28 shows the histology results performed on animal 3 (from Table 4-5) with a BL2T3 device implanted in the right hemisphere and a BL3T3 device implanted in the left hemisphere. The animal was euthanized 18 days post-implantation. After fixing the brain, the right hemisphere was sliced coronally in 20 μm sections, whereas the left hemisphere was sliced horizontally in 20 μm sections. The stains used were for ionized calcium binding adapter molecule 1 (iba1) (for microglia), glial fibrillary acidic protein (GFAP) (for astrocytes), and 4',6-diamidino-2-phenylindole (DAPI) (for cell nuclei). Additionally, the BL2T3 device in the right hemisphere had been painted with a Dil stain before implantation, which helps to identify the device outline in the histology images. These histology studies helped develop the expertise and protocols needed for histology on chronic implants in Gen 3 devices.
4.5.2 Intraoperative acute recordings in the rat brain in response to electrical stimulation

In order to artificially elicit neural activity in the region surrounding the implanted device electrode sites, an experiment was designed, in which Gen 2 devices were implanted in the motor cortex of anesthetized (ketamine/xylazine) adult female Long Evans rats. Tungsten wire electrodes were then implanted in the opposing brain hemispheres and the brain tissue was electrically stimulated using these wire electrodes. Figure 4-29 shows the recording from a BL3T4 device (this was the same device as the one used in section 4.4.4).

Figure 4-29 shows the recording from a BL3T4 device in a rat motor cortex recording local field potentials in response to a 9mA 40 μs/phase biphasic electrical stimulation in the opposing brain hemisphere. (a) Electrode site 1 (180 kΩ impedance). (b) Electrode site 2 (200 kΩ impedance). (c) Tungsten wire (240 kΩ impedance).
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Figure 4-29 shows that the device is successfully recording field potentials in response to the electrical stimulation. In order to achieve an even higher degree of confidence that the device is recording physiological signals (as opposed to some stimulation artefact), the devices were tested in an optogenetic mouse model (sections 4.5.3 and 4.5.4), where the stimulation was optical and, therefore, decoupled from the electrical output being measured.

4.5.3 Intraoperative acute recording in the optogenetic mouse model

Gen 2 devices were also tested in the optogenetic mouse model. As discussed previously in section 1.1.1.4, optogenetics is a very useful engineering tool for testing of neural electrodes with two key advantages. First, neural activity can be elicited on demand in the region surrounding the neural electrodes. This is useful because resting neurons near an electrode site might not always be electrically active at the time of recording. Optogenetics techniques can force neurons around the electrode to fire on-demand. Second, by decoupling the mode of the input (optical illumination) into the neural system from the mode of output (electrical response of neurons), there can be a higher degree of certainty that the recorded signals are physiological in origin (as opposed to an artefact of the recording system).

Since Gen 2 was the first time this device was tested in an optogenetic mouse model, the recording-only capability of the device was tested first (Figure 4-30), before testing the triggering capability in vivo. The device was implanted approximately 1.3 mm into the prefrontal cortex of an anesthetized (ketamine/xylazine) transgenic Thy1-ChR2-YFP mouse. A laser waveguide was then placed above the skull and a 473 nm wavelength laser was used to optically stimulate the brain. Ideally, the laser waveguide would be attached to the device such that the laser probe tip penetrates brain tissue along with the device and lies approximately 100 µm above the electrode site, which maximizes the efficiency of the optical stimulation. However, for ease of packaging and connectorization, the laser waveguide was placed outside the skull and the light intensity was increased to levels high enough to stimulate regions of the brain around the electrode sites. The ground and reference wires were attached to a skull screw. Pictures of the experimental setup are shown in Figure 4-31.
Two male Thy1-ChR2-YFP mice were implanted with a Gen 2 device each. Figure 4-32 shows representative neural activity (with reference to the reference wire) recorded from one of the animals closely following 20 laser stimulation trains (each train consisting of 10 pulses at 10 Hz with 5 ms pulse duration, and an inter-train interval of 5 s) delivered using the optical fiber. Figure 4-32a shows multi-unit activity (filter settings 0.3 - 8 kHz, ~50 kHz sampling frequency), and Figure 4-32b shows histograms for quantification across trials. To ensure physiological origin of the recorded activity, the stimulation frequency was raised to 100 Hz (Figure 4-33) resulting in loss of the correlation between stimulation and neural response [109]. The stimulation frequency was then reduced back to 10 Hz (Figure 4-34), resulting in the recovery of neural activity that correlated to the optical stimulation.

This experiment successfully demonstrated the ability of the device to record neural data in an optogenetic mouse model, paving the way for testing of the integrated recording+deployment capability of the device in the optogenetic mouse model (section 4.5.4).
Figure 4-32 In vivo acute recordings of neural activity in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse in response to a 10 Hz laser stimulation train. (a) Multi-unit activity and (b) Multi-unit activity histogram.

Figure 4-33 In vivo acute recordings of neural activity in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse in response to a 100 Hz laser stimulation train. (a) Multi-unit activity and (b) Multi-unit activity histogram.

Figure 4-34 In vivo acute recordings of neural activity in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse in response to a 10 Hz laser stimulation train. (a) Multi-unit activity and (b) Multi-unit activity histogram.
Intraoperative acute recording+triggering in the optogenetic mouse model

The integrated electrical and mechanical functionality of the probes was confirmed under acute *in vivo* conditions in the medial prefrontal cortex of two anesthetized (ketamine/xylazine) transgenic Thy1-ChR2-YFP mice (Figure 4-35). The ground and reference wires were placed under the skin in the neck of the animal. The device was inserted in the primed configuration (step 1 in Figure 4-35, Figure 4-36a), and neural activity was recorded closely following 20 laser (473 nm wavelength) stimulation trains (each train consisting of 10 pulses at 10 Hz with 5 ms pulse duration, and an inter-train interval of 5 s) delivered using a stationary optic fiber inserted adjacent to the device (step 2 in Figure 4-35, Figure 4-37). The device was then deployed by applying saline to the PEG glue causing the tip to pierce ~50 μm deeper into the brain (step 3 in Figure 4-35, Figure 4-36b). Neural activity was recorded again in response to similar laser stimulation (Step 4 in Figure 4-35, Figure 4-38).

1. Insert device into optogenetic mouse
2. Stimulate with laser and record
3. Deploy probes
4. Stimulate with laser and record

---

**Figure 4-35** Schematic of overview of experimental design

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**Figure 4-36** Device implanted in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse. (a) Untriggered device. (b) Device triggered by dissolving PEG with saline. Scale bar = 500 μm.
Local field potentials (LFPs, 1-1000 Hz filter settings, 3 kHz sampling frequency) (Figure 4-37a and Figure 4-38a) indicated successful electrical recordings before and after deployment (colored lines indicate individual recordings from each of the 20 laser trains, while the black line indicates the average of these 20 recordings). The LFP amplitude changed after deployment (Figure 4-38a) as expected, since the probe tip moved deeper into the brain and further away from the stationary source of laser stimulation. Simultaneously with LFPs, multi-unit activity (MUA) was successfully recorded in response to laser pulses both before and after probe deployment (raw traces in Figure 4-37b and Figure 4-38b, filter settings 0.3 - 8 kHz, ~50 kHz sampling frequency). Histograms for quantification across trials (Figure 4-37c and Figure 4-38c) confirmed the reliability of the evoked activity over a total of the 20 laser stimulation trains.

Figure 4-37 In vivo acute recordings of neural activity in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse in response to a 10 Hz laser stimulation train for the pre-deployed Gen 2 device. (a) Local field potentials. (b) Multi-unit activity. (c) Multi-unit activity histogram.
Figure 4-38 *In vivo* acute recordings of neural activity in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse in response to a 10 Hz laser stimulation train for the post-deployed Gen 2 device. (a) Local field potentials. (b) Multi-unit activity. (c) Multi-unit activity histogram.

To ensure physiological origin of the recorded activity, the stimulation frequency was changed from 10 Hz (Figure 4-39) to 100 Hz (Figure 4-40) and back to 10 Hz (Figure 4-41). Increasing the stimulation frequency from 10 Hz to 100 Hz resulted in the loss of the correlation between stimulation and neural response and reducing the frequency again to 10 Hz resulted in a recovery of correlation, as expected based on previous studies [109]. This frequency cycling was repeated after device deployment (Figure 4-42, Figure 4-43 and Figure 4-44). A negative control experiment was also performed following the animal tests by immersing the probes in 0.9% sodium chloride solution; no response was detected following optical stimulation (data not shown).
Figure 4-39 In vivo acute recordings of neural activity in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse in response to a 10 Hz laser stimulation train for the device in the pre-deployed configuration. (a) Multi-unit activity and (b) Multi-unit activity histogram.

Figure 4-40 In vivo acute recordings of neural activity in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse in response to a 100 Hz laser stimulation train for the device in the pre-deployed configuration. (a) Multi-unit activity and (b) Multi-unit activity histogram.

Figure 4-41 In vivo acute recordings of neural activity in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse in response to a 10 Hz laser stimulation train for the device in the pre-deployed configuration. (a) Multi-unit activity and (b) Multi-unit activity histogram.
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Figure 4-42 *In vivo* acute recordings of neural activity in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse in response to a 10 Hz laser stimulation train for the device in the post-deployed configuration. (a) Multi-unit activity and (b) Multi-unit activity histogram.

Figure 4-43 *In vivo* acute recordings of neural activity in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse in response to a 100 Hz laser stimulation train for the device in the post-deployed configuration. (a) Multi-unit activity and (b) Multi-unit activity histogram.

Figure 4-44 *In vivo* acute recordings of neural activity in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse in response to a 10 Hz laser stimulation train for the device in the post-deployed configuration. (a) Multi-unit activity and (b) Multi-unit activity histogram.
Note that these this set of experiments was performed independently on two mice (each implanted with a primed BL1T4 device), with the second implant performed 2 weeks after the first. Both surgeries (each lasting 2-5 hours) were terminal and the devices were deployed intraoperatively during the course of each surgery. For each animal, 1-3 blocks of data were recorded at a 10 Hz laser stimulation, followed by 1-3 blocks of data at a 100 Hz laser stimulation, followed by 1-3 blocks of data again at a 10 Hz laser stimulation. After device deployment, 1-3 blocks of data were again recorded at a 10 Hz laser stimulation, followed by 1-3 blocks of data at a 100 Hz laser stimulation, followed by 1-3 blocks of data at a 10 Hz laser stimulation. Each block of data consisted of 20 laser stimulation trains (pulse duration 5 ms, inter-train interval of 5 s). All recordings shown above were from the large electrode sites of each device.

These tests demonstrate successful in vivo triggering and electrical recording of neural activity in acute conditions.

4.6 Conclusions

While the major accomplishments of Gen 1 were the design of a new microfabrication process flow and the successful validation of the electrical functionality of the device in an in vivo testing, the major accomplishment of Gen 2 was the successful validation of the integrated functionality (recording+deployment) of the device in an acute in vivo setting (section 4.5.4).

Other key accomplishments of Gen 2 devices were:

1. **Device geometry design for compatibility with in vivo setup**
   
   Gen 2 devices were designed to have a large portion of the device (~1.5 mm) sticking out beyond the coverslip support (sections 4.2.1, 4.3.2). This eliminated the “wings” issue of Gen 1 (Figure 3-14), which was an impediment to the ease of device implantation during Gen 1 in vivo studies.

2. **Electrode design improvements**
   
   Gen 2 device tips had three electrode sites (vs. two in Gen 1). Additionally, based on impedance tests (section 3.4.3) and in vivo studies (section 3.5.2) of Gen 1 devices, a subset of Gen 2 devices were designed to have larger electrode sites (site areas as high as 3150 μm² compared to a maximum area of 750 μm² for Gen 1 electrode sites).

3. **Fabrication-related improvements**
   
   a. Gen 1 device fabrication (section 3.3) had indicated a need for added redundancy in the electrode traces in narrow regions such as the device leg region, in order to protect against imperfect gold liftoff (step 4 in Figure 3-4). Therefore, Gen 2 devices had 5-6 redundant traces over each leg (Figure 4-1), as opposed to a single trace (Figure 3-1). This approach worked well in preserving electrical connectivity of the electrode traces even when the gold liftoff was not perfect. The electrode trace redundancy was, therefore, also used in the Gen 3 design (section 5.2).

   b. A post-fabrication nanostrip soak was introduced (section 4.3.1.1) to clear any undesired polyimide residue from narrow regions, such as between the device legs.
c. Release holes were introduced in the Gen 2 B-type devices (section 4.2, Figure 4-7) to reduce the time taken for the final sacrificial etch (step 6 in Figure 3-4). It was found that B-type devices did release from the wafer significantly faster than other devices that did not have release holes (3-4 days vs. 1 week). Therefore, Gen 3 devices were all designed to have release holes (section 5.2).

4. Interfacing with the optogenetic mouse model
While Gen 1 worked to establish a working interface with the rat model test setup, Gen 2 also established a working interface with the optogenetic mouse model setup (sections 4.5.3 and 4.5.4). This is useful in providing options for future devices generations to be tested in either the rat or the optogenetic mouse model.

5. Histology studies
Gen 2 devices were the first set of devices for which histology studies were performed (section 4.5.1), thereby, establishing histology protocols that could be easily adapted for future device generations.

Gen 2 device tests highlighted three key areas of improvement to enable chronic in vivo studies with the device:

1. Remote on-demand deployment for chronic experiments
Gen 1 and Gen 2 devices used a water-soluble glue, PEG, which was dissolved by manually applying saline to the device head region (sections 3.4.2 and 4.4.2). This approach is not very compatible with chronic studies, where manual application of saline to deploy the device would require physical access to the device, which might not be easy if the device is sealed inside the animal skullcap to maintain a sterile environment. A way to deploy the device remotely, without, having to physically access the device post-implantation would be ideally suited for chronic studies.

2. Sensing of deployment in a chronic setting
Since the Gen 2 recording+triggering study (section 4.5.4) was performed intraoperatively, it was easy to visually confirm the deployment of the device as the craniotomy had not been sealed at the time of device deployment (Figure 4-36). For chronic studies, however, it would be useful to have a way to confirm device deployment, even after the device has been sealed inside the skullcap post-implantation.

3. Increasing number of electrode sites
Increasing the number of electrode sites per device implant would increase the probability of one of the electrode sites being in close proximity to an electrically active neuron, and would be particularly useful from chronic studies in non-optogenetic rodents. Such a multiplexed electrode approach is common in many neural probe technologies such as the Utah array [60].

Thus, Gen 2 devices built on what was learned from Gen 0 and Gen 1 devices, in order to enable the first in vivo demonstration of the integrated functionality of the device in an acute setting, paving the way for devices that would be compatible with chronic in vivo studies.
Chapter 5: Gen 3

5 GEN 3

This chapter describes the Gen 3 devices, which built on what was learned from Gen 1 and Gen 2 (chapters 3 and 4) to incorporate various improvements in the device design, fabrication and packaging:

1. Gen 3 devices were designed to have remote on-demand device deployment by incorporating integrated resistive heaters. This eliminated having to manually deploy the device, as was done in Gen 1 and Gen 2.
2. Gen 3 devices incorporated two new features to sense device deployment: a new packaging setup that allowed for visual access to the device even after implantation, and a capacitance sensor pad design to sense device deployment by sensing changes in capacitance.
3. Gen 3 devices were designed to have up to 27 electrode sites per implant package (versus 2 sites for Gen 1 and 3 sites for Gen 2).

While Gen 1 and Gen 2 devices tested the in vivo functionality of the device in an acute setting, Gen 3 devices were designed to test various design features that would eventually enable chronic recording with these devices.

