

A method to monitor spatiotemporal neuroplastic changes in the motor cortex due to ischemic infarcts using real-time multi-electrode recording in free-moving rats

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Abstract:

Gross neuroplastic changes in the brain following cortical infarction have been detailed with imaging techniques such as functional Magnetic Resonance Imaging (fMRI), Positron Emission Tomography (PET), and Electroencephalography (EEG).¹⁻⁵ However, no combination of these noninvasive modalities can provide the high spatial and temporal resolution needed to track neuroplastic changes on the level of individual neurons. Rapid Prototyping (RP) allow for precise, quick, cost effective component design, and was utilized to create a platform from which the spatiotemporal neuron level changes in a Sprague Dawley rodent model could be chronically monitored both pre and post stroke. RP was used to fabricate a jig that is designed to facilitate the production of a specialized array of chronically implantable tungsten microwires (50um in diameter, 390um interelectrode spacing). The array includes a port permitting precise placement of a fiber optic probe onto the brain surface to induce a localized 1.54mm² infarct in the motor cortex via photothrombosis following array implantation. Preliminary device evaluation showed that firing rates of neurons around the infarction site decreased approximately 50% in the hours following the stroke. Though the method has some limitations, RP is an excellent low-cost method for neural engineering applications.

Introduction:

Stroke is both the third leading cause of death, and a leading source of motor related disability in the United States. Over 65 billion dollars are spent each year in the form of medical interventions and the lost wages of those disabled by stroke.² For both quality of life and economic reasons, recovery of motor function post stroke remains a highly active field of research.

Many studies have documented the post stroke recovery process using non invasive modalities such as fMRI, PET, and EEG.^{1,3-6} These studies have confirmed that neural activity in the area around the infarct site decreases sharply after stroke. It has been noted that some patients who suffer loss of motor function in a particular limb sometimes are able to regain that function after a recovery period.⁷⁻⁹ The brain undergoes plastic changes which alters the function of select areas of the cortex. fMRI studies have shown that recovery of a previously paralyzed limb correlates with increased cortex activity both ipsilaterally and contralaterally to the infarct site.⁷⁻¹⁰

Furthermore, other studies have shown that repetitive movements, such as those involved in physical therapy, affect both neural activity patterns and functional recovery rate of stroke patients.¹¹ A recent study has indicated that using robotic assisted therapy for patients whose primary motor deficit is in the hand can greatly improve functionality in the affected hand.¹⁰ fMRI images from the study showed greater activity in the motor cortex after the robot assisted therapeutic course had been completed. However, the exact mechanism for the increased motor and neural function could not be determined from the study. The therapeutic robot used was set to provide active assistance if the subject had not completed their intended

movement in the time allotted. The force and duration of the active assistance was constant for each subject throughout the study.

In order to better understand neural reorganization in real time on a single unit level, and to improve the effectiveness of robot assisted therapies for stroke patients, we are investigating neural firing rates of cell populations surrounding an infarct. Whereas previous studies can obtain reasonable spatial resolution using fMRI and satisfactory temporal resolution using EEG, no one noninvasive modality (or combinations of modalities) can provide the same depth of information that can be obtained from direct intracortical recordings. Intracortical recordings have been used to monitor individual cell firing rates since 1969, and are still a primary method of acquiring data for brain computer interfaces (BCI), auditory prosthetics, and visual prosthetic devices.¹²

To monitor the spatiotemporal neural changes that occur with a stroke, a rodent is implanted with an intracortical electrode array, a stroke is induced via photothrombosis M1 forelimb region, and the firing rates of the neurons surrounding the infarct are monitored over time. In later studies, robot assisted therapy will be added to the recovery process in an attempt to decrease recovery time and observe the underlying neurophysiological changes that take place day to day. With an increased understanding of the underlying neural organization, robotic therapy can translate into the clinical setting in a form that actively monitors the patient and changes its output depending on the decoded neural patterns.

Methods:

Electrodes:

Microwire electrodes are produced by creating an array of small insulated metal wires (<50um) affixed in some specific pattern. Using a sophisticated 5 DSP based amplifier (Tucker Davis Technologies, Rx7) the voltage potential with reference to a biological ground is recorded the tip of each lead in the microwire array in real time. This voltage potential correlates directly with the depolarization, or firing, of a neuron. Although current research suggests that the stiffness mismatch between the hard metals traditionally used in microwire arrays and the brain may be a principle cause of severe immune response, however they are still one of the cheapest and most reliable method of obtaining data in many acute and chronic animal studies lasting under 12 months.

