Spatiotemporal electrophysiology of cerebral ischemia observed using chronic electrode array in auditory cortex

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Stroke research is of considerable societal value in an age in which the scourge is a leading cause of disability and the third-leading cause of death in the United States.\textsuperscript{1,2} While previous studies investigate the electrophysiology of stroke, none examine the long-term time-course of stroke recovery in the auditory cortex, the objective of this study. An electrode was implanted in the auditory cortex of two anesthetized Sprague-Dawley rats, stroke was induced in one of the subjects using photothermolysis, and daily electrical recordings were made while each subject was presented with a click stimulus every 500 ms. Peri-stimulus time histograms reveal that in the control subject, the second stimulus-evoked burst’s peak decreased the day following implantation (Day 1) but returned almost to its Day 0 (day of surgery) value by Day 5, representing recovery from implantation trauma. The mean firing rate decreased logarithmically from its Day 0 value of 90 Hz to 10 Hz by Day 8, revealing decreasing electrode viability. In the stroke subject, the second stimulus-evoked burst’s peak was undetected Day 1, but was detected again on Day 4, elucidating that the rat auditory cortex regains function as stroke recovery progresses.\textsuperscript{3}

Introduction

Stroke Background

Once every forty seconds, someone in the United States suffers a stroke.\textsuperscript{4} In 2006, cerebrovascular disease claimed the lives of 137,265 Americans, making it the third-leading cause of death among Americans.\textsuperscript{2} Those who survive a stroke are far from entirely fortunate, though, as the terrible disease is also a leading cause of disability in the U.S., costing Americans an estimated $65.5 billion in 2008 alone.\textsuperscript{1,4} The social and economic impact of stroke is clear and the search for a cure of tantamount importance. Basic neuroscience investigation plays a critical role in any such search.

Delving deeper, two types of stroke exist: ischemic and hemorrhagic. An ischemic stroke occurs when a clot forms in a cerebral blood vessel, depriving the upstream neural tissue of oxygen and vital nutrients. Hemorrhagic strokes occur when a cerebral blood vessel ruptures, leaking blood, toxic to the surrounding neural tissue, into the brain and preventing it from reaching neurons further upstream. The proposed study focuses on ischemic strokes, which account for 87 percent of all strokes.\textsuperscript{4}

Current treatment options for ischemic stroke remain relatively limited.\textsuperscript{5} The only Food and Drug Administration (FDA)-approved pharmaceutical intervention is intravenous tissue plasminogen activator (tPA).\textsuperscript{5} Yet, many stroke patients do not present to the emergency department within the drug’s three-hour treatment window.\textsuperscript{5} For patients ineligible for tPA therapy or treated with tPA to no success, the FDA has approved mechanical thrombolysis.\textsuperscript{5} Unfortunately, this latter treatment option is carried out by interventional neuroradiologists who perform the necessary endovascular catheterization, subspecialists often not available twenty-four hours a day at small or rural medical centers.\textsuperscript{5}

Purpose

The authors conducted the proposed study to reveal the spatiotemporal electrophysiology of the rat auditory cortex (ACx) following cerebral ischemia induction employing the photothermolysis technique and recorded in response to auditory click stimuli using a chronically-implanted, multi-channel electrode array. Though this is a basic neuroscience investigation, the authors’ goal was to illuminate how neural tissue at different distances in the ACx from the infarct core was altered electrophysiologically as stroke recovery progressed through the chronic stages. This and subsequent studies may lead to novel new ways to assess stroke recovery in human patients using electrophysiology tests and could lead to the development of neuroprosthetics aimed at restoring function to regions of the brain devastated by stroke.

Previous Studies

Early studies into the electrophysiological changes that take place in the brain following a stroke were performed by using the photothermolysis technique to create a lesion in the parietal cortex or the border of the motor and occipital cortices of Wistar rats and then making in vitro electrical recordings in thin, post-mortem brain slices.\textsuperscript{6,7}
A more recent study made electrical activity recordings in vivo in Wistar rats immediately following and one week after stroke induction, this time using the middle cerebral artery occlusion (MCAo) technique. Yet, this latter study did not provide a stimulus to the rat during the electrical recording.