This chapter will discuss the relevant models (section 5.1) that were used to guide the Gen 3 device design, the proposed device dimensions and model predictions (section 5.2), the microfabrication process used to manufacture these devices (section 5.3), benchtop characterization of the device tips, the device shuttle structure and the electrode sites (section 5.4), and in vivo tests (section 5.5) in the rodent model.

5.1 Modeling
A new feature in Gen 3 devices was a resistive heater mechanism to melt the glue holding the device back in order to remotely deploy the probes on-demand. Therefore, in addition to the structural models described previously in sections 2.1.1 and 2.1.2, thermal and electrical models were used to model this heater structure and trigger mechanism of Gen 3 devices.

Thermal models were used to determine the amount of power the heater needs to be capable of delivering in order to melt the glue during the triggering step. Electrical models were used to determine the appropriate electrode material and dimensions that can deliver this required power.

Five important considerations for the device heater are:

1. It should be capable of delivering enough power during the triggering step such that the temperature of the heater island rises enough to melt the glue being used to hold the shuttle body in the primed position.
2. The voltages and currents required to achieve this desired power should be in a range that can be handled by benchtop laboratory power supplies.
3. The current density resulting from the applied currents should be smaller than the failure current density of the heater material.
4. The heater material should be compatible with the microfabrication facilities available.
5. Since the heater material also doubles as the electrode, it should have characteristics that make it suitable for recording physiological activity from the brain.

The thermal model in section 5.1.1 informs the desired power in consideration 1. Considerations 2 and 3 are then used in section 5.1.2 to design a heater with a considerable factor of safety that can deliver the required currents/voltages repeatably without failure.

5.1.1 Thermal model

5.1.1.1 Thermal model – determining required power input

The device heater structure is modeled using a thermal model, which predicts the power input required into the system in order to melt the glue in the device trigger mechanism during device deployment.

To maximize safety and heater efficiency, it is desirable to have the heater as thermally insulated from the rest of the device structure as possible. This localizes the temperature rise to the heater region (thereby minimizing the temperature rise in other areas of the device, such as the device tip, which is desirable from the point of view of safety), and also reduces the power input required to achieve the desired temperatures to melt the glue. In order to thermally insulate the heater, a device structure, as shown in Figure 5-1, is envisioned.

![Figure 5-1 Schematic of heater island structure.](image)

In order to simplify the analysis, the island structure housing the heater is assumed to be isothermal. This is justified by comparing convective and conductive resistances experienced by the central heater island. The ratio of the conductive and convective thermal resistances seen by the heater is given by

\[
\frac{\text{Conductive thermal resistance}}{\text{Convective thermal resistance}} = \frac{l_{\text{characteristic}} / (k_{\text{heater-island}})}{1 / (h_c A_{\text{heater-island}})} = \frac{h_c l_{\text{characteristic}}}{k}, \quad \text{Equation 5-1}
\]

where \(l_{\text{characteristic}}\) is the characteristic length for conduction heat transfer within the heater island, \(A_{\text{heater-island}}\) is the area of each of the top and bottom surfaces of the heater island, \(h_c\) is the heat transfer coefficient for convection from the heater island to the ambient, and \(k\) is the conductive heat transfer coefficient of the structural material of the heater island.
Chapter 5: Gen 3

As will be seen later, the heater island is designed to have dimensions on the order of 1 mm. Moreover, typical convection heat transfer coefficients for free convection in air are on the order of 10 Wm⁻²K⁻¹, and conductive heat transfer coefficients for polyimide (chosen material based on the structural model – section 2.1.2.1) is on the order of 0.1 Wm⁻¹K⁻¹. Using these typical values in equation 5.1 above, a ratio of conductive resistance to convective resistance is calculated to be 0.1. Thus, heat transfer within the heater island structure via conduction is much easier than convection from the island structure to the surroundings via convection. Therefore, the assumption of an isothermal heater island structure is valid.

A simplified heater model is shown in Figure 5-2 below.

![Figure 5-2 Simplified model with heater resistances.](image)

In this simplified model then, the total power input is the sum of the heat flows \( q_1, q_2, \) and \( q_3. \)

**Estimating \( q_1 \)**

The heat flow in this branch is due to free convection from the top and bottom surfaces of the isothermal heater island. This heat flow in the steady-state is given by [110]

\[
q_1 = 2h_c A_{heater-island} (T_A - T_{ambient}).
\]  

**Equation 5-2**

As will be seen later in section 5.2, typical areas of the heater island are 1 mm X 1 mm, and typical temperatures \( T_A \) that the heater island needs to reach are 50 K higher than ambient temperatures. Assuming a heat transfer coefficient for free convection to be 10 Wm⁻²K⁻¹, it can be estimated that \( q_1 \) will be on the order of 1 mW.

**Estimating \( q_2 \)**

The heat flow in this branch will be due to fin-type cooling through the device shuttle and front half of the device. This heat flow is more difficult to model exactly using analytical methods; however, some simplifying assumptions can be made. If the heat flow in this path is modeled similar to the heat flow in
Chapter 5: Gen 3

an infinite fin with width equal to the width of the device shuttle, then the heat flow \( q_2 \) can be estimated to be \([110]\)

\[
q_2 = \sqrt{h_c P_2 k_{c,2} (T_A - T_{ambient})}. \tag{5-3}
\]

As will be seen in section 5.2, typical widths of the device shuttle are on the order of 200-500 \( \mu m \), and typical thicknesses are on the order of 20 \( \mu m \). Therefore, typical values of the perimeter \( P \) can be assumed to be on the order of 1 \( mm \), and typical values of the cross-sectional area \( A_c \) can be assumed to be 500 \( \mu m \times 20 \mu m = 10^{-8} \) \( m^2 \). Assuming the heat transfer coefficient \( h \) to be 10 \( Wm^{-2}K^{-1} \) (typical for free convection in air), the conduction coefficient \( k \) to be 0.1 \( Wm^{-1}K^{-1} \), \( q_2 \) can be estimated to be 0.1 mW.

Estimating \( q_3 \)

The heat flow in this branch will be due to fin-type cooling through the neck of the heater island and then through the back of the device. This heat flow is also more difficult to model exactly using analytical methods; however, some simplifying assumptions can be made. If the heat flow in this path is modeled similar to the heat flow in an infinite fin with width equal to half the width of the entire device, then the heat flow \( q_3 \) can be estimated to be \([110]\)

\[
q_3 = \sqrt{h_c P_3 k_{c,3} (T_A - T_{ambient})}. \tag{5-4}
\]

As will be seen in section 5.2, typical widths of the device widths are on the order of 5 \( mm \), and typical thicknesses are on the order of 20 \( \mu m \). Therefore, typical values of the perimeter \( P_3 \) can be assumed to be on the order of 10 \( mm \), and typical values of the cross-sectional area \( A_{c,3} \) can be assumed to be 5 \( mm \times 20 \mu m = 10^{-7} \) \( m^2 \). Assuming the heat transfer coefficient \( h \) to be 10 \( Wm^{-2}K^{-1} \) (typical for free convection in air), the conduction coefficient \( k \) to be 0.1 \( Wm^{-1}K^{-1} \), \( q_3 \) can be estimated to be 1.5 mW.

Therefore, the total power input into the system can be estimated to be the sum of \( q_1 \), \( q_2 \), and \( q_3 \), which is on the order of 2.5 mW. Since this is an extremely simplified model of the actual system, a considerable factor of safety needs to be added when accounting for the power generation capacity of the device heater (section 5.1.2).

Model assumptions

The model makes the following assumptions, which affect the accuracy of its predictions:

1. The model assumes a 1-D fin in calculating heat flows \( q_2 \) and \( q_3 \) above. This assumes infinite conduction in one of the horizontal directions, which is an inaccurate assumption. In reality, there will be a temperature drop in all horizontal directions on the device as one moves further away radially from the central heater island. Therefore, the model overestimates the heat transfer by assuming a 1D fin.

2. For ease of calculation, the model assumes that the entire polyimide structure is free standing in air. In reality, a large portion of the polyimide structure will be in contact with the packaging – therefore, instead of free convection, heat will be conducted out from the surfaces of the device in contact with the device packaging. Depending on the contact resistances between the device and the packaging, and depending on the conductive heat transfer coefficient of the packaging,
the actual power input into the system might be different than that predicted by the model. The ratio of the thermal resistance due to conduction through the device packaging in contact with the device (actual conditions) to the thermal resistance due to free convection from a free standing device (model conditions) is given by

\[
\frac{\text{Conductive thermal resistance through packaging}}{\text{Convective thermal resistance (free convection)}} = \frac{h_{\text{device}} l_{\text{characteristic,package}}}{k_{\text{package}}}, \quad \text{Equation 5-5}
\]

where \(l_{\text{characteristic,package}}\) is the characteristic length for conduction heat transfer through the device package, \(h_{\text{device}}\) is the heat transfer coefficient for convection from the device to the ambient, and \(k_{\text{package}}\) is the conductive heat transfer coefficient of the structural material of the heater island. Assuming a heat transfer coefficient \(h_{\text{device}}\) for free convection to be 10 \(\text{Wm}^{-2}\text{K}^{-1}\), a conduction heat transfer coefficient \(k_{\text{package}}\) to be 0.5 \(\text{Wm}^{-1}\text{K}^{-1}\) (typical for most plastics), and a characteristics package length of \(l_{\text{characteristic,package}}\) to be 1 cm, this gives a ratio of conductive resistance to convective resistance of 0.2. Thus, conduction through the device package can be significant, and the model underestimates the power input into the system by neglecting this heat flow path.

3. For ease of calculation, the model also neglects the glue which is placed on the heater island. Incorporation of the glue could affect model predictions in two ways – the glue could affect the steady-state heat flows depicted in Figure 5-2 and it could affect time taken by the system to reach steady state. Glues used in this work such as polyethylene glycol typically have thermal conductivities on the order of 0.2 \(\text{Wm}^{-1}\text{K}^{-1}\), similar to that of the polyimide heater island on which the glue is placed. The addition of the glue, therefore, should not change the isothermal heater island assumption of the model that was validated using equation 5.1. Therefore, the steady-state predictions of the model will be valid even with the incorporation of the glue. The addition of the glue will, however, increase the time taken by the system to reach steady state, since the glue adds some thermal mass to the system. The time taken to melt the glue will be given by \([110]\)

\[
q_{\text{glue}} t_{\text{glue}} = m_{\text{glue}} c_{p,\text{glue}} \Delta T_{\text{glue}} + m_{\text{glue}} c_{\text{fusion,glue}}, \quad \text{Equation 5-6}
\]

where \(q_{\text{glue}}\) is the power input into the glue, \(t_{\text{glue}}\) is the time taken to melt the glue, \(m_{\text{glue}}\) is the mass of the glue, \(c_{p,\text{glue}}\) is the heat capacity of the glue, \(\Delta T_{\text{glue}}\) is the temperature difference between the ambient and the melting point of the glue, and \(c_{\text{fusion,glue}}\) is the heat of fusion of the glue. The value of \(q_{\text{glue}}\) can be assumed to be \(q_i\) (the heat flow from the thermal island to the ambient), which was estimated using equation 5.2 earlier to be on the order of 1 mW. Material properties for a typical glue such as polyethylene glycol are: \(c_{p,\text{glue}} = 2000 \text{Jkg}^{-1}\text{K}^{-1}\), \(c_{\text{fusion,glue}} = 160 \text{kJ/kg}\), \(m_{\text{glue}} = 1 \text{mg}\) (1 mm X 1 mm X 1 mm volume at a density of 1 gcm\(^{-3}\)). For a value of \(\Delta T_{\text{glue}}\) of 30-50K, the above values indicate that the time taken to melt the glue is dominated by the melting process itself (versus heating the glue from ambient temperature to its melting point) and is approximately 160 s.
It is hard to predict which of the effects in points 1 and 2 above will ultimately dominate. To be safe, device heaters were designed to have the capability to produce up to 250 mW of power at the failure current density of the chosen electrode material. At this upper limit of power input (assuming all heat flow paths grow proportionately), the time required to melt the glue will also be significantly reduced to a few seconds.

5.1.1.2 Thermal model - biological safety consideration

It is important that during the trigger deployment step, the levels of heat delivered to the brain remain within reasonable limits. In order to estimate the rise in temperature of the portion of the device that will be in contact with brain tissue, the system is modeled as shown in Figure 5-3 below.

![Diagram of device schematic and thermal model](image)

The system is modeled as a thermal fin with a heat source at the location of the gold resistive heater. The tip of the fin is located at the hottest part of the device that will be in contact with brain tissue. The tip is assumed to be insulated. For such a fin, the temperature $T$ at any location $x$ is given by

$$
\frac{T(x) - T_{\text{ambient}}}{T(x) - T_{\text{base}}} = \frac{\cosh m(L-x)}{\cosh mL}.
$$

Equation 5-7

This overestimates the temperature rise across the device in two ways:

1. It neglects heat flow from the base of the fin to the rest of the device through the numerous shuttle legs. Thus, the actual temperature rise along the device will be lower than what is predicted by this model.
2. By forcing an insulating boundary condition on the plane where the device contacts the brain ($x = L$), this model neglects heat flow from the part of the device submerged in brain tissue to the surrounding brain tissue. This further overestimates the temperature rise in the device.

Thus, if the model then predicts a temperature change that is physiologically safe, the actual device should have an even lower temperature rise.
5.1.2 Electrical model – failure current density consideration

Consider a resistive heater of rectangular dimensions with a width $w_{\text{heater}}$, thickness $t_{\text{heater}}$, and total length $l_{\text{heater}}$. Its cross-section area $A_{\text{cross-section}}$ is then given by

$$A_{\text{cross-section}} = w_{\text{heater}} t_{\text{heater}}.$$  \hspace{1cm} \text{Equation 5-8}

The device heater is modeled as a linear resistor with a uniform cross-section and a uniform flow of electric current. Its resistance $R$ is given by

$$R = \frac{\rho_{\text{heater}} l_{\text{heater}}}{A_{\text{cross-section}}},$$  \hspace{1cm} \text{Equation 5-9}

where $\rho$ is the electrical resistivity of the heater material, and $l_{\text{heater}}$ is the total length of the heater.

The power consumed by a heater of resistance $R$ is given

$$\text{Power} = i^2 R,$$  \hspace{1cm} \text{Equation 5-10}

where $i$ is the current flowing through the heater.

For a microscale heater such as this, an important electrical consideration is ensuring that the current densities the heater encounters are smaller than the failure current density of the material used. If the current densities are too high, it can lead to heater failure due to electromigration or melting [111], [113]. Current density is given by

$$J = \frac{i}{A_{\text{cross-section}}}.$$  \hspace{1cm} \text{Equation 5-11}

Using equations 5.8, 5.10 and 5.11, we can re-write the current density as

$$J = \sqrt{\frac{\text{Power}}{R(w_{\text{heater}} t_{\text{heater}})^2}}.$$  \hspace{1cm} \text{Equation 5-12}

Based on the analysis in section 5.1.1.1, we chose the maximum power generation capacity of the heater to be 250 mW. The heater material (which determines the resistivity $\rho$) and dimensions then need to be chosen such that the current density predicted by equation 5.12 is at or below the failure current density of the chosen heater material when the heater generates 250 mW of power.