In order to generate a spatial map of firing rates around an infarct site, an electrode had to be designed with a high channel density and an open pathway to the cortex. A previous actuate study performed in our lab concluded that on a neuronal level, a stroke is both a highly spatial and temporal event. In that study, firing rates from directly around the infarct site dropped off quickly after the stroke was induced, where as electrodes 600 microns away from the site experienced a much slower and gradual reduction in firing frequency.¹³

The microwire electrodes for this study are made up of a hollow central guide tube, surrounded by microwires set in a specific spacing and geometric pattern. A wide array of interchannel spacings and overall array geometry could be employed for this study. It is impossible to determine at this time what sort of parameters would provide the most useful information about neuroplasticity. As such, three prototype designs were created to test how varying the electrode itself altered the recording results.

First is a concentric array (Fig.1 Top R), in which circular paths of microwires radiate out away from the central stroke guide. The concentric pattern will provide firing rate information on all sides of the infarct zone. The second design has the microwire array offset from the infarction site (Fig.1 Top L) in which the microwires are placed all on one side of the infarct zone. Offsetting the array will increase the farthest distance from the infarct that can be recorded, and will help to generate a good spatial map in one direction. However it cannot be assumed that firing rates will change symmetrically on all sides of an infarct. The last design employs an extremely high channel density in a square pattern with the infarct occurring in the center of the array (Fig.1 Bottom). This high density array will give the highest spatial resolution out of any other design, however it will be difficult to insert without damaging the surrounding tissue.