The study by Chiganos et al. was the first to use the photothrombosis technique to induce cerebral ischemia in the ACs of a Sprague-Dawley (SD) rat and make subsequent electrical recordings while simultaneously providing an auditory stimulus. However, this study concluded electrical recording after the first 800 seconds following stroke induction, the initial beginning of the hyperacute stage of stroke recovery; each animal was euthanized immediately following surgery and electrical recording. The proposed study is quite original in that it focuses on what Kreisel et al. classifies as the acute, subacute, consolidation, and chronic stages of stroke recovery, stages typically collectively referred to as chronic in neural engineering parlance, while similarly using the photothrombosis technique in SD rats and making electrical recordings in the presence of an auditory stimulus.

Furthermore, though the aforementioned Chiganos et al. study explored the electrophysiology of cerebral ischemia from a temporal perspective, it did not examine stroke recovery from a spatial perspective, using only a single-channel electrode array implanted in the infarct core. The proposed study uses a linear, four-channel microwire electrode array for recording the electrical activity within the AC at four different distances from the middle of the infarct, allowing the authors to draw conclusions about how the electrophysiology of stroke recovery varies spatially within the AC. Previous studies show that focal cerebral ischemia causes remote functional changes in the brain, an effect termed diaschisis. Employing a linear, multi-channel electrode array with acceptable spatial resolution implanted chronically allows for the observation of diaschisis and both its spatial and temporal dynamics in a rat model of stroke.

Finally, by implanting an electrode array that places electrodes in both the infarct core and the surrounding region, neural engineers can interrogate the spatiotemporal electrophysiological dynamics of the penumbra, a pathological region of tissue surrounding the infarct core that is temporarily deprived of oxygen and vital nutrients during the infarction, but experiences ischemic damage that is sometimes reversible. The penumbra is of prominent interest as a site for potential therapeutic interventions.

**Key Experimental Design Decisions**

Two leading models of cerebral ischemia in the rat are MCAo and photothrombosis. Cerebral ischemia resulting from MCAo varies greatly in volume due to collateral blood flow; its extent is also not limited to any one particular portion of the brain. This renders the MCAo model very clinically relevant, but the fact that the extent of ischemia it produces is largely uncontrollable, unpredictable, and usually not uniform from trial to trial limits its worth to neural engineers. Though the MCAo model has its merits, photothrombosis is much more well suited to the proposed study. Photothrombosis is a model and a technique that photochemically induces clot formation in surface cortical tissue, forming a region of focal cerebral ischemia on the scale of millimeters. This is invaluable to the neural engineer, who can now produce a lesion in a very specific locale on the brain surface, such as the motor cortex (MCx), and assess motor impairment, or, in the proposed study, produce a lesion in the ACx and make electrical activity recordings in the presence of an auditory stimulus.

In a spatiotemporal electrophysiology study of this kind, producing a tightly controlled focal infarction in the ACx, rather than the MCx, is ideal. MCx experiments often involve intensive training of the rat to perform a certain motor behavior or series of motor behaviors prior to stroke induction and then observing differences in the rats performance of the behavior(s). Additionally, quantitative analysis is at the heart of engineering. Researchers have a very difficult time quantifying rat behavior. Lastly, the question of how experimenters can make meaningful electrical recordings in freely-moving rats without generating too large of an electromyographic artifact or having their recording apparatus become accidentally disconnected from an animal implant remains.

This is a question the proposed study will not attempt to answer, opting instead to investigate ACx electrophysiology. The previously-addressed Chiganos et al. study stands as a proof of concept that meaningful, high-level, quantitative electrophysiological data is obtainable from the rat ACx following photochemically-induced focal cerebral ischemia.

A final, key experimental design decision facing investigators in a study of this kind is how to restrain the rat during daily electrical recording. Restraint is highly desirable, as it eliminates the data acquisition concerns presented by freely-moving animals and inherent in MCx recording. Rather than physically restrain their rats, such as through the use of a restraint hammock, the authors of the proposed study have elected to anesthetize their rats just prior to recording, using what they feel is a less traumatic and more humane form of restraint. A new question that arises, however, is whether the ketamine-based general anesthetic used in the proposed study and described later will have an effect on the results. Rennaker et al. found that while ketamine-based anesthesia greatly influenced ACx electrical activity in rats following the presentation of temporally-complex auditory stimuli when compared to awake rats exposed to the same stimuli, the two groups of subjects exhibited similar response dynamics whenever the stimulus presentation rate (SPR) was less than 20 Hz. The proposed study only makes electrical recordings at an SPR of 2 Hz (one click stimulus every 500 ms).
Rennaker et al. also reported that ketamine-based anesthesia induces stimulus-driven oscillations not exhibited by unanesthetized rats, but this oscillatory activity was not observed at SPRs below 111 Hz.\textsuperscript{17}