5.1.3 Capacitive sensor model

Gen 3 devices have integrated metal pads to sense capacitive changes as the device changes from the primed to the deployed configurations. A capacitance model is developed to design the optimal size, shape, and location of these metal pads that would maximize the capacitance change that is being detected. The model is show in Figure 5-4 below.
The metal pads are modeled as parallel plate capacitors. Thus, the capacitance between plates 1 and 2 is given by

$$C_{1-2} = \frac{\varepsilon A}{d_{1-2}},$$

Equation 5-13

where \(\varepsilon\) is the permittivity of the medium between the two pads, \(A\) is the area of each pad, and \(d_{1-2}\) is the distance between pads 1 & 2. Similarly, the capacitance between pads 1 & 3, and pads 2 & 3 is given by,

$$C_{1-3} = \frac{\varepsilon A}{d_{1-3}}, \text{ and}$$

Equation 5-14

$$C_{2-3} = \frac{\varepsilon A}{d_{2-3}}.$$  

Equation 5-15

As the device is deployed, pad 1 will move relative to pads 2 and 3. Thus, the changes in the three capacitances will be given by

$$\Delta C_{1-2} = \frac{\varepsilon A}{d'_{1-2}} - \frac{\varepsilon A}{d_{1-2}},$$

Equation 5-16

$$\Delta C_{1-3} = \frac{\varepsilon A}{d'_{1-3}} - \frac{\varepsilon A}{d_{1-3}}, \text{ and}$$

Equation 5-17

$$\Delta C_{2-3} = \frac{\varepsilon A}{d_{2-3}} - \frac{\varepsilon A}{d_{2-3}} = 0,$$

Equation 5-18

where \(d'_{1-2}\) and \(d'_{1-3}\) are the new distances between pads 1 & 2, and pads 1 & 3 respectively. Pads 2 & 3 do not move relative to each other, and hence, the capacitance \(C_{2-3}\) measured between these pads should not change as the device is deployed. The measured capacitance \(\Delta C_{2-3}\) can, thus, be used a control to account for any capacitance drifts or noise in the system that might be external to the device deployment event.
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Equations 5.16 and 5.17 are used to optimize the areas and distances between the capacitance pads such that the predicted differences in the capacitance are in the range of values that can be detected by commercially available benchtop capacitance measurement chips. The chip chosen for our experiments was the Analog Devices 7746 chip, which has a stated resolution down to 4 aF and is accurate to within 4 fF. Since our capacitance setup is measuring the change in capacitance, the accuracy of the chip is not as important as the resolution and repeatability. The capacitance pads in our device, therefore, need to be designed such that the difference in capacitances predicted between the primed and deployed states of the device are $>> 4$ aF.

Note that the analysis above uses a parallel plate capacitor model (in which the plates face each other) to model plates that lie in the same plane. This model is, thus, an oversimplification of the actual system for the purpose of ease of calculation. It, nonetheless, gives us insights into the design of the capacitance pads.

Note also that the measured capacitance changes would have to be much higher than any noise in the measurement system, in order to be detectable with reasonable repeatability. While it is hard to predict the levels of noise that might affect the system, this consideration implies that steps should be taken to eliminate any potential sources of noise from the measurement system.

5.2 Proposed design and model predictions

Gen 3 devices were designed based on analytical model predictions (sections 2.1, 5.1) in conjunction with benchtop (sections 3.4, 4.4) and in vivo (sections 3.5, 4.5) test results of Gen 1 and Gen 2 devices. A typical Gen 3 device is shown in Figure 5-5 below.

![Figure 5-5 Schematic of a typical Gen 3 device. Green lines indicate the edge of the polyimide structure, blue lines indicate the metal trace, and magenta lines indicate the exposed areas of the metal.](image)

Gen 3 devices have three major style variations (Figure 5-6) – PS-type devices with a single shuttle and a single tip per shuttle, TT-type devices with two shuttles and a single tip per shuttle, and JT-type devices...
with a single shuttle and 3 tips per shuttle. PS-type devices are the most similar to Gen 1 and Gen 2 devices; TT-type devices are intended to increase the number of electrode sites available, while providing the ability to deploy each device tip independently (since each shuttle has its own trigger mechanism); and JT-type devices are intended to increase the number of electrode sites, while still maintaining a small footprint.

Another important variation of Gen 3 devices is the device length (Figure 5-7). As will be seen in section 5.3.2, these varying lengths allow for stacking multiple devices on top of each other to increase the number of electrode sites per implant package.

Approximately half of Gen 3 devices were made with an OH-type head geometry similar to Gen 1 and Gen 2 devices, where the head is at one end of the moveable shuttle structure (Figure 5-8). The other half of Gen 3 devices were made with a new type of head geometry, where the head is in the middle of the moveable shuttle structure. These devices with the new NH-type heads were designed to reduce the stress induced in the head region by making the geometry more symmetric.

Figure 5-6 Schematic of major shape/style variations of Gen 3 devices - (a) PS-type devices, (b) TT-type devices, (c) JT-type devices. Green lines indicate the edge of the polyimide structure, blue lines indicate the metal trace, and magenta lines indicate the exposed areas of the metal.
Figure 5-7 Schematic showing the three lengths of devices – (a) short devices, (b) medium devices, and (c) long devices. Green lines indicate the edge of the polyimide structure, blue lines indicate the metal trace, and magenta lines indicate the exposed areas of the metal.

Figure 5-8 (a) NH-type device head, with the head in the middle of the movable shuttle structure. (b) OH-type device head similar to Gen 1 and Gen 2 device heads.

5.2.1 Gen 3 – proposed tip dimensions and model predictions
Gen 3 devices were designed to have two major tip variations (Figure 5-9). T20-type tips are larger with a 20 μm tip width and three electrode sites per tip, whereas T10-type tips are smaller with a 10 μm tip width and a single electrode site per tip.
Figure 5-9 Schematic of the two leg/tip types – (a),(b) T20-type device, and (c),(d) T10-type device. Green lines indicate the edge of the polyimide structure, blue lines indicate the metal trace, and magenta lines indicate the exposed areas of the metal.

The predicted buckling forces of these Gen 3 tips are listed in Table 5-1 below. Note that Gen 3 tips are even longer than Gen 2 tips (Figure 4-3, Table 4-1), with values of \( l_{\text{tip},2} \) over 3 mm vs. \( \sim 1.5 \) mm for Gen 2 tips. This change was motivated by the need to move the device leg region further up from the surface of the brain, for easier surgical implantations. Gen 2 and Gen 3 devices were both designed for tips to penetrate approximately 1.5 mm into brain tissue to reach the target neurons. For Gen 2 devices, this meant that the device leg region lay just above brain tissue. However, the additional 1.5 mm in Gen 3 tip lengths results in the device leg region lying above the surface of the skull (since the skull is about 1 mm or so above the surface of the brain in rats). This has two advantages over the Gen 2 design: (1) it allows for thicker packaging to be used since the packaging can now sit outside the skull and is not restricted by the size of the craniotomy; and (2) it reduces the probability of fluid wicking up from the brain to the device leg region by having space between the leg region and the moist surface of the brain.

Because Gen 3 device tips were designed to be longer than Gen 2 tips, they are floppier with the predicted buckling forces ranging from 0.5 \( \mu \text{N} \) to 1.1 \( \mu \text{N} \) at the lower limit (Table 5-1), compared to 13 to 18 \( \mu \text{N} \) for Gen 2 (Table 4-1). While Gen 0 benchtop tests had indicated that forces on the order of a few \( \mu \text{N} \) were sufficient to pierce explanted brain tissue, no live tissue piercing tests had been done with devices with predicted buckling forces as low as 13 to 18 \( \mu \text{N} \). It was acknowledged that this could be a potential issue for Gen 3 device implantation; however, it was a tradeoff that had to be made for the reasons mentioned in the preceding paragraph. Additionally, it was assumed that if the Gen 3 tips ended up not piercing brain tissue easily, they could be reinforced with PEG during device insertion, as was done for some of the curled Gen 2 devices (section 4.3.1.2).
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Table 5-1 Proposed tip dimensions for 10 µm thick Gen 3 devices and corresponding buckling force predictions calculated using equation 2.1. The upper limit is calculated using \( l_{tip,1} \) as the tip length, and the lower limit is calculated using \( l_{tip,2} \) as the tip length. Actual dimensions may differ from these designed dimensions by a few µm due to microfabrication processing limitations.

<table>
<thead>
<tr>
<th>Device type</th>
<th>Tip length - limit 1 ( l_{tip,1} ) (µm)</th>
<th>Tip length - limit 2 ( l_{tip,2} ) (µm)</th>
<th>Tip width ( b_{tip} ) (µm)</th>
<th>Predicted buckling force - upper limit (mN)</th>
<th>Predicted buckling force - lower limit (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>xx-xx-T10</td>
<td>108</td>
<td>3081</td>
<td>10</td>
<td>0.4</td>
<td>0.0005</td>
</tr>
<tr>
<td>xx-xx-T20</td>
<td>113</td>
<td>3086</td>
<td>20</td>
<td>0.8</td>
<td>0.0011</td>
</tr>
</tbody>
</table>

5.2.2 Gen 3 – proposed electrode site dimensions

In vivo tests with Gen 2 devices (section 4.5) had indicated that the larger electrodes sites (with site areas 1500-3000 µm²), designed for recording field potentials, were very effective at doing so. However, it was found to be more challenging to record endogenous neural activity with the smaller electrode sites (with sites areas of 440 µm²), likely because of polyimide residue on the electrode sites (section 4.4.3). Therefore, Gen 3 electrode sites (optimized for the detection of single units with an area of 500 µm² (Table 5-2)) had two improvements over the small sites of Gen 2 devices:

1. Gen 3 T20-type sites were comprised of 13 µm wide rectangles vs. the narrower 5 µm wide rectangles in the Gen 2 small sites, making it less likely for polyimide residue to remain during the microfabrication of the devices (since undesired residue is more likely in narrower features). The T10-type electrode sites had to be narrower than their T20-type counterparts due to the smaller width of the T10-type tips. However, even these sites were designed to be slightly wider than the Gen 2 small sites, with a width of 6 µm.

2. Gen 3 device fabrication incorporated an additional plasma cleaning step (section 5.3.1.1) in the fabrication process flow, in order to remove any polyimide residue.

Table 5-2 Proposed electrode site area variations for Gen 3 devices. Actual dimensions may differ from these designed dimensions by a few µm due to microfabrication processing limitations.

<table>
<thead>
<tr>
<th>Tip type</th>
<th>Electrode site dimensions</th>
<th>Electrode site area (µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T10</td>
<td>6 µm X 85 µm</td>
<td>510</td>
</tr>
<tr>
<td>T20</td>
<td>13 µm X 39 µm</td>
<td>507</td>
</tr>
</tbody>
</table>
5.2.3 Gen 3 – proposed leg dimensions and model predictions

The legs in the T20 devices were wider but also longer than legs in the T10 devices (Figure 5-9), in order to achieve similar values for the predicted induced stresses and forces delivered during deployment (Table 5-3). For a 50 µm leg deflection, the T20 devices were designed to have a predicted maximum induced stress of 30 MPa and predicted total force of ~10 mN, whereas the T10 devices were designed to have a predicted maximum induced stress of 42 MPa and predicted total force of ~15 mN. For a 100 µm leg deflection, the T20 devices were designed to have a predicted maximum induced stress of 60 MPa and predicted total force of ~20 mN, whereas the T10 devices were designed to have a predicted maximum induced stress of 83 MPa and predicted total force of ~30 mN. These dimensions were chosen based on benchtop (section 4.4.2) tests with Gen 2 devices, which had indicated that L2 devices, with predicted maximum induced stress of 83 MPa and total force delivered of 30 mN at 50 µm deflection (Table 4-3), were easy to prime and deploy. Note that while Gen 2 devices were designed to be primed up to a 50 µm leg deflection, Gen 3 devices are designed such to be primed up to a leg deflection of 100 µm. This was done to ensure that the device tips would fully penetrate any glial scar in chronic in vivo tests, given the uncertainty in the exact thickness of the glial scar that might develop in response to these devices.

Table 5-3 Proposed leg dimensions for 10 µm thick Gen 3 devices and corresponding predictions of force delivered (calculated using equation 2.5) and maximum induced stress (calculated using equation 2.6). Actual dimensions may differ from these designed dimensions by a few µm due to microfabrication processing limitations.

<table>
<thead>
<tr>
<th>Device type</th>
<th>Leg width (µm)</th>
<th>Leg length (µm)</th>
<th>Number of legs per shuttle</th>
<th>Maximum stress induced (MPa)</th>
<th>Force per leg (mN)</th>
<th>Total force (mN)</th>
<th>Maximum stress induced (MPa)</th>
<th>Force delivered (mN)</th>
<th>Total force (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH-PS-T20</td>
<td>20</td>
<td>500</td>
<td>120</td>
<td>30</td>
<td>0.08</td>
<td>10</td>
<td>60</td>
<td>0.16</td>
<td>19</td>
</tr>
<tr>
<td>OH-JT-T20</td>
<td>20</td>
<td>500</td>
<td>120</td>
<td>30</td>
<td>0.08</td>
<td>10</td>
<td>60</td>
<td>0.16</td>
<td>19</td>
</tr>
<tr>
<td>OH-PS-T10</td>
<td>10</td>
<td>300</td>
<td>340</td>
<td>42</td>
<td>0.05</td>
<td>16</td>
<td>83</td>
<td>0.09</td>
<td>31</td>
</tr>
<tr>
<td>OH-JT-T10</td>
<td>10</td>
<td>300</td>
<td>340</td>
<td>42</td>
<td>0.05</td>
<td>16</td>
<td>83</td>
<td>0.09</td>
<td>31</td>
</tr>
<tr>
<td>NH-PS-T20</td>
<td>20</td>
<td>500</td>
<td>116</td>
<td>30</td>
<td>0.08</td>
<td>9</td>
<td>60</td>
<td>0.16</td>
<td>19</td>
</tr>
<tr>
<td>NH-JT-T20</td>
<td>20</td>
<td>500</td>
<td>116</td>
<td>30</td>
<td>0.08</td>
<td>9</td>
<td>60</td>
<td>0.16</td>
<td>19</td>
</tr>
<tr>
<td>NH-PS-T10</td>
<td>10</td>
<td>300</td>
<td>320</td>
<td>42</td>
<td>0.05</td>
<td>15</td>
<td>83</td>
<td>0.09</td>
<td>30</td>
</tr>
<tr>
<td>NH-JT-T10</td>
<td>10</td>
<td>300</td>
<td>340</td>
<td>42</td>
<td>0.05</td>
<td>16</td>
<td>83</td>
<td>0.09</td>
<td>31</td>
</tr>
</tbody>
</table>

5.2.4 Gen 3 – Proposed heater dimensions and model predictions

Gen3 devices are of three different lengths (Figure 5-7) and therefore, their respective heaters also have varying lengths. Each device heater can be broken down into three sections as shown in Figure 5-10 below, with section A having a heater line width of 20 µm, section B having a heater line width of 40 µm,
and section C having a line width of 100 μm. While all heaters have the same lengths of sections A and B, the short, medium and long heaters vary in the lengths of their section C as shown in Table 5-4 below.

![Figure 5-10 Schematic showing the different sections that make up the resistive heater.](image)

Assuming a gold resistivity of $2.2E-8 \, \Omega \cdot m$, and a heater thickness of 200 nm, then the predicted electrical resistances of the short, medium and long heaters are calculated using equation 5.9 and listed in Table 5-4 below.

### Table 5-4 Table showing the proposed heater dimensions for the 3 types of gold heaters and their predicted electrical resistances (calculated using equation 5.9), assuming an electrical resistivity $\rho = 2.2E-8 \, \Omega \cdot m$, and a gold thickness of 200 nm. Actual dimensions may differ from these designed dimensions by a few μm due to microfabrication processing limitations. Current densities (calculated using equation 5.12) in section A of the heaters for a 250 mW power are also listed.