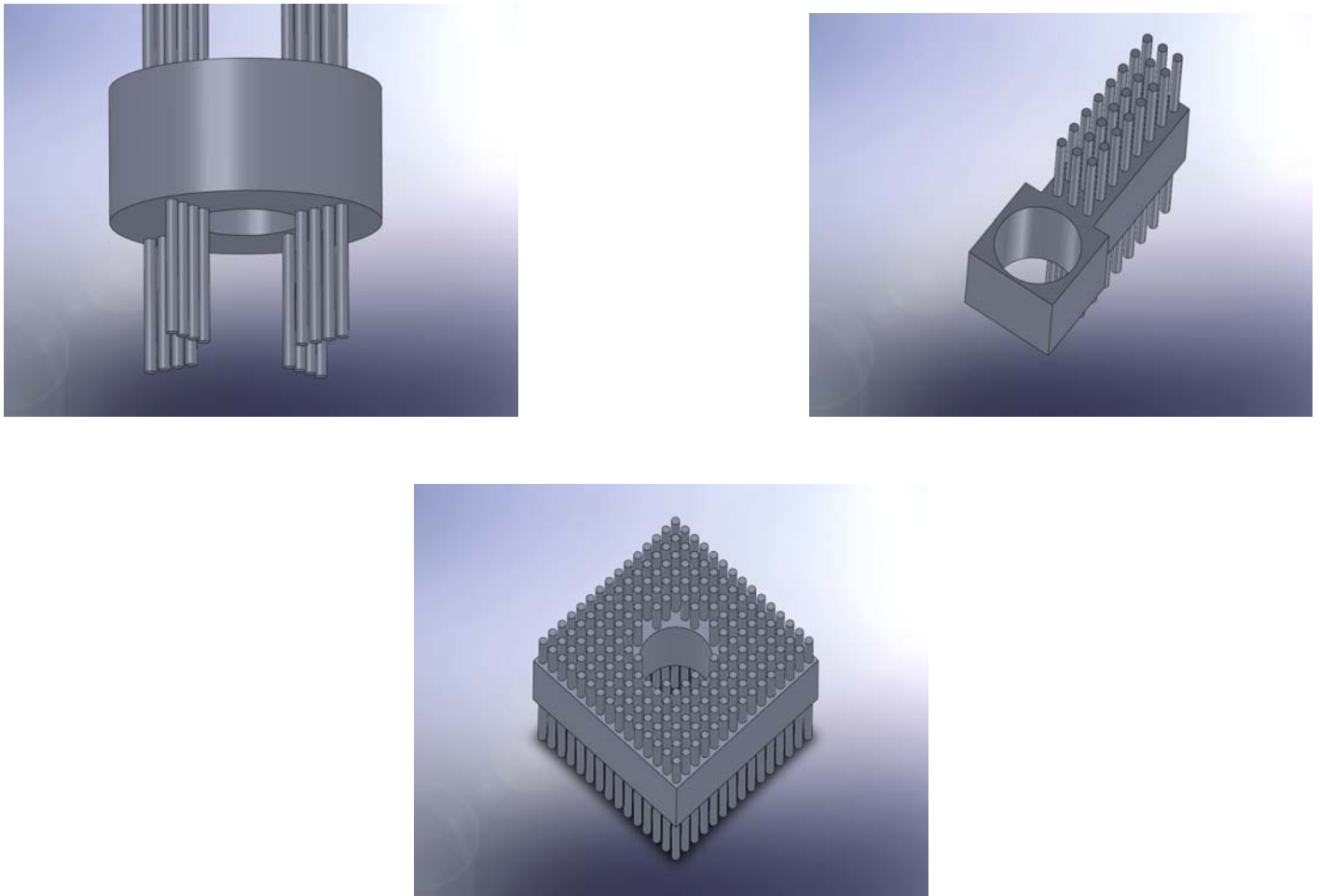


Fig. 1 Renderings of microwire electrode designs that would be compatible with the photothrombosis technique to induce an infarct. Top Left- A concentric array, providing good special information from all sides of the infarct. Top Right- An offset array that would allow neurplastic changes to be monitored far away from the infarct site. Bottom- An array made up of a high density of microwires. This array would give the most complete picture of neuroplasticity, but would be difficult to fabricate and to implant.

Many investigators continue to hand fabricate their own microwire electrodes despite the availability of commercially produced arrays. For this study, no commercially available array exists that would be completely compatible with the photothrombosis technique. The photothrombosis compatible electrodes described in the previous paragraph could never be hand fabricated in a manor that would ensure equal channel spacing and distances from the infarct site. Additionally, hand fabricating such an array would require the investment of many hours of labor.

Rapid prototyping is a technology that has traditionally been used to quickly visualize design concepts and produce small batches of mechanical parts. Recently, however, rapid prototyping technology has been suggested for use in biomedical applications.¹⁴⁻¹⁶ Intracortical microwire array production is one area of Biomedical engineering that could greatly benefit from the speed and precision that rapid prototyping techniques provide. Rapid prototyping was used to produce a set of jigs that allow closer tolerances in channel spacing and geometry than what can be achieved using hand production techniques alone. Furthermore, since rapid prototyping can quickly produce a complicated part for a low cost, new electrodes designs can be tested quickly and cheaply.

Surgical:

Male Sprague Dali rodents were chosen as subjects for this study based on their well documented neuroanatomy and relative level of intelligence. All animal experiments were carried out in full compliance with the policies of Animal Care Committee at the University of Illinois Chicago protocol 08013.

The animals were first anesthetized with a 3% isoflourine and oxygen mixture to promote a relaxed state for the injections of the main anesthetic. A 40:2:1 part mixture of