### Materials and Methods

#### Electrode Array Fabrication

Each rat was chronically implanted with a single-channel microwire electrode array following a design modified from another study and delineated in Figure 1.\textsuperscript{18}

Fabrication began with an eight socket-by-two socket black plastic connector base (2.00 mm PitchMilli-Grid\textsuperscript{TM} Receptacle from Molex Inc.) that was split into two four socket-by-two socket connector bases by gripping each end simultaneously with a needle-nose pliers and applying torque. Next, four individual 10.00 mm segments were cut from a 304.80 mm-long polyimide-coated tungsten microwire with a diameter of 0.13 mm (Plastics One Inc.) using wire cutters. 2.00 mm of one end of each microwire segment was held in the flame of a disposable flint lighter (Zhuoye Lighter Mfg. Co. Ltd.) for one second to melt off the insulative polyimide coating. A scalpel with a Size #11 surgical blade (Bard-Parker\textsuperscript{TM} from Becton, Dickinson, and Co.) was then run three times across the newly-exposed tungsten tip of each microwire sitting atop the workbench. Pressure was applied when using the scalpel to ensure that the black carbonaceous layer that formed on each microwire tip following melting was completely removed. Special care was taken not to snap off the delicate tip of the microwire during this process.

Each microwire was in turn soldered (Radioshack two-part, lead-free, 96%-Sn, 4%-Ag solder) to its own individual silver pin on the underside of the connector base such that all four microwire electrodes were only in one of the base’s two fourocket rows. Care was taken not to inadvertently bridge any two of the connector pins through inattention to proper soldering technique. The disposable flint lighter was now used to melt away the polyimide coating from the final 2.00 mm of the previously unaltered end of each microwire and the scalpel technique employed again to ensure a clean tungsten surface. At this point, an autoranging multimeter (Craftsmen) was used to make sure current flow was possible between each connector pin and its attached microwire electrode and that a functional electrical connection existed.

A 7.00 mm segment of red plastic tubing (diameter = 2.00 mm), was cut using a scissors. Fabrication continued with the thorough stirring of a 50:50 mixture of the liquid and solid powder constituents of coral Teets cold-cure polymethyl methacrylate (PMMA) denture material (CO-ORAL ITE Dental Mfg. Co.) in an aluminum dish using a wooden toothpick. Latex gloves (Fisher-brand) were worn when handling the uncured PMMA and its ingredients as they are toxic to the skin. The liquid constituent of the PMMA mixture is also flammable and volatile, so extra attention was paid not to use the soldering iron or disposable flint lighter while any of the liquid was exposed to the open atmosphere of the laboratory. Quick, dexterous action was used to mold the uncured PMMA into an island that extended 5.00 mm down from the underside of the connector base, 2.00 mm across the short side of the base, and the width of the base plus an additional 3.00 mm off to one side into which the red plastic tubing was inserted, serving as a port for the fiber-optic probe described in the following subsection. Caution was exercised to ensure that none of the uncured PMMA made its way up into the connector base’s ports, adhered to the length of the electrodes extending below the desired PMMA island, or clogged the fiber-optic light port. The electrodes were again tested to certify that each one conducted an electrical current.

#### Surgical Implantation and Stroke Procedure

The animal experiments in the proposed study were performed in compliance with a protocol approved by the Association for Assessment and Accreditation of Labora-
tory Animal Care-accredited Animal Care Committee of the University of Illinois at Chicago. Five male SD rats (350-450 g; Taconic Inc., n = 5) were first exposed to 0.3% halothane gas in a closed anesthesia chamber for 10 min. After shaving a rat’s head and the region over its left femoral vein, the rat was given a double bolus intramuscular injection of ketamine (100 mg/kg), xylazine (5 mg/kg), and acepromazine (2.5 mg/kg) (KXA) to more deeply anesthetize the animal. Throughout surgery, pulse rate and oxygen saturation were electronically monitored and body temperature was maintained using a warming blanket. Every 20 min, the paw-pinch reflex test was performed and additional KXA was administered as needed to maintain a constant plane of anesthesia.

Surgery began with an incision above the left femoral vein and typically lasted 5.0-5.5 h. Vessel clips were applied to the vein to temporarily halt blood flow while a catheter with an outside diameter of 0.762 mm (SAI Inc.) was inserted. A suture was used to hold the catheter in the vein and the vessel clips removed. The catheter was used to intravenously administer a 0.9%-NaCl solution (Baxter) for fluid replacement and also for Rose Bengal (RB) dye solution administration, detailed later. The incision was carefully sutured close to ensure that the catheter did not come out of the vein in the process.