<table>
<thead>
<tr>
<th>Device type</th>
<th>Device length</th>
<th>Section A</th>
<th>Section B</th>
<th>Section C</th>
<th>TOTAL</th>
<th>Max current density for a 250 mW power input (A/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JT-T10, PS-T10, PS-T20</td>
<td>Short</td>
<td>21</td>
<td>20</td>
<td>2.5</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>21</td>
<td>20</td>
<td>2.5</td>
<td>40</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>21</td>
<td>20</td>
<td>2.5</td>
<td>40</td>
<td>34</td>
</tr>
<tr>
<td>JT-T20</td>
<td>Short</td>
<td>21</td>
<td>20</td>
<td>2.5</td>
<td>40</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>21</td>
<td>20</td>
<td>2.5</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>21</td>
<td>20</td>
<td>2.5</td>
<td>40</td>
<td>42</td>
</tr>
</tbody>
</table>
Note that the above calculations neglect the 50 nm titanium adhesion layer. This is a valid approximation since the electrical resistivity of titanium (4.2 E-8 Ω-m) is almost 20 times that of gold, and its cross-sectional area is a fourth of that of the gold trace. The resistance of the titanium trace is, therefore, almost two orders of magnitude higher than that of the gold trace, and can be neglected.

The thermal model had estimated a power input into the system of 250 mW to melt the glue during the deployment step. The predicted maximum current density for this power input is on the order of $10^{10}$ A/m² for all the heater variations designed. The failure current density for gold, based on other structures with similar dimensions, is $10^{11}$ to $10^{12}$ A/m² [114]. Therefore, the proposed heater dimensions are reasonable and meet all the design criteria discussed at the beginning of section 5.1.

5.2.5 Gen 3 – Proposed capacitive sensor dimensions and model predictions

Figure 5-11 Gen 3 capacitance pad design. (a) NH-type devices. (b) OH-type devices.

The capacitance pads are similarly designed for both NH- and OH-type devices (Figure 5-11). One capacitance pad is located on the moveable shuttle head, while two additional capacitance pads are location on the stationary device body. A fourth non-functional metal pad is located on the device head but designed solely for the purpose of symmetry (to minimize any stresses that might have arisen in the device head due to asymmetric placement of the functional metal pad). All capacitance pads are designed to be square-shaped with sides of 300 μm. For a device deflection of 100 μm, the initial interpad distances are $d_{1,2} = 598$ μm, $d_{1,3} = 480$ μm, $d_{2,3} = 470$ μm, and the final interpad distances are $d_{1,2} = 665$ μm, $d_{1,3} = 470$ μm, $d_{2,3} = 470$ μm. From equations 5.16, 5.17 and 5.18, the predicted capacitances and difference in capacitances between the primed and deployed states is given by $C_{1,2} = 1.33$ fF, $C_{1,3} = 1.66$ fF, $\Delta C_{1,2} = -0.134$ fF, and $\Delta C_{1,3} = 0.035$ fF. These values are much higher than the 4 aF resolution of the capacitance measurement chip in use in our setup and, therefore, should be reasonably detectable (assuming noise levels lesser than these values).

5.3 Fabrication and packaging

5.3.1 Fabrication

The fabrication process flow for the third generation of devices was similar to the process described in section 3.3.1, with three modifications before the final release etch (step 6 in Figure 3-4):

1. An additional oxygen plasma cleaning step was performed to remove any polyimide residue from the electrode sites (section 5.3.1.1).
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2. Polyimide thicknesses were measured using an optical profilometer (section 5.3.1.2).

3. Wafer heaters were characterized using a probe station to measure the electrical resistance (section 5.4.2.1).

5 batches were fabricated; however, only batches 2, 3 and 5 ended up yielding functional devices. Batch 1 devices failed due to excessive oxygen plasma etching which resulted in the devices being etched away. Oxygen plasma descumming on subsequent batches was performed in small time increments in order to avoid this. Batch 4 devices passed visual inspection; however, benchtop characterization revealed that most devices had issues with electrical connectivity in the gold traces resulting in open heaters (infinite resistance) and non-functional electrodes. This was likely due to an issue with the gold deposition step in this batch, possibly due to tool malfunctioning, which also led to deviations from theory for the heater resistances of batches 2, 3 and 5 (section 5.4.2.1).

Batches 2 and 3 were similar to Gen 2 devices in the range of thicknesses for the polyimide and metal layers. Batch 5 consisted of 6 wafers – wafers 1 through 3 were designed to similar to Gen 2 devices with 200 nm of deposited gold for the metal layer, but wafers 4 through 6 were designed to have 600 nm of gold.

Figure 5-12 shows a PS-type device (single tip per shuttle) and Figure 5-13 shows a JT-type device (three tips per shuttle). Zoomed-in microscope pictures of various features of the device are shown in the insets. The T20- and T10-type tips and corresponding electrode tips can be seen in the microscope pictures in Figure 5-14. A zoomed-in SEM (FEI/Philips XL30 Field Emission Gun (FEG) Environmental Electron Scanning Microscope (ESEM)) photograph of the electrode site on a T20-type tip is shown in Figure 5-15.
Figure 5-12 Stereoscope picture of a PS-T10 device with a single tip per moveable shuttle and a single electrode site per tip. All metal traces end in exposed contact pads (not shown). Scale bar = 1 mm. Top inset shows a zoomed-in microscope picture of the capacitance pad region of a different device. Bottom inset shows a zoomed-in microscope picture of the gold resistive heater of a different device.
Figure 5-13 Stereoscope picture of a JT-T20 device with three tips on a single moveable shuttle and with three electrode sites per tip. All metal traces end in exposed contact pads (not shown). Scale bar = 1 mm. Inset shows a zoomed-in stereoscope picture of the tip region.

(a)

Figure 5-14 Microscope pictures of the device tips and electrodes sites. (a) A T20-type tip with three electrode sites. Scale bar = 100 μm. (b) A T10-type tip with a single electrode site. Scale bar = 50 μm.
5.3.1.1 Oxygen plasma descumming step

The issue of webbed legs that was seen in Gen 2 devices (4.3.1.1) was eliminated in Gen 3 devices by two modifications in the design and fabrication process:

1. Increasing inter-leg distance

   The leg spacing was 80 μm for Gen 3 T20-type devices and 25 μm for Gen 3 T10-type devices (compared to 20 μm for Gen 2 devices).

2. Tuning the exposure settings in the photolithography step

   One way to reduce undesirable residue is to reduce the light exposure time during the photolithography step – this gives less time for light to diffuse into unwanted areas. However, reducing the exposure time by too much prevents complete cross-linking of polymers in areas that are desired to remain on the wafer, especially if the photoresist coating is thick (more than 1-3 μm). This can make such areas partially soluble in the photoresist developer and lead to unwanted removal of photoresist from some areas. This problem is then commonly addressed by reducing the
exposure time but exposing for multiple intervals – this allows for complete cross-linking in areas that are desired to remain in the wafer, while preventing unwanted diffusion of light into areas that are to be etched away.

The two modifications above helped eliminate the issue of webbed legs in Gen 3 devices. However, the issue of undesired polyimide residue on some of the electrode sites persisted in Gen 3 devices, as can be seen in Figure 5-16.

![Microscope images of electrode sites of some fully cured devices, showing polyimide residue on the sites. Scale bars = 10 μm.](image)

Figure 5-16 Microscope images of electrode sites of some fully cured devices, showing polyimide residue on the sites. Scale bars = 10 μm.

Usually, an acetone rinse followed by a very short oxygen plasma etch is standard to remove any unwanted photoresist residues after any photolithography step. However, polyimide is more resistant to etching than standard photoresists used for patterning. As a result, acid etches (such as microstrip) or oxygen plasma etches are required to remove any unwanted polyimide residue that remains in the electrode site areas after photolithography. Oxygen plasma cleaning (Matrix 106 Asher®, RF Forward power 250 W, pressure 0.5 Torr, MFC1 5%) on Gen 3 devices was successful in removing undesired polyimide residue from the electrode sites (Figure 5-17). As can be seen, each successive plasma cleaning step removes increasing amounts of the polyimide residue until the sites are cleared. The plasma etch is more directional than wet etches after device release, so it preferentially thins out the top polyimide layer by some amount. However, the thickness of the top polyimide layer in Gen 3 devices
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was also tuned to account for any thinning that might be caused by the oxygen plasma cleaning step – as a result, Gen 3 devices are not overly thinned due to the cleaning step, which was an issue with Gen2 devices that were subject to post-fabrication nanostrip cleaning (section 4.3.1.1). The thinning of Gen 3 devices due to plasma cleaning was estimated (based on microscope images) to be not more than 1-2 μm.

Figure 5-17 Electrode sites from a Gen 3 device showing the effect of oxygen plasma cleaning in removing polyimide residue on the sites. The device shown above is from a wafer that was cleaned in steps of 4 mins. (a) Total plasma etch time = 4 mins, (b) Total plasma etch time = 12 mins, (c) Total plasma etch time = 20 mins, (d) Total plasma etch time = 32 mins. Scale bars = 10 μm.

5.3.1.2 Polyimide thickness tuning and stress-induced curling

Gen 2 devices had exhibited some curling (section 4.3.1.2), which made the devices harder to implant during in vivo tests. It is hypothesized that the observed curling was due to a mismatch in residual stresses in the two polyimide layers. Such a residual stress mismatch is common in microfabricated thin films and can lead to curling of thin films [81]. Therefore, the thicknesses of the top and bottom polyimide layers in Gen 3 devices were tuned such that their residual stresses would cancel each other out. To determine the optimal relative thicknesses of the top and bottom polyimide layers needed to do this, one of the fabrication batches of Gen 3 varied the thickness of the top polyimide layer while

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keeping the thickness of the bottom polyimide layer constant. Polyimide layer thicknesses as measured by an optical profilometer (Wyko Veeco®) are shown in Figure 5-18. Table 5-5 shows the spin speeds that were used for the polyimide layers, the etch times of the oxygen plasma cleaning step, as well as the level of observed curling in the devices. It was found that wafer 1 devices curled downward (device tips pointing in the direction of the bottom polyimide layer), wafer 2 was relatively flat, wafer 3 had a slight upward curl (device tips pointing in the direction of the top polyimide layer), and wafers 4 and 5 had a significant upward curl. Thus, it seems that a top polyimide layer that is slightly thicker than the bottom polyimide layer seems to be optimal in producing devices with minimal curling. For subsequent batches, the spin recipes of the top and bottom polyimide layers were modeled after wafer 2 of this batch.

Note that these thicknesses were measured after the oxygen plasma de-scumming step, and just before the final aluminum etchant release etch. Since the oxygen plasma selectively etches the top polyimide layer (the bottom layer is mostly protected from the plasma by the top layer), the thicknesses of the top polyimide layer right after the spin-on step will be higher than those depicted in Figure 5-18.

<table>
<thead>
<tr>
<th>Wafer</th>
<th>Bottom layer thickness</th>
<th>Top layer thickness</th>
<th>Oxygen plasma clean time</th>
<th>Observed curling</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3000</td>
<td>1500</td>
<td>32 mins</td>
<td>Significant curling downward</td>
</tr>
<tr>
<td>2</td>
<td>3000</td>
<td>2000</td>
<td>4 mins</td>
<td>Minimal curling</td>
</tr>
<tr>
<td>3</td>
<td>3000</td>
<td>2500</td>
<td>8 mins</td>
<td>Slight curling upward</td>
</tr>
</tbody>
</table>

Figure 5-18 Optical profilometer measurements of the top and bottom polyimide layer thicknesses for Gen 3 Batch 3 devices. The error bars indicate one standard deviation in either direction.

The polyimide spin speeds for the wafers described in Figure 5-18 are listed in Table 5-5 below.
Chapter 5: Gen 3

<table>
<thead>
<tr>
<th>4</th>
<th>3000</th>
<th>3000</th>
<th>4 mins</th>
<th>Significant curling upward</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3000</td>
<td>3500</td>
<td>4 mins</td>
<td>Significant curling upward</td>
</tr>
</tbody>
</table>

Results from wafer 3 were used to inform spin speeds for subsequent fabrication batches that would result in minimal curling as well total thicknesses close to 10 µm. Spin speeds for batch 5 are listed in Table 5-6.

Table 5-6 Spin speeds for the top and bottom polyimide layers for Gen3 Batch 5 devices. A slower speed results in a thicker polyimide coating.

<table>
<thead>
<tr>
<th>Wafer</th>
<th>Spin speeds (rpm)</th>
<th>Oxygen plasma clean</th>
<th>Observed curling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bottom layer</td>
<td>Top layer</td>
<td>time</td>
</tr>
<tr>
<td>1</td>
<td>3000</td>
<td>2000</td>
<td>32 mins</td>
</tr>
<tr>
<td>2</td>
<td>2500</td>
<td>2000</td>
<td>8 mins</td>
</tr>
<tr>
<td>3</td>
<td>2000</td>
<td>1500</td>
<td>20 mins</td>
</tr>
<tr>
<td>4</td>
<td>3000</td>
<td>2000</td>
<td>20 mins</td>
</tr>
<tr>
<td>5</td>
<td>2500</td>
<td>2000</td>
<td>36 mins</td>
</tr>
<tr>
<td>6</td>
<td>2000</td>
<td>1500</td>
<td>76 mins</td>
</tr>
</tbody>
</table>

Devices in batch 5 all exhibited minimal curling, which was then further reduced, as needed, using a post-fabrication weight clamp (section 5.3.1.3).

Figure 5-19 Optical profilometer measurements of the top and bottom polyimide layer thicknesses for Gen 3 Batch 5 devices. The errors bars indicate one standard deviation in either direction.
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5.3.1.3 Post-fabrication weight clamp to uncurl devices

Figure 5-20 below shows the post-fabrication setup used to further straighten devices.

(a) (b) (c)

Figure 5-20 Weight clamp setup. (a) Devices are first placed between two flat silicon wafers. (b) The wafers are clasped together. (c) An aluminum block is placed on the wafers and the setup is heated on a hot plate at around 130°C for a few hours.

5.3.2 Packaging and connectorization

Significant improvements were made in Gen 3 packaging to allow for multiplexing as well as visual confirmation of device deployment during chronic animal studies. In order to multiplex devices, custom PCBs were designed and manufactured (Figure 5-21) by Michael Sunshine, who is part of Chet Moritz’ research group in the University of Washington. These PCBs are improvements over those from previous generations in three important ways:

1. The PCB vias were designed to have the same spacing and dimensions as the gold contact pads of the Gen 3 devices. This allows for the devices to be attached directly onto the PCB using silver conductive epoxy, thereby, bypassing the need for using wires to connect the device to the PCB (as was done with previous generations). This makes the resulting package more compact, and also significantly reduces the packaging assembly time.

2. The PCB dimensions and vias were designed to obviate the need for using an additional cover slip for device support (as was done with previous generations). Instead, the PCB and corresponding 3D-printed package (Figure 5-22) serve as a structural support for the Gen3 devices. This, again, makes the package more robust and compact, and also significantly reduces packaging assembly time, as compared with Gen2 packaging.

3. The PCB was designed to have three sets of vias, each set corresponding to the short, medium and long lengths of the fabricated devices respectively. Thus, up to three devices can be stacked, one on top of the other, to create an array of electrode tips. PCBs with a single set of vias (not shown in Figure 5-21) were also designed to package single devices.

In addition to the PCBs, custom packaging material was designed and 3D printed (Figure 5-22). These 3D-printed parts were used to package both single devices, as well as multiple devices into a single implant package.
Chapter 5: Gen 3

Figure 5-21 Custom-made PCBs for multiplexed Gen3 devices. (a) Picture of PCB. (b) Schematic of PCB showing the electrical connections.