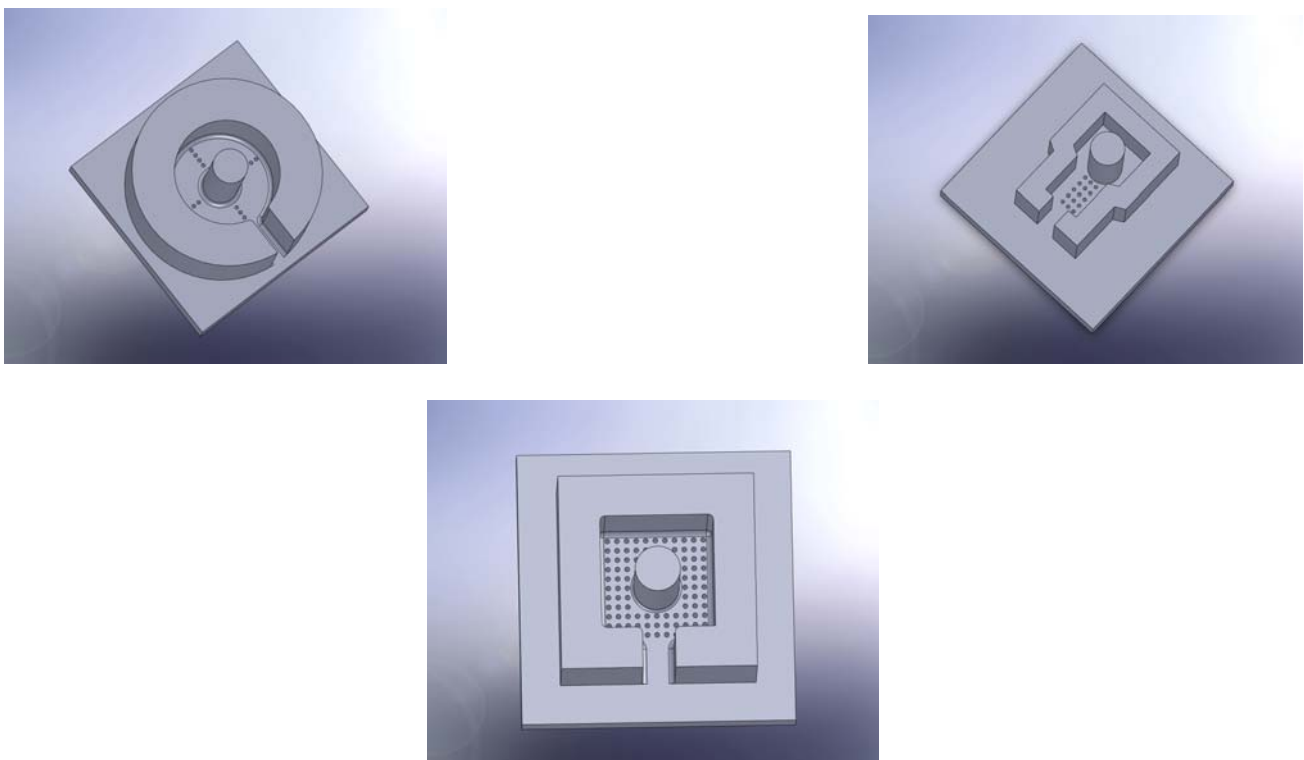


Fig 2. The molds that can be made using RP techniques that would allow the creation of the electrodes from Fig. 1

ketamine, xylazine, and acepromazine was injected intramuscularly throughout the surgical procedure to maintain a state of unconscious. Reflex responsiveness was tested throughout the procedure using a standard paw pinch test.

After opening, a 3-5mm craniotomy was performed in the approximate M1 forlimb region. The craniotomy we performed as far away from the major sutures as possible, in an attempt to minimize vascular damage. Once the skull was removed, the dura was picked off the cortex using a fine pair of forceps. After examining the exposed vasculature, the microwire electrode is orientated into a position that will minimally interfere with the exposed vessels. The array is then lowered quickly (approximately .5 mm/s) down into the cortex until the brain is penetrated by the microwires. After initial penetration, the array is brought back up in depth so that it rests in the approximate layer V region (1.1m-1.9mm from the surface of the cortex) The array is then affixed to the skull using Teets Cold Cure Dental Acrylic (CO-ORAL Rite Dental).

Since the electrode array is designed with an open path to the cortex, the infarct does not have to be induced on the same day as the electrode implantation. The rodent is allowed to recover from surgery, and baseline recordings are taken every day after the rodent fully recovers.



Fig.3 A six channel microwire recording electrode made of 50um tungsten wires encased in 140um polyimide tubes. Each wire is spaced 400um center to center apart. The guide tube for the stroke induction can be observed on the right side of the array.

Infarct Induction:

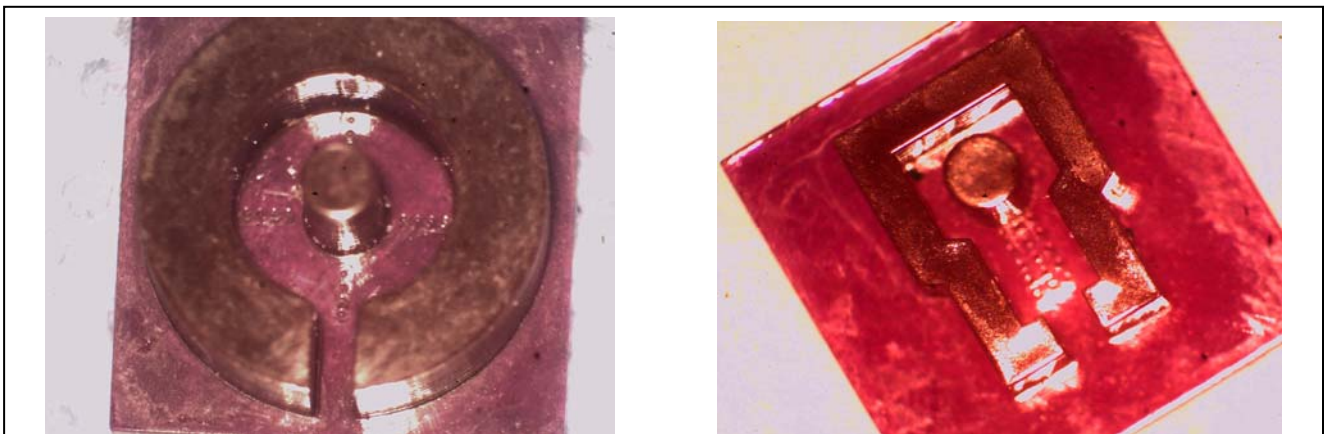
After a stable baseline firing rate can be established for a rodent and maintained for several days, the infarct is induced. The rodent is again anesthetized using the same procedure described in the previous subsection. Once unconscious, a tail vein catheter is inserted into to dorsal vein running along the tail of the rodent. A light reactive dye (Aldrich Chemicals) was infused through the tail vein at a rate of 1.0 ml/min. After waiting for the dye to perfuse for 3-5 minutes, a fiber optic probe was lowered through the guide tube in the microwire electrode. During the clotting process, simultaneous neural recordings are being made to document the infarct development. The probe was supported approximately 1 mm above the surface of the cortex for 20 minutes. After 20 minutes, the dye is no longer reactive and no more platelet aggregation can be initiated.