Next, a 2.00 cm incision was made above the midline cranial suture and connective tissue cut to expose the cranial surface. A craniectomy was performed using a 3.00 mm drill bit (Small Parts Inc.) at the same stereotactic coordinates employed in Chiganos et al. and the exposed dura mater was removed. An electrode array was attached to a micromanipulator (Kopf) via an alligator clip and lowered into the primary ACx to a depth of 550 μm, corresponding to layers IV and V of the rat ACx.

The first rat functioned as the control group in the proposed study (n = 1). After an electrode array was implanted in the rat’s ACx, no stroke was induced and the head was closed by the method detailed later. In the experimental group (n = 1), cerebral ischemia was induced using the photorthrombosis technique following electrode array implantation as depicted in Figure 2.

The RB dye solution (Aldrich Chemicals, 10 mg/mL 0.9% saline solution, 2 mg/100 mg body weight) was infused at 1.0 mL/min. Light sources in the laboratory were eliminated and a fiber-optic light probe (Intralux 6000, Volpi Inc.) with a heat filter (Ealing Inc.) was inserted into its port in the electrode array and lowered to approximately 1 mm above the brain surface. The primary ACx was illuminated for 20 min to ensure complete clot formation and then the fiber-optic light probe removed.

Oxidized regenerated cellulose-based absorbable hemostat (SURGICEL Fibrillar, Johnson & Johnson Medical Inc.) and bone wax (Ethicon) were used to fill the craniectomy. A small dollop of PMMA prepared and handled as described in the previous subsection was placed on top of the hemostat and bone wax to lock the electrode array in place and the scalp was sutured shut.

Surgery concluded with the removal of the catheter. The earlier suture holding the catheter-insertion site closed was removed and the catheter removed. A suture was used to reclose the operative site. Triple antibiotic ointment consisting of neomycin, polymyxin B sulfates and bacitracin zinc (Fougera) was given prophylactically immediately following surgery and one and two days after surgery and therapeutically as indicated following visual examination of the recovering rat during the daily data acquisition sessions.

Data Acquisition

Recording sessions were held at the same time every morning for the duration of the study on all rats with implants up to that time. Each session began with a visual examination of each recovering rat to check for symptoms of infection and to ensure that no implant had become dislodged. The animals were then anesthetized using the same halothane-KXA method detailed in the previous subsection.

During recording and stimulus presentation, the ear of each animal opposite the side of the brain hemisphere in which the infarction was induced was placed 0.91 m (36 inches) from a speaker (Injected Series, Blaupunkt) approximately on the same vertical plane as the animal subject’s ears. The external portion of the ear on the side of the ischemic hemisphere was filled with cotton gauze to prevent the stimulus from entering it and thereby mitigating any cross-hemisphere auditory activity. Effort was also made to eliminate any ambient noise present in the laboratory.

The data acquisition system (System 3, Tucker-Davis Technologies) connected to the speaker interfaced with a personal computer through a Peripheral Component Interconnect (PCI) card (Gigabit, Tucker-Davis Technologies). This system was programmed to produce a 100 dB-click stimulus once every 500 milliseconds and to
record data at a 1.5 signal-to-noise ratio. During each recording session, the system was set to establish an automatic threshold. Every session consisted of five minutes of recording during silence followed by five minutes of recording during presentation of the click stimulus.

**Control Data**

To begin, the prominent second local maximum on the graphs in Figure 3 is the peak of the second stimulus-evoked burst. In Figure 3(a), this peak has a y-axis value of 625 counts/bin (1 bin = 10 ms). This value indicates that during the 10 ms bin representing the peak, 625 spikes in the electrical activity data were counted. On Day 1 (Figure 3(b); one day post-operation), this peak drops to 275 counts/bin. The y-axis value of this peak slowly increases from Day 2 (Figure 3(c)) onward, reaching 525 counts/bin on Day 5 (Figure 3(e)), only 100 counts/bin less than the original Day 0 value. This sudden decrease in the peak value following the implantation surgery succeeded by the slower increase in the peak value reveals that implantation site within the ACx is recovering from the penetrating trauma associated with implanting the microwire electrode array. Meaningful auditory cortex electrical activity is slowly restored.