Figure 5-22 Pictures and corresponding schematics of the 3-D printed pieces. (a) Bottom piece. (b) Middle piece. (c) Top piece. Scale bars = 5 mm.
In order to package a single device, the device is first electrically connected to the PCB by solder bumping the PCB vias, manually adding dabs of silver conductive epoxy (MG chemical #8331) onto the PCB vias, and subsequently, aligning the device gold contact pads with the PCB vias and pressing the device onto the PCB. After the silver epoxy has cured, the bottom 3D printed part is attached to the PCB using Loctite® superglue. The device edges are then attached to the bottom 3D printed part using superglue. Next, the top 3D printed part is attached onto the bottom 3D printed part, using superglue. This serves as an additional clamp to keep the device edges in place during the subsequent priming step. To complete the packaging assembly, glass coverslips are glued onto both sides of the package using epoxy (DevCon 2-Ton epoxy). After the epoxy cures, unwanted areas of the coverslips are removed using a Dremel® tool. An un-primed device, ready for implantation, is shown in Figure 5-24, whereas a primed device, ready for implantation, is shown in Figure 5-25.

Figure 5-23 Schematic showing an exploded view of part of the assembly.
Figure 5-24 Gen 3 implant package with a single PS-T20 device, prior to priming. (a) Front of device. Gold contacts pads of the device are soldered onto vias on this side (not visible through the epoxy). (b) Back of device. Scale bars = 2 mm.
Figure 5-25 (a) Gen 3 implant package with a single PS-T20 device, after priming. (b) Zoom-in of the head region showing the deflected legs. Scale bars = 1 mm.

Gen3 devices can also be arrayed to have multiple probe tips. The packaging assembly process is similar to that of the single devices, with some minor modifications. The first part of the packaging process is identical to that of the single device assembly. The device is first electrically connected to the PCB by solder bumping the PCB vias, manually adding dabs of silver conductive epoxy (MG chemical #8331) onto the PCB vias, and subsequently, aligning the device gold contact pads with the PCB vias and pressing the device onto the PCB. After the silver epoxy has cured, the bottom 3D printed part is attached to the PCB using Loctite® superglue. The device edges are then attached to the bottom 3D printed part using superglue. Next, the 3D printed middle piece is attached onto the bottom PCB part. This connector piece acts as the spacer between the first polyimide device layer and the next one, and is designed to be ~0.5 mm thick to provide an inter-tip distance comparable to that in other technologies like the Utah Array, which has an inter-tip distance of 400 μm [60]. This helps ensure that each device tip is recording physiological signals that are relatively independent of each other. A second polyimide device is then superglued onto the existing package, followed by another middle piece. The third polyimide device is then superglued on top of this, and finally, the top 3D printed part is attached using superglue. The final packaged devices prior to triggering are shown in Figure 5-26 and Figure 5-27 below. To complete the packaging assembly, glass coverslips are glued onto both sides of the package using epoxy (DevCon 2-Ton epoxy). After the epoxy cures, unwanted areas of the coverslips are removed using a Dremel® tool.
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Figure 5-26 Gen3 implant package with 3 PS-T1b devices stacked on top of each other. Scale bar = 1 mm.

Figure 5-27 Different views of a Gen 3 implant packages with 3 JT-T10 devices. Scale bar = 2 mm.
This custom 3-D printed package serves three important functions:

1. It clamps the device edges (so the central device shuttle can be pulled back during the priming step), while keeping the central device shuttle and the supporting device legs suspended in free space. This is in contrast to the Gen2 device package, where the device edges had to be glued onto the supporting glass coverslip (section 4.3.2), leaving the device shuttle and/or legs susceptible to being stuck to the underlying glass coverslip in case of presence of moisture. While this was not an issue in the Gen2 benchtop or in vivo triggered tests, the new 3D printed package for the Gen3 devices is more robust.

2. It allows for visual inspection of the device legs, even after implantation of the device in an animal. Such visual inspection after implantation was not possible with packages for previous generations, where the device was covered up in skull cement after implantation, with no direct visual access to any part of the microfabricated device.

3. By allowing for multiple devices to be stacked on top of each other (Figure 5-26, Figure 5-27, Figure 5-28), this new packaging setup greatly increases the number of electrodes sites per implant package. Up to 27 electrode sites can be incorporated into a single implant package (with three JT-T20 devices stacked on top of each other) versus 2 sites for Gen 1 and 3 sites for Gen 2 device packages.
5.4 Benchtop characterization

Benchtop tests were used to characterize Gen 3 device features before testing the devices in vivo (section 5.5). Gen 3 devices have two new features compared to previous generations: the resistive heater part of the trigger mechanism, and the capacitive sensor pads that detect device deployment. The resistive heater was characterized extensively (section 5.4.2.1) and preliminary benchtop tests with the capacitive sensor pads were also performed (section 5.4.4). Similar to Gen 1 and Gen 2 devices, the trigger mechanism and the electrode sites were also characterized (sections 5.4.2.2 and 5.4.3 respectively).

5.4.1 Tip buckling tests

Because Gen 2 devices were successful at piercing brain tissue without buckling and because Gen 3 device tips were not too much more flexible than Gen 2 device tips (predicted buckling forces of 5-11 μN compared to 13-18 μN for Gen 2 tips), Gen 3 device tips were not tested separately in benchtop tip buckling tests before in vivo testing.

5.4.2 Resistive heater and trigger mechanism characterization

Heaters were characterized by first measuring the electrical resistances (section 5.4.2.1), followed by benchtop tests to melt different kinds of glue materials by flowing a current through the heaters (section 5.4.2.2).

5.4.2.1 Heater resistance measurements

Heater resistances were measured by using a probe station on the devices right before the etch step (step 6 in Figure 3-4). Preliminary heater measurements were made for batch 2, followed by more extensive measurement for batch 3 (Table 5-7), and even more extensive measurements for batch 5 (Table 5-8 and Table 5-9).

For batch 2, preliminary resistance measurements indicated heater resistances on the order of 1 kΩ, which is an order of magnitude higher than predicted by theory (equation 5.9). To investigate this further, more extensive measurements were performed on batch 3 and batch 5.

For batch 3, heaters were tested for each type of device (short, medium, long) in each wafer (Table 5-7). Device types (e.g., JT-T20, PS-T10 etc.) were not recorded. Measured resistances are from 3 devices of each type (for example, 3 short heaters from wafer 1, 3 medium heaters from wafer 1, and so on). Resistances predicted by equation 5.9 for 200 nm thick gold heaters, assuming an electrical resistivity of 2.2E-8 Ω-m, are also listed in the table. Similar to batch 2, resistance measurements for batch 3 are an order of magnitude higher than predicted by theory.

Table 5-7 Measured electrical resistances of heaters of batch 3. Measurements are from 3 devices of each heater type. Predicted resistances are calculated using equation 5.9 for a 200 nm gold film, assuming an electrical resistivity of 2.2E-8 Ω-m.

<table>
<thead>
<tr>
<th>Heater Length</th>
<th>Predicted Resistance (Ω) for JT-T20 t = 200 nm</th>
<th>Predicted Resistance (Ω) for all other devices t = 200 nm</th>
<th>Average measured resistance (kΩ)</th>
</tr>
</thead>
</table>

147
For batch 5, almost all devices in each wafer were probed. Table 5-8 lists the average and standard deviations of the measured resistances of heaters from wafers 1, 2, and 3 of batch 5, which were all designed to have a gold thickness of 200 nm. Heaters of all the devices, except the JT-T20 devices were of the same dimensions and, hence, are lumped together in the table; heaters in the JT-T20 devices were slightly longer. Predicted resistances based on equation 5.9 are also listed in the table. Similarly, Table 5-9 lists the average and standard deviations of the measured resistances of heaters from wafers 4 and 5 of batch 5, which were both designed to have a gold thickness of 600 nm. Heaters of all the devices, except the JT-T20 devices were of the same dimensions and, hence, are lumped together in the table; heaters in the JT-T20 devices were slightly longer. Predicted resistances based on equation 5.9 are also listed in the table.

Table 5-8 Measured electrical resistances of heaters from wafers 1, 2, and 3, of batch 5. Measurements are from 25 short devices for wafer 1, 24 for wafer 2, 16 for wafer 3; 22 medium devices for wafer 1, 20 for wafer 2, 19 for wafer 3; 19 long devices for wafer 1, 20 for wafer 2, 17 for wafer 3; 5 JT-T20 short devices for wafer 1, 6 for wafer 2, 5 for wafer 3; 6 JT-T20 medium devices for wafer 1, 6 for wafer 2, 6 for wafer 3; and 5 JT-T20 long devices for wafer 1, 6 for wafer 2, 4 for wafer 3. Predicted resistances are calculated using equation 5.9 for a 200 nm gold film, assuming an electrical resistivity of 2.2E-8 Ω-m.

<table>
<thead>
<tr>
<th>Device type</th>
<th>Heater Length</th>
<th>Predicted resistance (Ω) ( t = 200 \text{ nm} ) ( \rho = 2.2E-8 \text{ Ω-m} )</th>
<th>Measured resistance (Ω)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wafer 1</td>
<td>Wafer 2</td>
</tr>
<tr>
<td>PS-T20, TT-T20, PS-T10, TT-T10, JT-T10</td>
<td>Short</td>
<td>133</td>
<td>507</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>147</td>
<td>570</td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>160</td>
<td>626</td>
</tr>
<tr>
<td>JT-T20</td>
<td>Short</td>
<td>142</td>
<td>540</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>155</td>
<td>588</td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>169</td>
<td>653</td>
</tr>
</tbody>
</table>
Table 5-9 Measured electrical resistances of heaters from wafers 4 and 5 of batch 5. Measurements are from 13 short devices for wafer 4, 18 for wafer 5; 14 medium devices for wafer 4, 20 for wafer 5; 11 long devices for wafer 4, 15 for wafer 5; 3 JT-T20 short devices for wafer 4, 7 for wafer 5; 5 JT-T20 medium devices for wafer 4, 8 for wafer 5; and 3 JT-T20 long devices for wafer 4, 5 for wafer 5. Predicted resistances are calculated using equation 5.9 for a 600 nm gold film, assuming an electrical resistivity of 2.2E-8 Ω-m.

<table>
<thead>
<tr>
<th>Device type</th>
<th>Heater Length</th>
<th>Predicted resistance (Ω)</th>
<th>Measured resistance (Ω)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>t = 600 nm ( \rho = 2.2E-8 ) Ω-m</td>
<td>Batch 5 Wafer 4</td>
</tr>
<tr>
<td>PS-T20, TT-T20, PS-T10, TT-T10, JT-T10</td>
<td>Short</td>
<td>44</td>
<td>73</td>
</tr>
<tr>
<td>JT-T20</td>
<td>Medium</td>
<td>49</td>
<td>78</td>
</tr>
<tr>
<td>JT-T20</td>
<td>Long</td>
<td>53</td>
<td>84</td>
</tr>
<tr>
<td>JT-T20</td>
<td>Short</td>
<td>47</td>
<td>77</td>
</tr>
<tr>
<td>JT-T20</td>
<td>Medium</td>
<td>52</td>
<td>83</td>
</tr>
<tr>
<td>JT-T20</td>
<td>Long</td>
<td>56</td>
<td>92</td>
</tr>
</tbody>
</table>

There are two important observations that can be made from the information above:

1. Resistances measured in batch 2 and batch 3 heaters are approximately an order of magnitude higher than predicted by theory.
2. Resistances measured in batch 5 are higher than predicted by theory, but only by 1 to 3 times.

There are two possible reasons for the higher-than-expected resistances across all batches:

1. Electrical resistivity of the as-deposited films could be higher than the resistivity of bulk gold. This can be a result of the deposition method where micro-cracks might be introduced into the gold traces, either due to a thermal mismatch between the gold and the underlying polyimide substrate or due to an uneven polyimide substrate.
2. The actual thicknesses of the deposited films could be different from the expected thicknesses—a lower-than-expected film thickness would result in a higher-than-predicted resistance.

The change in measured resistances from batches 2 and 3 to batch 5 was likely caused by miscalibration of the metal deposition tool. Around the time of fabrication of batch 5, reports from other users in the microfabrication facility had suggested that the metal deposition tool was depositing film thicknesses 3-4 times higher than the target thickness value.

Therefore, the discrepancies observed between the measured and predicted resistances of Gen 3 heaters are explained as follows. It is hypothesized that our deposited films have a higher electrical resistivity than that of bulk gold. This explains the general higher-than-expected measured resistances. Additionally, it is hypothesized that tool miscalibration led to batch 5 heater thicknesses being approximately 4 times the target thicknesses. This theory is supported by Table 5-10. Variations 1 and 2 show the conditions for the original predictions. However, if the electrical resistivity of the deposited films is 1.65E-7 Ω-m (instead of 2.2E-8 Ω-m for bulk gold), then variation 3 shows the predicted heater
resistances for batches 2 and 3, which match well with the ranges of measured values (Table 5-7). If the gold thickness for batch 5 is four times the target thickness, then variation 4 shows the predicted heater resistances for wafers 1, 2 and 3 of batch 5, which match with the ranges of measured values (Table 5-8) better than the original predictions. Similarly, variation 5 shows the predicted heater resistances for wafers 4 and 5 of batch 5, which match with the ranges of measured values (Table 5-9) better than the original predictions. Note that the wafer-to-wafer variation in the measured heater resistances seen in Table 5-7, Table 5-8 and Table 5-9 might have been because of variations in the electrical resistivity of the deposited gold in each wafer, which might have been due to micro-cracks in the gold film, as discussed earlier. The difference in polyimide thicknesses in between wafers might have resulted in variations in the levels of microcracks, leading to variations in the electrical resistivity of the deposited gold films from wafer to wafer.

Table 5-10 Predicted resistance for the device heaters for different electrical resistivities and heater thicknesses.

<table>
<thead>
<tr>
<th>Variation 1 (Original predictions for batches 2, 3 and batch 5 – wafers 1, 2, 3)</th>
<th>Variation 2 (Original predictions for batch 5 – wafers 4, 5)</th>
<th>Variation 3 (Hypothesized approximate conditions for batches 2, 3)</th>
<th>Variation 4 (Hypothesized approximate conditions for batch 5 – wafers 1, 2, 3)</th>
<th>Variation 5 (Hypothesized approximate conditions for batch 5 – wafers 4, 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho = 2.2E-8 \ \Omega\cdot m$ $t= 200 \ nm$</td>
<td>$\rho = 2.2E-8 \ \Omega\cdot m$ $t= 600 \ nm$</td>
<td>$\rho = 1.65E-7 \ \Omega\cdot m$ $t= 200 \ nm$</td>
<td>$\rho = 1.65E-7 \ \Omega\cdot m$ $t= 800 \ nm$</td>
<td>$\rho = 1.65E-7 \ \Omega\cdot m$ $t= 2.4 \ \mu m$</td>
</tr>
<tr>
<td>JT-T10, PS-T10, PS-T20</td>
<td>JT-T20</td>
<td>JT-T20</td>
<td>JT-T20</td>
<td>JT-T20</td>
</tr>
<tr>
<td>S</td>
<td>133</td>
<td>44</td>
<td>1000</td>
<td>250</td>
</tr>
<tr>
<td>M</td>
<td>147</td>
<td>49</td>
<td>1099</td>
<td>275</td>
</tr>
<tr>
<td>L</td>
<td>160</td>
<td>53</td>
<td>1198</td>
<td>300</td>
</tr>
<tr>
<td>S</td>
<td>142</td>
<td>47</td>
<td>1066</td>
<td>267</td>
</tr>
<tr>
<td>M</td>
<td>155</td>
<td>52</td>
<td>1165</td>
<td>291</td>
</tr>
<tr>
<td>L</td>
<td>169</td>
<td>56</td>
<td>1264</td>
<td>316</td>
</tr>
</tbody>
</table>

Note that the above discussion is not meant to infer the exact values of the electrical resistivity or the thickness of the deposited metal film. Rather, it is meant to illustrate that the experimental observations (higher-than-predicted heater resistances and the lower resistance of batch 5) are likely caused due to a higher-than-expected electrical resistivity of the deposited gold films for all batches and a higher-than-expected gold layer thickness for batch 5.