After the allotted time, the probe is removed and the channel in the microwire electrode used to induce the infarct is permanently sealed with dental acrylic. The neuropotentials of the rodent are then recorded at regular intervals in order to capture the death of the neurons closest to the infarct site. When recordings seem to become stable, the recordings are stopped and the rodent is allowed to recover from anesthesia. After approximately 24 hours, the rodent is observed qualitatively for any movement related artifacts that may exist, and a block of neural recordings is taken. A block of recordings will be made every day, at approximately the same time, for the duration of the study.

Results:

Electrode:

The first batch of RP molds were ordered from RJ Manufacturing, a third party company. The designs were produced on a Sterolithography Apparatus (SLA) that was specially modified by the company to allow for higher resolutions (Fig. 4). All planer surfaces in the X, Y and Z direction had a smooth, mirror like finish to them. A smooth sidewall promotes less adhesion with the dental acrylic and facilitates an easy electrode removal. However, upon closer inspection, the 180 um guide holes in the parts were found to be filled in with resin (Fig.5 TL). This resin proved to be impossible to clear out of the hole path because it was completely adhered to the surrounding sidewalls. A fourth test piece was produced using hole features spanning from 120um-300um in diameter. Only the 300um holes, the largest holes tested, came back clear of debris (Fig 5. TR). When the 300um holes were further inspected, their actual diameter proved to be approximately 50 um smaller than what was intended (Fig 5 Btm).