Another important observation drawn from Figure 3 is the changing morphology of the second stimulus-evoked burst. Burst data from Days 0, 1, and 2 (Figures 3(a), 3(b) and 3(c), respectively) possesses a Gaussian- or normal distribution-like shape that is replaced on Days 4 and 5 (Figure 3(d) and 3(e), respectively) with second stimulus-evoked burst bins that form a narrow, sharply-pointed second stimulus-evoked burst on the corresponding PSTHs. This indicates that while the implant site in the ACx is recovering from the implantation trauma, the auditory neuronal units there have undergone a fundamental change in their firing characteristics. Additional data collection is required in order to determine if this change is permanent.

Delving deeper, Figure 4 presents a challenge in data analysis. The time elapsed between the first and second stimulus-evoked burst peaks as a function of number of days post-op. Control subject presented with click stimulus.

![FIG. 4: Time elapsed between first and second stimulus-evoked burst peaks as a function of number of days post-op. Control subject presented with click stimulus.](image)
in Figure 4 is correct. An investigation of this interesting graph’s physiological underpinnings and correlates could also prove fruitful.

![Graph of mean firing rate vs. time](image1)

**FIG. 5:** Mean firing rate in control subject as a function of number of days post-op. Vertical standard error bars shown. Logarithmic trend line shown with equation and R^2 value. Notice how R^2 value greatly increases when Day 5 data point excluded (Graphs c and d). a) No stimulus. b) Stimulus. c) No stimulus. d) Stimulus.

Continuing, Figure 5(a) and 5(b), graphs of mean firing rate vs. time, show a general decrease in mean firing rate as a function of the increasing time elapsed since surgical implantation. Decreasing from 90 Hz to 10 Hz in Figure 5(b) between Days 0 and 8, the line graph represents a very rough approximation of a decreasing logarithmic trend line as evidenced by an R^2 value of only 0.6565. Upon visual inspection of Figure 5(a) and 5(b), the data point generated from the Day 5 data deviates quite far from the proposed logarithmic mathematical model and causes the data set as a whole to deviate as well. Thus, in Figure 5(c) and 5(d), the Day 5 data point was excluded. On Figure 5(c), the R^2 value is now 0.8954, more firmly corroborating that the decay in the mean firing rate with respect to time is closely modeled using a logarithmic equation. The rate decays over time due to scar tissue formation around the electrode.

**Experimental Data**

Now considering the data collected from the stroke subject, Figure 6 clearly illuminates that the focal infarction led to aberrant ACx electrical activity. Comparison of Figure 6(a) and 6(b) to Figure 6(c) quickly reveals that by Day 1, already, the second stimulus-evoked burst either became too small to detect or is completely gone at present. Yet, by Day 4 (Figure 6(d)) the second stimulus-evoked burst has begun to return and now has a peak at 150 counts/bin (1 bin = 10 ms). This data is very encouraging as it strongly suggests that while some ACx electrical activity was lost following stroke induction, it was slowly restored (at least partially) over the course of three days as stroke recovery commenced.

**Summary**

Briefly reviewing, the electrical activity of the ACx is altered at the implant site following the implantation surgery, but previous electrical activity levels are almost restored within four days.

Both the morphology of the PSTHs in Figure 3 and the time elapsed between the first and second stimulus-evoked burst peaks change with respect to time, but additional data and electrophysiological insight is needed to suggest explanations for these phenomena.

Figure 5 established that mean firing rate decreased logarithmically, an observation best explained by the author’s proposal that electrode recording viability decreases logarithmically as scar tissue forms around the electrode. The sudden disappearance of the second stimulus-evoked burst in the stroke subject one day after stroke induction and its gradual reappearance three days later leads the authors to conclude that the second stimulus-evoked burst is restored because the rat ACx recovers functionally as stroke recovery progresses.
Future Directions

Four important avenues to pursue in this line of research that are natural extensions of the proposed study are:

1. Carry out more surgeries to increase sample size and continue recording for broadened temporal picture.

2. Employ multi-channel electrodes to generate spatial data. While this was one of the chief-most objectives of the proposed study, the difficulty of chronic implantation surgeries proved to damper this line of investigation, now leaving it available for other researchers to attempt to undertake.

3. Develop a logarithmic mathematical model of the erosion of electrode viability to quantitatively take electrode viability into account in later electrical recording studies.

4. Perform studies that will characterize and explain the physiological phenomenon responsible for the findings of the proposed study.

Basic neuroscience studies like these will provide the firm foundation for novel stroke therapies that may have not yet even entered investigators’ imaginations. Humanity is relying on them.

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