Note also that even if the higher-than-predicted heater resistances are caused due to microcracks in the deposited gold, this should not significantly affect the impedances of the electrode sites since these site impedances are much higher (~100 kΩ to 1 MΩ at 1 kHz) than any increase in trace impedance (~1 kΩ) that might result from microcracks in the deposited gold films.
5.4.2.2 Trigger mechanism testing

Despite discrepancies between predicted and measured values of Gen 3 resistive heaters (section 5.4.2.1), benchtop tests indicated that the heaters could successfully deploy the devices. Because Gen 3 devices are designed for triggering with a resistive heater approach (versus by manually melting a glue as in Gen 1 and 2), they can be primed and deployed using a water-soluble glue such as PEG (Figure 5-29), or a water-insoluble glue such as bonewax (Figure 5-30).

![Figure 5-29 TT-T20 Gen 3 device (batch 2 wafer 1) triggering with PEG. (a) Device is primed with approximately 50 μm of leg deflection and held back with PEG (mol. wt. 8000). (b) A 7 mA current through the resistive heater (measured resistance of 820 Ω) at 6 V causes the PEG (M.P. ~59-64°C) to melt and deploy the device. Scale bars = 1 mm.](image1)

![Figure 5-30 JT-T20 Gen 3 device (batch 2 wafer 3) triggering with bonewax. (a) Device is primed with approximately 80 μm of leg deflection and held back with bonewax. (b) An 11 mA current through the resistive heater (measured resistance of 1.15 kΩ) at 13 V causes the bonewax (M.P. ~65°C) to melt and deploy the device. Scale bars = 500 μm.](image2)

Information from the triggering tests of select representative devices is shown in Table 5-11 below. It was found that PEG 8000 (M.P. 59-64°C) typically melted for input powers of 40 to 70 mW, whereas bonewax (M.P. ~65°C) typically melted for input powers of ~150 mW. Moreover, it was found that heaters could be used repeatably to melt the PEG/bonewax (benchtop heater tests were performed 2-5 times on a single device across 10-15 devices), indicating that the currents in the heater are below the failure current density values. For the upper limit power of 150 mW, typical 1 kΩ heaters (batches 2, 3) had currents on the order of 10 mA (as seen in Table 5-11), which translates to a maximum current density of 2.5E9 A/m². Similarly, typical 100 Ω heaters (batch 5) experienced currents on the order of 40 mA, which translates to a maximum current density of 10¹⁰ A/m². These values are below the failure current density of gold microstructures, which is typically on the order of 10¹¹ to 10¹² A/m² [114].
Table 5-11 Measured voltages and currents during device deployment, for select Gen 3 devices. Resistances measured prior the deployment tests are also listed.

<table>
<thead>
<tr>
<th>Device type</th>
<th>Measured resistance (Ω)</th>
<th>Glue used</th>
<th>Glue melting point (°C)</th>
<th>Voltage (V)</th>
<th>Current (mA)</th>
<th>Power (mW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JT-T20</td>
<td>1150</td>
<td>Bonewax</td>
<td>65</td>
<td>13</td>
<td>11</td>
<td>143</td>
</tr>
<tr>
<td>PS-T20</td>
<td>1140</td>
<td>PEG 8000</td>
<td>59-64</td>
<td>9</td>
<td>8</td>
<td>72</td>
</tr>
<tr>
<td>TT-T20 left</td>
<td>820</td>
<td>PEG 8000</td>
<td>59-64</td>
<td>6</td>
<td>7</td>
<td>42</td>
</tr>
<tr>
<td>TT-T20 right</td>
<td>830</td>
<td>PEG 8000</td>
<td>59-64</td>
<td>8</td>
<td>9</td>
<td>72</td>
</tr>
</tbody>
</table>

There are three important conclusions based on these heater characterization results:

1. Similar to the benchtop trigger tests with Gen 1 and Gen 2 devices (sections 3.4.2 and 4.4.2), these tests confirm that the central shuttle structure can be pulled back by 50-100 μm, held in place with a glue such as PEG or bonewax, and can be deployed to move the tip back to the original position.
2. The measured input power values (40 to 140 mW) required to melt the glue are on the same order of magnitude as the thermal model predictions (250 mW) made in section 5.1.1 which seems reasonable given that the simplifying assumptions employed in the thermal model.
3. The repeatable functioning of the heater indicates that the current density considerations taken into account in section 5.1.2 are accurate.

5.4.3 Electrode impedance testing

Gen 3 device electrode site impedances were characterized with a setup similar to the one used for Gen 2 devices (section 4.4.3). Device tips were submerged in saline and an Agilent 4294A precision impedance analyzer was used to sweep a 10 mV AC signal between 40 Hz and 10 MHz. Typical impedances at the physiologically relevant frequency of 1 kHz ranged between 300 kΩ and 2 MΩ. The narrower electrode sites of the T10 tips had a larger impedance than the wider electrode sites of the T20 tips.

Note that while the electrode sites on the Gen 3 T20 tips have a similar area as the tip sites of Gen 2 devices (Table 4-4), the Gen 3 electrode sites have a much lower impedance (few hundred kΩ) compared to the impedance of Gen 2 electrode sites (typically a few MΩ). This supports the polyimide residue theory discussed in section 5.3.1.1 and points to the effectiveness of the oxygen plasma descumming step in removing this undesired residue from the electrode sites.
Table 5-12 Typical measured impedances at 1 kHz for Gen 3 devices. Measured values represent the range of single measurements from multiple (n ≥ 4) devices of each tip type.

<table>
<thead>
<tr>
<th>Tip type</th>
<th>Site dimensions</th>
<th>Site area (μm²)</th>
<th>Typical measured impedances (MΩ) at 1 kHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>T10</td>
<td>6 μm X 85 μm</td>
<td>510</td>
<td>1 to 2</td>
</tr>
<tr>
<td>T20</td>
<td>13 μm X 39 μm</td>
<td>507</td>
<td>0.3 to 1</td>
</tr>
</tbody>
</table>

5.4.4 Capacitive sensor characterization

The capacitance pads of the Gen 3 probes (section 5.2.5) were characterized in preliminary benchtop tests by Abubakar Abid in Prof. Joel Voldman’s research group at MIT. Devices were epoxied onto a glass coverslip for support and the metal contact pads corresponding to the capacitance pads were connected to 50 μm diameter stainless steel wires using silver epoxy. These wires were connected to an Analog Devices AD7746 24-bit capacitance sensor evaluation board. The device was then pulled back using the micromanipulator priming setup (as in section 5.4.2.2), some PEG was melted onto the device head region to hold it in place in the primed configuration, and the micromanipulator was then removed. The capacitance between the pad on the device head and one of the stationary pads on the device body was then measured. The device was deployed by applying a voltage to the device gold resistive heater to melt the PEG. The capacitance was measured again.

Results from two sample device tests are shown below. Figure 5-31 shows that the measured capacitance differs by approximately -1 fF when the device is deployed. This corresponds to ΔC₁₂, which was predicted to be -0.035 fF (section 5.2.5). Figure 5-32 shows that the measured capacitance differs by approximately 5 fF. This represents ΔC₁₃, which was predicted to be 0.134 fF. Note that difference in direction of the measured capacitance values between the two figures comes from the fact that the first setup measures capacitance between pads 1 and 2, which increase in spacing as the device deploys, whereas the second setup measures capacitance between pads 1 and 3, which decrease in spacing as the device deploys (see Figure 5-11 for capacitance pad setup). Note also that the actual measurements are approximately 50 times higher than predicted by theory (section 5.2.5). This might be because the parallel plate capacitor model used was an oversimplification of the actual experimental conditions, or because parasitic capacitances in the measurement system dominated over the capacitances between the sensor pads on the device.

Figure 5-31 Capacitance pad characterization, with x axis showing the measured capacitance in fF. The device was primed by approximately 100 μm and deployed three consecutive times before failure due to tearing of the head during the priming step. The three red stars denote the capacitance measured with the device in the primed configuration, while the three blue dots (extremely close together) denote the capacitance measured with the device in the deployed configuration. The yellow dot indicates the capacitance measurement after the device after device head failure.
These are very preliminary results and indicate that the capacitance pads on the devices could potentially be used to detect device deployment in vivo. However, more benchtop tests with these capacitance sensor pads would be needed to translate this sensing mechanism to be used in chronic in vivo studies.

5.5 In vivo studies

Various in vivo studies were performed in the rat and mouse models to validate the functionality of the devices in an in vivo setting. Since Gen 3 electrode sites had different geometries and impedances compared to Gen 2 devices, the in vivo recording-only capability of Gen 3 devices was first tested (section 5.5.1) to confirm that the new Gen 3 sites can successfully record electrical activity. Preliminary chronic studies and histology were also performed with Gen 3 devices (sections 5.5.2 and 5.5.4).

For the optogenetic mouse studies (section 5.5.1), all surgeries and electrophysiology data analyses were performed by Dr. Ulrich Froriep in Prof. Polina Anikeeva’s research lab at MIT. For the rat studies (section 5.5.2), all surgeries and electrophysiology data analyses were performed by Michael Sunshine and Aiva levins in Prof. Chet Moritz’ research lab in University of Washington. All histology studies (section 5.5.4) were performed by Michael Sunshine.

5.5.1 Intraoperative acute recording in the optogenetic mouse model

The recording functionality of Gen 3 devices was first tested in an acute in vivo setting. The devices were implanted approximately 1.3 mm into the prefrontal cortex of an anesthetized (ketamine/xylazine) transgenic Thy1-ChR2-YFP mouse, along with a optic fiber placed adjacent to the device (Figure 5-33). The ground and reference wires were attached to a skull screw. Neural activity was recorded closely following 20 laser (473 nm wavelength) stimulation trains (each train consisting of 10 pulses at 10 Hz with 5 ms pulse duration, and an inter-train interval of 5 s) delivered using the optic fiber.
Four Thy1-ChR2-YFP mice were implanted (with one device per animal). Local field potentials (LFPs, 1-1000 Hz filter settings, 3 kHz sampling frequency) and multi-unit activity (MUA, filter settings 0.3 to 8 kHz, 50 kHz sampling frequency) in response to laser stimulation were successfully recorded from all implanted animals. Figure 5-34 shows LFPs and Figure 5-35 shows MUA recorded from one representative animal. Endogenous recordings were found to be more challenging, with a potential single-units being successfully recorded in only one of the implanted animals (Figure 5-36).
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Figure 5-34 In vivo acute recordings of neural activity (filtered for local field potentials) in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse in response to a 10 Hz laser stimulation train. In the absence of laser stimulation, the brain reverts back to delta wave patterns characteristics of sleep activity.

Figure 5-35 In vivo acute recordings of neural activity (filtered for multi-unit activity) in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse in response to a 10 Hz laser stimulation train.
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Figure 5-36 In vivo acute recordings of neural activity (filtered for local field potentials) in the medial prefrontal cortex of a Thy1-ChR2-YFP optogenetically active mouse in response to a 10 Hz laser stimulation train. A possible single-unit recording is observed at approximately t = 12.745s.

These acute experiments in the optogenetic mouse model confirm the electrical functionality of the Gen 3 devices and validate the new device packaging for acute in vivo use in the mouse model. For chronic studies (section 5.5.2), the rat model was chosen because of the larger skull size of the rat and because of prior expertise in our collaborating research groups with chronic rat models.

5.5.2 Preliminary chronic studies in the rat model – study 1

5.5.2.1 Study details
A chronic study was performed in the rat model and involved implanting 9 adult female Long Evans rats with PS-T20-type Gen 3 devices. To reduce complexity associated with multiplexing devices, each implant package in this study had one PS-T20 device (similar to Figure 5-24 and Figure 5-25).

5 of the rats were implanted with un-primed devices, which served as controls; 4 of the rats were implanted with primed devices. 2 of the primed devices were scheduled to be deployed 2 weeks post-implantation and the remaining 2 were scheduled to be deployed 4 weeks post-implantation.

The goals of this study were:

1. To validate the new package in a chronic rat model.
2. To test recording and triggering functionality in the rat model.
3. To compare electrical recordings of the control devices versus devices that were deployed 2 weeks post-implantation and 4 weeks post-implantation.
5.5.2.2 Device package and implantation procedure

The experimental setup for these implants is shown in Figure 5-37. Gen 3 device packaging was different from previous generations in a number of ways that necessitated three important new considerations:

1. Package size
   As described in section 5.3.2, Gen 3 packaging allows for multiplexed devices. However, this also results in a thicker implant package (since the 3D printed pieces add thickness to the package) and in a taller implant package (since the Gen 3 PCBs (Figure 5-21) need to be taller to incorporate three sets of vias instead of just one set of vias as was the case with previous generations (Figure 4-14)). An important consideration for Gen 3 in vivo studies, therefore, was ensuring that the new packaging would fit well in the craniotomy, and be mechanically stable after the surgery. Both these considerations were met; however, a reduction in the package size in the future will help prevent unintentional headcap failure, as discussed in section 5.5.2.3.

2. Glass window for visual access
   A new feature in Gen 3 packaging was the glass window that allows for visual access to the device while maintaining a sealed device environment. This allows visual confirmation of device deployment post-implantation without compromising the sterility of the device.

3. Compatibility of primed devices with sterilization processes
   A new consideration for Gen 3 was ensuring proper priming of the devices designed to be deployed post-implantation. Previous Gen 1 and Gen 2 in vivo studies included devices that were either not primed or only deployed intraoperatively. Sterilizing primed Gen 3 devices for post-implantation deployment meant that the glue used to hold the Gen 3 devices back in the primed position needed to be compatible with the sterilization process. Even though a low-temperature oxygen plasma sterilization process was used, it was found that Gen 3 devices primed with PEG 2000 (M.P. ~45-50°C) deployed unintentionally during the sterilization process. Bonewax (M.P. 65°C), was found to survive the sterilization process; however, bonewax was found not to be strong enough to hold the devices primed beyond approximately 50 μm of deflection. Therefore, cyanoacrylate (M.P. ~90°C) was used in the 4 primed devices implanted in this study, since it was found to survive the sterilization process and was capable of holding primed devices deflected up to ~100 μm. An alternative glue that could also be potentially used in the future is a higher molecular weight PEG, such as PEG 20,000 (M.P. ~65°C); however, this approach was not tested in this study. An added benefit to using cyanoacrylate or bonewax over PEG is that these glues are water insoluble and take longer to break down (e.g. it takes 3-6 weeks for cyanoacrylate to break down in water). This provides added protection against unintentional deployment of the device post-implantation due to any moisture inside the implant package.
5.5.2.3 Recording results

While animals were successfully implanted with the package shown in Figure 5-37, all animals in this experiment, unfortunately, had to be prematurely euthanized due to headcap failures within a few days after implantation. In most cases, these headcap failures occurred overnight when the animals were left unsupervised in their cages. It was hypothesized that animal movement in the cages resulted in implant packages getting caught in the animal cages, leading to traumatic headcap failure. An important factor that might have contributed to this was the large height of the Gen 3 implant packages (sticking out 2-3 cm above the skull versus < 1 cm for Gen 1 and Gen 2 devices).