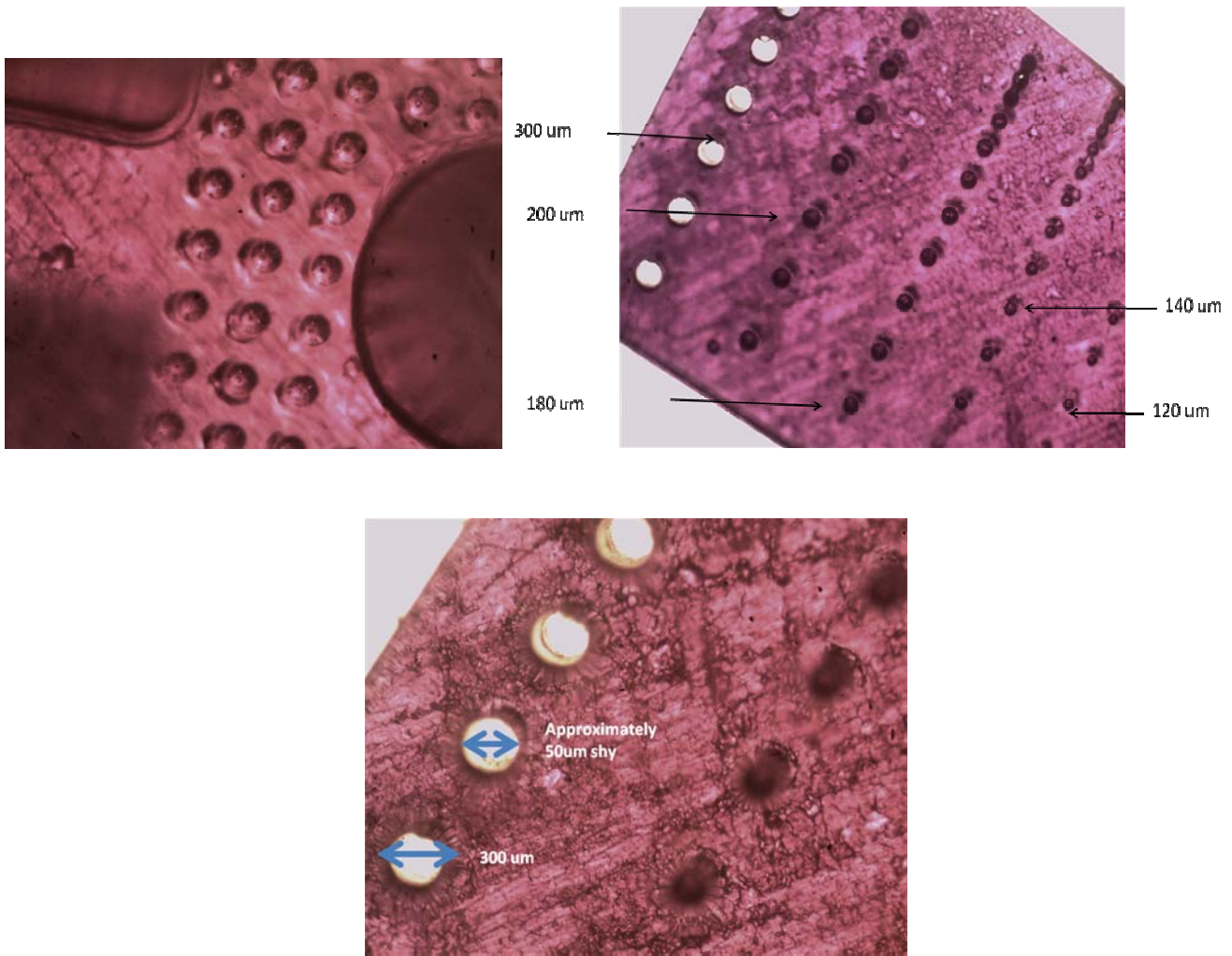


Fig. 5 Upper Left- The 180µm filled in holes on the SLA jig.
 Upper Right- A test piece on which only 300µm holes came back free of debris
 Bottom- The actual diameter of the holes drafted to be 300 µm were around 250µm

Another set of RP parts was ordered from RJM, using a Perfactory RP machine with 300µm holes instead of an SLA with 180µm holes. The Perfactory parts were warped in the Z direction, and had rough top and bottom planes. However, the holes in these pieces came in free of any cured resin. A thin film of Teflon was stretched over the piece to prevent the dental acrylic from

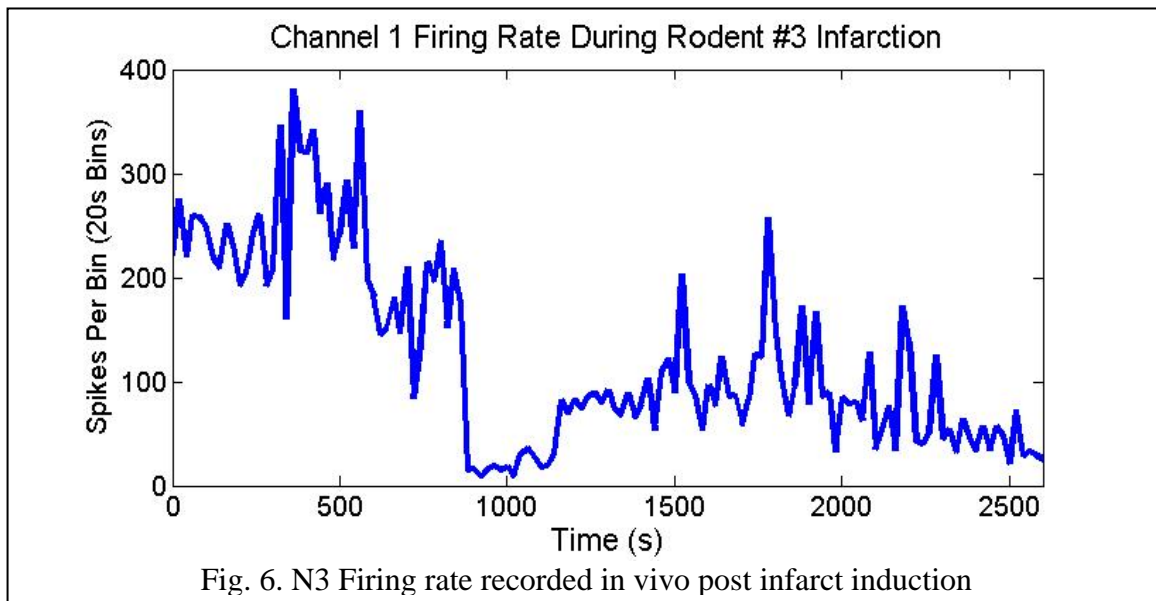
sticking to the jig during electrode assembly. A similar phenomenon was observed in which the actual diameter of the small holes (250 μm) were about 50 μm smaller than then intended size. A different polyimide tube was chosen to accommodate the different sized hole, and several electrodes were then produced.

Neurological Potentials

In N1, there were technical complications with our data acquisition system resulting in no usable data pre-stroke. From previous studies in our lab, it was expected that after a stroke was induced, neurons close to infarct side would quickly die and stop producing signals. However, in the incremental recordings taken as the infarct was induced in N1, activity remained relatively constant across all channels (Appendix 1).

N2 provided strong baseline recordings data, however before a stroke could be induced, the microwire array stopped picking up clear neuronal signals. Since the signal characteristics had completely changed overnight and continued to change in an unpredictable manor, that animal could no longer provide useful neural data.

N3 provided the first stable baseline recordings pre stroke, and showed the predicted pattern of activity decline post infarct. Data from one channel of the N3 shows an overall trend toward less neuronal firing as time increases (Fig. 6)



Discussion:

Sterolithography should have provided the most accurate RP parts in this study. The beam resolution on the Viper SLA machine used was 3 μ m, therefore a feature in the 100 μ m range is well within the capabilities of the machine. However, upon investigation with RJM, it was discovered that the beam diameter phases out of a perfect circle and begins to deform over time. The only way to correct this error is to recalibrate both the laser and the stepper motors that move the laser back and forth across the part. Recalibration is a long, arduous process but afterward the feature resolution of the machine should be greatly increased.

Even though a less accurate prefactory system was used to produce the final RP jigs, the hole sizing and spacing was smaller and than a similar jig produced on a CNC mill at Case Western Reserve University. The cost of the CNC part was over \$200 and took several weeks to design and fabricate, while the RP cost came to around \$28.00 and took only 1 day to produce and ship.

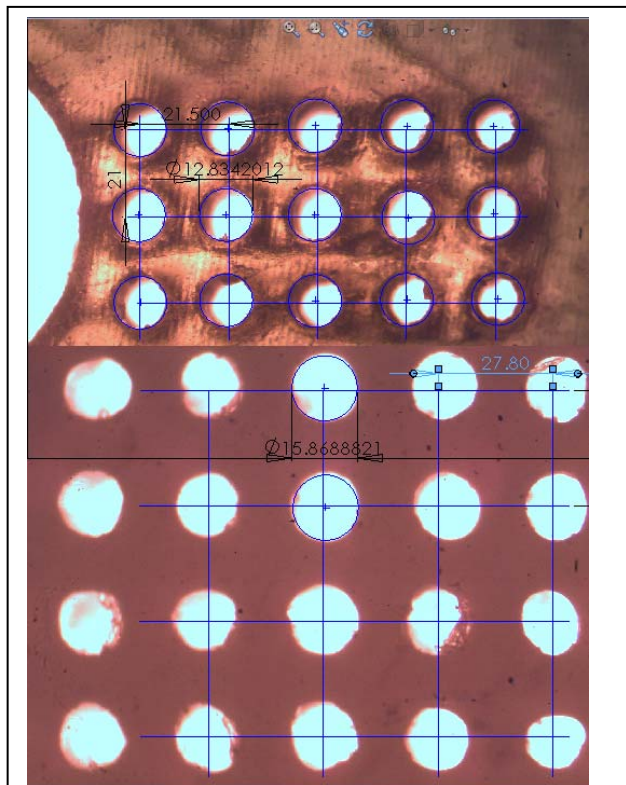


Fig. 7. The Prefactory produced RP jig (top) with 245 μ m holes spaced 380 μ m apart center to center. Below is the Teflon jig produced using CNC milling methods that incorporates 300 μ m holes spaced 450 μ m apart from one another

More rodents will need to be implanted and have strokes induced before any concrete conclusions can be made about the effectiveness of our stroke technique in this chronic study. Results are expected to mirror those found in the previous accurate study.