Another unexpected challenge with recording from the animals in this batch (before they were euthanized) was that the animals were unusually aggressive, which meant that the usual post-implantation recording method of clipping on the recording cables to the device connectors in awake ambulatory animals, was challenging. This was likely because the animals in this batch had not been adequately handled before the surgeries. As a result, animals had to be anesthetized before recording from them. However, no reliable single unit recordings were obtained from such recordings or from intraoperative recordings. There are a number of potential reasons why single units were not recorded:

1. Neural activity in anesthetized animals is typically lower than that in awake ambulatory animals. This might explain the lack of successful recordings in the animals that were anesthetized before recording.
2. It is possible that the Gen 3 electrode sites were too small, or the impedances too large to successfully pick up single-unit activity. This might explain the lack of successful intraoperative recordings. Typical microwires that successfully record injury potentials during implantation or neural activity in an intraoperative setting, have an electrode site area of 2000 $\mu m^2$ and sites facing the direction of implantation. In comparison, Gen 3 electrode sites were designed to have areas of 500 $\mu m^2$, and sites perpendicular to the direction of implantation. In the future, increasing the area of electrode sites or applying surface coatings to reduce the site impedance might help resolve this issue.

3. It is also possible that the Gen 3 electrode sites that were recorded from were not in close enough proximity to active neurons. This is likely since the Gen 3 implant packages in this study consisted of a single device per package, with the tip electrode site ~10 $\mu m$ from the very tip and the other electrode sites 300-600 $\mu m$ from the very tip. Typical microwire arrays generally have ~16 electrode sites, with 25% of those sites typically resulting in successful intraoperative recordings. Additionally, microwire arrays typically employ flush-cut electrode sites that are at the very tip of the microwire shanks. Future studies with our device can employ multiple device tips per implant package or electrode sites closer to the very tip of the device, in order to maximize the chance of successful recordings.

5.5.2.4 Takeaways

Even though this study could not be executed as originally planned, some important lessons were learned that can incorporated into future studies:

1. The headcap failure issues in this study can be mitigated in the future by reducing the implant package height, by storing the animals in extra tall cages, and handling the animals as much as possible before the implant surgeries.

2. The device window of the new Gen 3 package (Figure 5-37), which is intended to allow visual access to the device after implantation, worked as designed. Implant packages inspected a few days post-surgery on all animals showed that the device legs were visible through the package window.

3. This study helped iron out many of the issues with sterilizing primed devices in a manner compatible with chronic implants. Future devices can be primed with cyanoacrylate and sterilized in a low-temperature oxygen plasma, as was done in this study. Devices could potentially also be primed with a very high molecular weight PEG or bonewax.

4. The issues with recording endogenous single-unit neural activity need to be resolved before attempting another systematic chronic rat study with these probes (section 6.2.1). Potential strategies are described below in section 5.5.3.4.

5.5.3 Preliminary chronic studies in the rat model – study 2

5.5.3.1 Study details

Another chronic study was performed in the rat model and involved implanting 2 adult female Long Evans rats. Each implant package had one primed JT-T20 device and one unprimed JT-T20 device for a total of 18 active electrode sites (3 sites per tip; 6 tips); however, only 16 of these electrode sites were
connected due to space restrictions on the PCB. The primed devices for each animal were scheduled to be deployed 5 weeks post-implantation. The goal of this study was to test the multiplexed device setup and test device triggering a few weeks post-implantation.

5.5.3.2 Device package and implantation procedure

The experimental setup for this study was similar to that described in Figure 5-37.

5.5.3.3 Results

Electrode site impedances were measured in benchtop tests before implantation and then at regular intervals post-implantation (Table 5-13 and Table 5-14). These impedance tests indicated that most electrode sites were functional and electrically connected to the measurement setup throughout the experiment. However, the measured impedances were found to decrease over time. Impedances ranged from 600 kΩ to 2 MΩ during benchtop tests, but had decreased to 100-150 kΩ for the first animal by 2 weeks post-implantation and to 20-70 kΩ for the second animal by 5 weeks post-implantation. Additionally, recording reliable endogenous neural activity (LFPs or MUA) was found to be challenging in this study as well.

Table 5-13 Progression of electrode site impedances (at 1 kHz) for the first animal over time.

<table>
<thead>
<tr>
<th>Electrode site number</th>
<th>Benchtop test pre-implantation</th>
<th>Immediately following implantation</th>
<th>1 week post-implantation</th>
<th>2 weeks post-implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>750</td>
<td>550</td>
<td>420</td>
<td>120</td>
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</table>
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Table 5-14 Progression of electrode site impedances (at 1 kHz) for the second animal over time.

<table>
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<th>Electrode site number</th>
<th>Benchtop test pre-implantation</th>
<th>Immediately following implantation</th>
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</tr>
</tbody>
</table>

Resistances of the gold resistive heaters of each of the two primed devices were measured immediately after implantation and then again 5 weeks post-implantation. These measurements indicated that the heaters were functional immediately following implantation, but the heaters were found to have an open-circuit resistance at 5 weeks post-implantation. As a result, the primed devices in both animals were unable to be deployed post-implantation.

5.5.3.4 Takeaways

Two major issues seen in this study were the inability to deploy the device after 5 weeks and the lack of reliable endogenous neural recordings.

There are four potential reasons that might have contributed to the inability of the device to be deployed post-implantation:

1. Delamination of the polyimide leading to malfunctioning heaters
   It is possible that the functionality of the device heaters degraded over time after device implantation. Heater functionality was confirmed during device implantation by measuring heater resistances. However, resistances were not measured again until 5 weeks after implantation (at the time device deployment was attempted). It is possible that the heater...
functionality degraded at some point after device implantation, leading to an open-circuit resistance reading when device deployment was attempted 5 weeks post-implantation. One reason for heater degradation over time might be the polyimide potentially delaminating in response to the moisture in the implant package and leading to cracks in the heater traces. It is also possible that the movement of the animal in the cage post-implantation ruptured a heater trace. Delamination of polyimide would also explain the decreasing electrode site impedances that were observed.

To confirm if delamination of the polyimide was the main cause of the deployment issues seen in this study, the following benchtop test can be performed before further chronic in vivo tests. Packaged devices can be left with tips immersed in saline and electrical resistances of the device heaters and impedances of the electrode sites can be measured at different time points (e.g. after 1 day, 1 week, after 2 weeks, after 4 weeks, etc.). If the heater resistances and electrode site impedances stay constant over time, then delamination of the polyimide can be eliminated as the cause of the device deployment issues and the decreasing electrode site impedances seen in this study. If the heaters show an open-circuit resistance at a certain time point or if the electrode site impedances decrease over time, then microscope and SEM images at various time points can be used to correlate any delamination in the polyimide with the issues being observed.

If polyimide delamination is found to be the issue with chronic device deployment, then strategies to reduce delamination would have to be employed in future chronic in vivo studies. This might include packaging improvements to prevent exposure of the heater region to moisture. If there are particular areas of the device (e.g. corners) that are more prone to delamination, then future device geometries can be modified to minimize such delamination-prone areas. Changes to the polyimide fabrication recipe might also help protect against delamination. For example, partially curing the first polyimide layer at a lower temperature than the current recipe in 3.3.1 might help increase the number of open chemical bonds at the surface, which might in turn help increase the bonding strength of the second polyimide layer to the first one.

2. Stress relaxation in the polyimide legs
Another potential reason for the difficulty in deploying the device post-implantation might be stress relaxation (also known as plastic creep) in the primed polyimide legs. This is a phenomenon in which the stress induced in a material reduces over time for a constant strain, primarily due to plastic deformations over time as the structure is kept in the strained configuration [106]. A study [115] on the viscoelastic properties of thin films made from polyimide PI2525 indicates that stress threshold beyond which creep sets in for this polyimide is 95 MPa (failure stress for this polyimide is ~130 MPa). However, the polyimide used in this work (HD-4100) might have different properties, and these properties might also be sensitive to the particular microfabrication recipes and conditions used. Hence, benchtop tests would be required to investigate the extent of stress relaxation in the devices used here.
To investigate if stress relaxation of the polyimide legs was the main cause of the deployment issues seen in this study, the following benchtop test can be performed. Packaged devices can be primed and then attempted to be deployed at different time points (e.g. after 1 day, 1 week, after 2 weeks, after 4 weeks, etc.). Microscope images of the device legs before and after deployment can be used to study the amount of plastic creep, if any, that sets in at different time points.

If stress relaxation is found to be the issue with chronic device deployment, then future devices can be designed to incur much lower stresses. This can be done by making the device legs longer, thinner or narrower. There is a lower limit (10 μm) on the thickness and width of the device legs set by the tip size dimensions that are desired to be tested. However, there is no upper limit on the length of the device legs (besides any packaging size considerations). Thus, future devices can be designed to have legs that are 10 μm wide and 10 μm thick (similar to Gen 3 T10-type devices; Table 5-3) but are longer than current Gen 3 device legs. Note that the stress induced in the device legs decreases as the square of the leg length (equation 2.6); however, this has the unintended side-effect of decreasing the force delivered by the device legs as the cube of the leg length (equation 2.5). To maintain the total force delivered by the device, the number of legs can be increased (total force delivered by the device increases linearly with the number of device legs). Increasing the number of legs would result in increasing the shuttle length, which would increase the package length. However, reducing inter-leg spacing would help minimize this increase in shuttle length. For example, a Gen 3 PS-T10 device, which has 340 legs that are each 10 μm wide (with an inter-leg spacing of 25 μm), 10 μm thick and 300 μm long (Table 5-3), is predicted to incur a maximum stress of 83 MPa for a 100 μm device leg deflection and to have a capability to deliver 31 mN of force. A device that has 1360 legs that are each 10 μm wide (with an inter-leg spacing of 10 μm), 10 μm thick and 600 μm long is predicted to incur a maximum stress of 21 MPa for a 100 μm device leg deflection and to have a capability to deliver 16 mN of force. The shuttle length for this device would be approximately twice the shuttle length for the current PS-T10 Gen 3 devices.

3. Films/bloodclots cementing the legs in place over time
   It is possible that fluids from the brain cavity entered the device package post-implantation. Any clots or films deposited by these fluids onto the device legs over time might have cemented the legs in place, preventing them from being deployed when device deployment was attempted 5 weeks post-implantation. This can be addressed through packaging improvements that keep the device legs and heater region as isolated as possible from the brain cavity.

4. Chronic glial scar tissue too strong to pierce with Gen 3 devices
   It is possible that the 5 week glial scar was too strong for the device to pierce through. This hypothesis can be tested with in vivo studies with Gen 3 devices that attempt device deployment at different time points (e.g. after 1 day, after 2 days, after 1 week, etc.). If the devices are unable to be deployed after a certain time point, the packaging window can be
examined to ensure that no films are sticking to the device legs and hindering their movement. If no films are observed, that would indicate that the glial scar is likely too stiff for the device to pierce through, and the devices would have to be redesigned so that the legs are capable of delivering a higher force.

There are three reasons that might have contributed to the inability of the device to reliably record endogenous neural activity in this and the previous study (section 5.5.2):

1. **No neurons in close enough proximity to the electrode sites** 
   It is possible that no neurons were present in close proximity to the electrode sites. If this hypothesis is correct, then this issue can be addressed by multiplexing electrodes to include more electrode sites per implant package and implanting more animals. This would increase the chance that an active neuron is present close enough to one of the electrode sites.

2. **Electrode shape and size** 
   It is possible that the shape and size of the electrode sites is better-suited for recording local field potentials (as evidenced by the ease of successful LFP recordings in the optogenetic mouse model and the rat model; sections 4.5.2, 4.5.3, 4.5.4, 5.5.1) but not endogenous multi-unit activity (as evidenced by the difficulty with reliable endogenous neural activity recordings in the rat model; sections 4.5.1, 5.5.1, 5.5.2, 5.5.3).

   Increasing the size of the electrode sites in future generations might help the electrodes reach an impedance value where single-units are detected more easily. Current Gen 3 sites are approximately 500 µm² in size, whereas Gen 2 small sites were approximately 450 µm² and Gen 2 large sites were approximately 3000 µm². Increasing Gen 3 electrode site areas to values between 500 µm² and 3000 µm² might help the electrodes record single-unit neural activity more effectively.

   The shape of the Gen 3 electrode sites might have also contributed to the difficulty in recording endogenous neural activity. Gen 3 electrodes were all rectangular in shape (13 µm X 39 µm for T20 sites and 6 µm X 85 µm for T10 sites; Table 5-2) similar in shape to the Gen 2 tip sites (5 µm X 88 µm or 5 µm X 288 µm; Table 4-2), which also had trouble recording endogenous neural activity. In comparison, Gen 2 non-tip sites that were better at recording neural activity than the Gen 2 tip sites were triangular in shape with a base of 85 µm and a height of 74 µm. Similarly, Michigan probes or microwire arrays have circular sites with diameters ranging from 700 to 2000 µm². It is possible that the narrow slit-like geometry of the Gen 3 sites was not compatible with recording endogenous neural activity. Testing different electrode site shapes (rectangular, triangular, circular, etc.) in future device generations could help investigate the effects of electrode shape on the quality of neural recordings with this device.

Another factor contributing to the difficulty in recording neural activity might have been the recessed nature of the electrodes in this work (Figure 5-15). Electrode surfaces are recessed by
5-10 μm below the top surface of the device tip – an artefact of the microfabrication process used. In comparison, electrode surfaces of flush-cut microwires, or the Utah array have no such recesses. Similarly, Michigan probes that are typically coated with PEDOT or other coatings have minimal or no recesses.

3. **Electrochemical properties of the electrode sites**

It is possible that the electrochemical properties of the material used in the devices are not ideal for recording endogenous neural activity. This can be addressed in two ways. First, surface coatings can be used to modify the electrode surfaces of the current devices. Surface coatings [74], [75], [80] are a commonly used method to increase the electrochemical surface area of electrode sites while maintaining a small geometrical footprint. Future generations of devices could explore the use of surface coatings to increase the effectiveness of recording endogenous single-unit neural activity. Second, different electrode materials (other than gold) can be explored for use in future device generations. The current devices use gold because of the ease of fabrication of gold and compatibility with polyimide. For future device generations, other metal choices (such as tungsten, etc.) can be explored as a potential strategy to improve quality of the recorded signals (keeping in mind any associated fabrication-related tradeoffs).

5.5.4 Preliminary Gen 3 histology – 5-day PS-T20 implant

Similar to Gen 2 (section 4.5.1), a preliminary histology study performed on Gen 3 devices to investigate the tissue response to these probes and to establish a working histology protocol for the new Gen 3 devices and packaging. The study was done on an un-primed PS-T20 Gen 3 device implanted in the motor cortex of a rat that was euthanized 5 days post-implantation. After fixing the brain, it was sliced horizontally in 20 μm sections. The stains used were NeuN (for neurons) and GFAP (for astrocytes). Figure 5-38 compares the tissue reaction at the surface of the brain (where the implanted device is largest in size) and at a depth of 1.5 mm from the surface of the brain (the approximate location of the device tip). At the surface of the brain, there is an approximately 150 μm X 50 μm region that contains minimal neurons/astrocytes and is surrounded by a dense region of astrocytes. This is as expected since the size of the implanted device is largest at the level of the brain surface and should elicit the largest glial reaction at that level. Moreover, a distinct glial scar, which usually takes about 4-6 weeks to form (section 1.4.2), has not yet formed. At the level of the device tip, a small rectangular region (indicated by the white arrow) shows the device location. There is no significant observable neuronal cell loss in the region immediately surrounding the electrode tip, nor is there any significant observable increase in astrocytic activity. This is, again, as expected since the size of the implanted device is the smallest at the level of the device tip. Figure 5-39 shows an alternative view with brain slices arranged in a way to easily visualize the progression of the tissue reaction from the surface of the brain to the device tip region.

The two important conclusions from this histology study are:

1. As discussed above, the results of this histology study are consistent with what is to be expected based on similar studies in literature (section 1.4). This is encouraging and provides a basis for longer-term histology studies with these devices.
2. This study helped adapt the histology protocols to the new Gen 3 devices and packaging.
Figure 5-38 Histology on a PS-T20 Gen 3 device explanted after 5 days, showing horizontal slices at the cortical surface and at the device tip (1.5 mm from the cortical surface). The GFAP stains for astrocytes and the NeuN stains for neurons. The white arrow indicates the location of the explanted electrode.

Figure 5-39 Histology on a PS-T20 Gen 3 device explanted after 5 days. Horizontal slices from the cortical surface to the device tip (1.5 mm from the cortical surface) show the progression of tissue reaction along the length of the device tip as it tapers down. GFAP stains for astrocytes and NeN stains for neurons.
5.6 Conclusions

Gen 3 devices built on what was learned from Gen 1 and Gen 2 (chapters 3 and 4). Benchtop characterization (section 5.4), acute \textit{in vivo} testing (section 5.5.1) and preliminary chronic \textit{in vivo} testing (section 5.5.2) with Gen 3 devices set the stage for a systematic chronic study in either the rat model or the optogenetic mouse model for future device generations.

Key accomplishments of Gen 3 devices are:

**Design-related**

1. **Remote on-demand triggering mechanism for chronic experiments**
   Both Gen 1 and Gen 2 devices were deployed by manually applying water to a water-soluble glue (sections 3.4.2 and 4.4.2) holding the device in the primed configuration. While this approach worked well acutely, it was not compatible with chronic studies where manual access to the device post-surgery is difficult since the device has to be sealed inside the skullcap in order to maintain a sterile environment. The Gen 3 device design incorporates a new resistive heater mechanism (section 5.2.4) designed, based on thermal and electrical models (sections 5.1.1 and 5.1.2), to deploy the devices on-demand by melting a glue holding the device back in the primed position. While chronic \textit{in vivo} device deployment with this heater has not yet been demonstrated, successful benchtop tests support heater functionality (section 5.4.2) and pave the way for chronic \textit{in vivo} tests with this heater.

2. **Sensing of triggering using integrated capacitance sensor pads**
   Previous device generations relied on visual access to the device to confirm device deployment intraoperatively (section 4.5.4). However, the device is sealed inside the skullcap package during chronic experiments in order to maintain a sterile environment, which limits visual access to the device. Therefore, Gen 3 devices included a new mechanism to remotely sense deployment by measuring changes in capacitance in metal pads on the device (section 5.2.5). Preliminary benchtop experiments (section 5.4.4) suggest that this approach holds promise, but requires more characterization to be incorporated into chronic \textit{in vivo} tests.

**Fabrication-related**

3. **Oxygen plasma descumming to clean electrode sites**
   In order to increase the probability of detecting single units, Gen 3 electrode sites were designed to be small in size (500 $\mu$m$^2$ area, similar size to the small sites of Gen 2 devices), while also having a smaller impedance (hundreds of k$\Omega$) at the physiologically relevant frequency of 1 kHz. This was achieved by adding an oxygen plasma descumming step to the fabrication process (section 5.3.1.1).

4. **Tuning polyimide thicknesses and post-fabrication clamping to minimize device curling**
   In order to minimize the issue of device curling (section 4.3.1.2), the thicknesses of the two polyimide layers of Gen 3 devices were tuned to minimize any residual stress mismatch that
might result in device curling (5.3.1.2). Additionally, a post-fabrication weighted clamping technique (section 5.3.1.3) was used to further reduce curling in any fabricated devices.

Packaging-interface-related

5. Sensing of triggering using an innovative packaging setup
   In addition to the capacitance sensing pads in the device, an innovative packaging system was designed in order to maintain visual access to an implanted device in the chronic setting while also maintaining a sterile device environment (section 5.3.2).

6. Increasing the number of electrode sites per implant package to increase probability of detecting endogeneous neural activity
   Gen 3 devices were designed to have up to 27 electrodes sites per implant package (section 5.3.2) compared to 3 electrodes per package for Gen 2 devices (section 4.3.2). This increases the probability of detecting neural activity. Additionally, the TT-type Gen 3 device offer the ability to control deployment times of individual tips within the same implant package. This gives the ability to investigate the effect of different deployment times in the same animal.

Animal-study-related

7. Towards chronic studies
   From the point of view of animal testing, an important contribution of Gen 3 devices was the design of a packaging interface and corresponding surgery protocols to take the devices a step closer to compatibility with chronic animal studies in the future.

8. Preliminary histology studies to establish working protocols
   Preliminary histology studies (section 5.5.4) helped establish working histology protocols for the new Gen 3 packaging.

A number of lessons were learned from Gen 3 devices, which can be used to improve future devices:

1. Modifying electrode site sizes, shapes and introducing surface coatings
   While the addition of oxygen plasma descumming step helped remove any undesired polyimide residue from electrode site tips to reduce electrode impedance while keeping a small geometrical area, in vivo studies showed that it was still challenging to record endogenous neural activity with Gen 3 probes. Increasing the electrode site sizes, changing the site shapes and introducing surface coatings might help address some of these issues, as discussed in section 5.5.3.4.

2. OH-type vs. NH-type device head
   Although NH-type devices were meant to make it easier to prime devices by distributing the stresses to a more central location of the device shuttle, benchtop and in vivo tests showed that the NH-type device heads were much more difficult to prime. This was likely due to unanticipated out-of-plane device curling in NH-type devices during the priming step. Switching
to OH-type device heads for future device generations would improve the ease of priming the devices.

3. **Amount of force delivered by device legs**
   Gen 3 device legs were designed to deliver a very large amount of force (section 5.2.3). While the device legs could withstand this force, as predicted by our models, an unanticipated failure mechanism was tearing of the device head during the priming step (section 5.4.2.2). To resolve this, 30 to 90% of the device legs had to be manually broken off before priming Gen 3 devices. Therefore, it is recommended that future generations of devices with similar device head geometries should be designed to deliver a smaller amount of force than Gen 3 devices. Alternatively, design improvements need to be incorporated to either reduce the stress concentrations in the device head region or reinforce the device head to protect against failure by tearing.

4. **Size of implant packages**
   *In vivo* tests with arrayed Gen 3 devices suggested that while the implant packages were compatible with chronic studies in the rat model, the packages were quite large and stuck out of the rat skull by a significant amount. In a number of animals implanted with Gen 3 devices, the tall device package frequently got caught in the rat cages after the animal recovered from the implantation surgery. In some animals, this resulted in traumatic skullcap failure a few days post-implant, and these animals had to be euthanized. To avoid this problem in the future, it would be important for future device generations to consider ways to reduce the height and size of the implant package while maintaining the ability to multiplex devices.

5. **Optimizing capacitive sensor pads for *in vivo* testing**
   While preliminary benchtop tests with the capacitive sensor pads showed promise, integration of these pads with the *in vivo* test setup was found to be challenging due to excessive noise in the system. If the capacitive sensing approach is continued in future device generations, the design of this sensor setup would have to be modified to make them more functional during *in vivo* tests.

6. **Strategies to improve robustness of device triggering mechanism in chronic implants**
   The difficulty in deploying the devices in the preliminary chronic tests (section 5.5.3) might have resulted from issues with the electrical connectivity with the resistive heaters, from plastic creep in the primed polyimide legs, from films sticking to the device legs and obstructing leg movements, or from the chronic glial scar being stronger than the expected. As discussed in section 5.5.3.4, various benchtop and *in vivo* tests can be performed to identify and resolve these issues.
Chapter 6: Conclusions & Future Directions

6 CONCLUSIONS & FUTURE DIRECTIONS

Over the course of four device generations, increasing complexity and features have been added to this new kind of reconfigurable neural probe that is designed to be deployed on-demand to pierce through the glial sheath post-implantation.

This chapter summarizes the main contributions of this work (section 6.1) and suggests some potential future directions (section 6.2).

6.1 Contributions

6.1.1 First in vivo recording+deployment demonstration of a reconfigurable neural probe with small dimensions

To our knowledge, this is the first in vivo demonstration of a self-contained, easy-to-fabricate, reconfigurable neural probe with small-sized (10-20 μm) electrode tips (section 4.5.4). Prior work in the field with reconfigurable probes has involved device features greater than 20 μm, a complex fabrication process, or bulky external microdrives [100], [103]. Similarly, prior small-sized neural probes (with probe tips smaller than 20 μm) have not shown the ability to be repositioned post-implantation [80]. While some groups have attempted to manufacture devices with potentially small features that can be reconfigured [104], no in vivo studies have been performed these devices. The acute in vivo demonstration of the device in this work and the extensive characterization of the device features are an important step towards enabling chronic recording studies with such small-sized reconfigurable probes.

6.1.2 A new polymer-based microactuator to store and deploy energy in microscale devices

This work presents a novel way to store and release mechanical energy in microstructures by deforming polymer beam structures, using a meltable glue to hold the beams in their deformed shape and then using a metal resistive heater to melt the glue to allow for the beams to elastically deform back to their original shape. With extensive models and tests and over multiple device generations, this work has fine-tuned this polymer-based microactuation mechanism, which can be adapted for use in a number of microsystems requiring self-contained device actuation of a few hundred microns. Moreover, the gold resistive heater design, combined with the thermal island isolation, can be used by itself in cases where self-contained heating is required for temperatures on the order of 50-70 °C. The heater designed can also be potentially modified to reach temperatures higher than these.

6.1.3 Recipe development for fabrication of polyimide-based microdevices

An important contribution of this work was the development of fabrication recipes (sections 3.3, 4.3.1, 5.3.1) for devices with a polyimide-metal-polyimide sandwich structure. This required extensive tuning of various fabrication parameters. The final recipes can be re-purposed by researchers with access to similar microfabrication facilities, in order to build microstructures with a polymer-metal-polymer sandwich structures.
6.2 Future directions
There are number of potential future directions for this work, some of which are described below.

6.2.1 Chronic rat study
An important next step for this work is a systematic long-term rat study to study the effect of device deployment at different time points on recording quality and on tissue reaction. A sample proposed study is depicted in Table 6-1 below. Devices in the B-type batches are not deployed in the brain and are designed to serve as controls for the corresponding A-type batches. This study investigates the effects of device deployment at 1, 2, 4 and 6 weeks after implantation. Moreover, some batches are designed such that the animals are euthanized right after device deployment, whereas some batches are designed to follow the animals out a few more weeks after device deployment. This can help compare the tissue reaction around the probe right after deployment versus a few weeks after deployment. Each batch can have 2-3 animals to protect against accidental headcap failures, or other unanticipated premature animal deaths. Additionally, each animal can be implanted with a set of 20 µm tip implants in one hemisphere and a set of 10 µm tip implants in another hemisphere (provided the implant packages can be made small enough to allow for bilateral implants). This can help investigate the effect of tip size on the recording quality and tissue reaction. The entire study, as proposed, would employ 40-60 animals, which is quite large. To minimize the number of animals involved, subsets of the batches below can be selected, based on the priorities for the research questions being considered.

Table 6-1 Timeline for potential chronic rat study

<table>
<thead>
<tr>
<th>Batch number</th>
<th>Deploy</th>
<th>Euthanize + histology</th>
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<tbody>
<tr>
<td>1A</td>
<td>Week 1</td>
<td>Week 1</td>
</tr>
<tr>
<td>1B</td>
<td>-</td>
<td>Week 1</td>
</tr>
<tr>
<td>2A</td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>2B</td>
<td>-</td>
<td>Week 2</td>
</tr>
<tr>
<td>3A</td>
<td>Week 1</td>
<td>Week 4</td>
</tr>
<tr>
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</tr>
<tr>
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<td>Week 2</td>
<td>Week 2</td>
</tr>
<tr>
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<td>-</td>
<td>Week 2</td>
</tr>
<tr>
<td>5A</td>
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<tr>
<td>5B</td>
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<td>Week 4</td>
</tr>
<tr>
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<tr>
<td>6B</td>
<td>-</td>
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</tr>
<tr>
<td>7A</td>
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<tr>
<td>7B</td>
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<tr>
<td>8B</td>
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<td>Week 6</td>
</tr>
<tr>
<td>9B</td>
<td>-</td>
<td>Week 6</td>
</tr>
<tr>
<td>10A</td>
<td>Week 6</td>
<td>Week 12</td>
</tr>
</tbody>
</table>
Chapter 6: Conclusions & Future Directions

The above proposed study can either be performed with the existing Gen 3 design or with a newer (Gen 4) design.

If the existing Gen 3 probes are used, the following recommendations can help maximize the chances of success:

1. Smaller implant packages might help mitigate some of the headcap failure issues in section 5.5.2.
2. Increasing the number of electrode sites per implant package would increase the probability of one of the sites being in close enough proximity of an active neuron for successful single unit recordings.

If new Gen 4 probes are designed, then the following recommendations (in addition to the two recommendations above) can help maximize the chances of success:

1. Designing electrode sites to be larger in size than the Gen 3 electrode sites (500 μm²) might increase the recording sphere of the sites, making it easier to record endogenous neural activity in the rat model.
2. Surface treatments on the electrode sites can help increase the electrochemical surface area for a given geometric area. This can help reduce the impedance of the sites without increasing the size of the sites, which can, in turn, improve the ability to record neural activity without compromising the selectivity of picking up single units (versus local field potentials).
3. If new probes are designed, there is an opportunity to revisit the capacitive sensing approach, which was tested in preliminary benchtop tests but was not tested \textit{in vivo}. More benchtop characterization, different pad geometries, different sensor chips, or new ways to reduce electrical noise in the measurement setup might help the capacitance sensing approach be better suited for the animal test setup. Alternatively, the capacitance sensors can be eliminated entirely and device deployment confirmation can be done solely based on visual inspection through the new packaging window designed in Gen 3.

6.2.2 Chronic optogenetic rodent study

The advantage of using an optogenetic rodent model over a non-optogenetic one is the ability to trigger neural activity on demand in the vicinity of the neural electrodes, thereby, eliminating the need to be in very close proximity to an active neuron. This might circumvent some of the issues Gen 3 probes experienced with recording endogenous neural activity in the rat model (section 5.5.2).

There are two potential future directions with this work in optogenetic rodents:

1. Optogenetic mice
   Optogenetic mice were already used in this work to test the neural probes in an acute setting (sections 4.5.3, 4.5.4, 5.5.1). This model can easily be extended to a chronic setting if the chronic implant package (section 5.3.2) can be reduced in size to fit more comfortably on a mouse skull,
which is smaller than the rat skull. A chronic study (e.g. as in Table 6-1) can then be performed in an optogenetic mouse model.

2. Optogenetic rats
The Gen 3 device package is designed to be compatible with chronic studies in the rat model. Therefore, another future direction for this work is testing the devices in an optogenetic rat model. While optogenetically active rats are harder to achieve than optogenetically active mice, recent progress in this field might enable testing our devices chronically in an optogenetic rat model [116].

6.2.3 Probing the mechanical properties of a chronic glial scar
While we know that the glial cells play a very important role in the brain [3] and are a very important reason for neural electrode failure in the chronic setting [68], not many studies have focused on the mechanical properties of a glial sheath that forms around neural probes in a chronic setting. The reconfigurable probes in this work (or variations) can be used to probe the mechanical properties of glial scars in various ways:

1. Force required to pierce the glial scar
Probe tips with different predicted buckling forces can be implanted in the brain and deployed at different time points. Post-surgery histology studies can show which probe tips successfully pierced through the glial sheath. The predicted buckling force of the probe tip that just pierced through the glial sheath at each time point post-implantation can be used to estimate the force required to pierce the glial sheath at that time point.

2. Biomechanics of the scar
Once the force required to pierce the scar at different time points has been established, the same force can be delivered in different ways to investigate the mechanical properties of the glial scar. For example, probe tips can be deployed very quickly or very slowly.
REFERENCES