Conclusions:

Results gathered so far have tended to support the goal of being able to record chronically from rodents with infarcts induced via photothrombosis. The microwire electrode compatible with the photothrombosis technique has proven to be a stable, reliable platform from which to record neuropotentials. Furthermore, the photothrombosis technique itself has shown to work in previous studies, and preliminary data is following the previously observed pattern.¹³

Rapid Prototyping has proven to be a cheap, quick, and precise method of creating jigs to aid in the fabrication of microwire electrodes. RP has shown similar resolution to parts produced using traditional

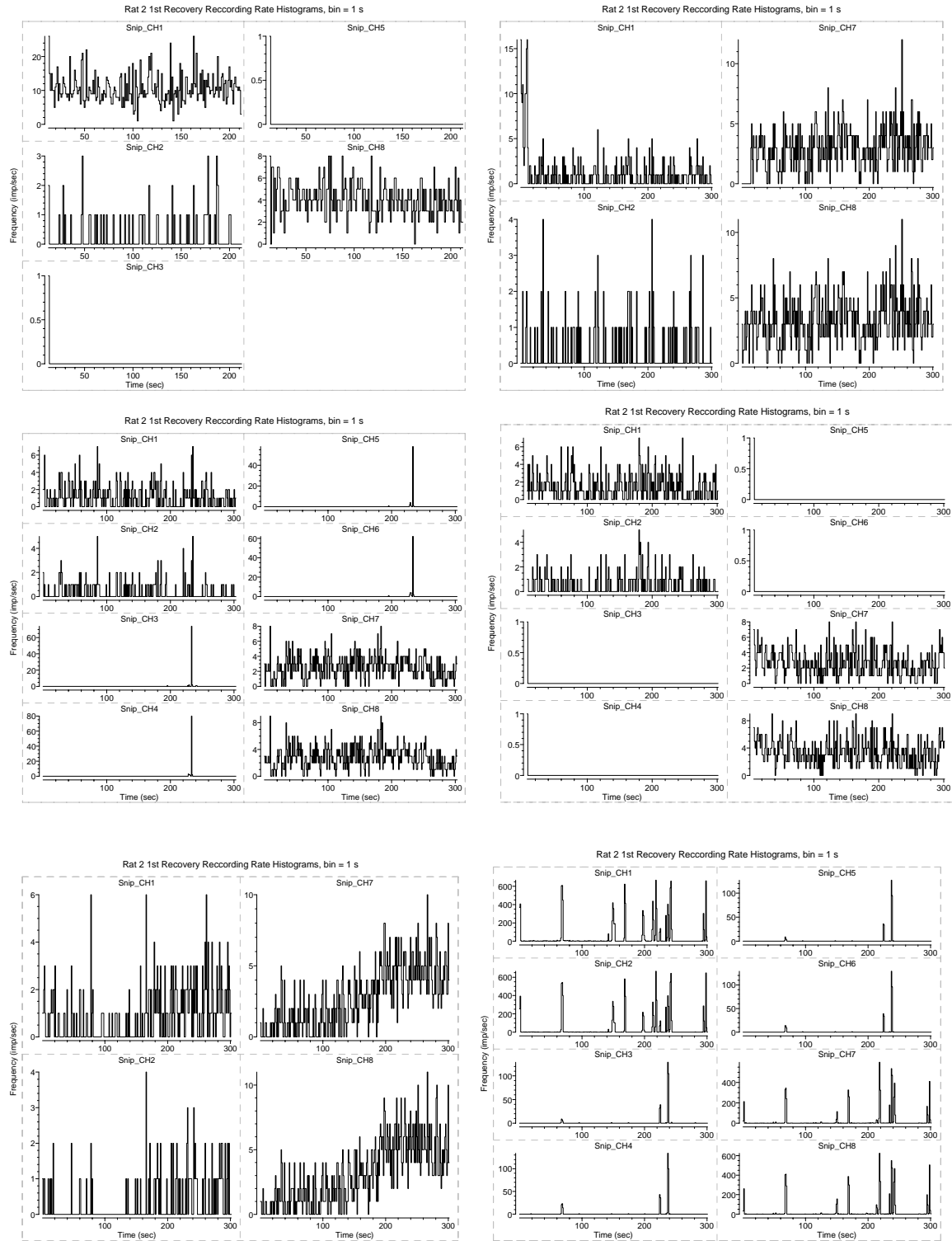
micromachining techniques. Now that the technique to produce the RP jigs has been proven, many different styles of electrodes can quickly be created.

More rodents will be implanted to provide a statically significant source of data for our pre-stroke, intra stroke, and post stroke recordings. Over the phase of the study, we will continue to implant every rodent with an microwire array, and monitor their neural firing patterns on set intervals. In parallel to this work, an obstacle course has been designed to quantify the rodent's motor/behavior recovery post stroke. When the neural data is combined with the functional motor data, an accurate spatiotemporal model of ischemic infarct will be produced that will lead to quicker, more effective forms of recovery post stroke.

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Appendix 1:



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