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A method for monitoring intra-cortical motor cortex responses in an animal model of ischemic stroke

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Abstract— **Neuroplasticity is believed to play a key role in functional recovery after stroke [1;2]. Neuroplastic effects can be monitored at the cellular level via e.g. neurotransmitter assessment, but these studies require sacrifice of the animal. FMRI can be used to assess functional neuronal performance, but the spatial and temporal resolution is far from the single cell level. The objective was to establish an effective method for short-term analysis of single and multi-unit electrophysiologial function before, during and after stroke. We instrumented one rat with a 16-ch array in the primary motor cortex (100 µm wire diameter) to monitor cortical activity. A bipolar cuff electrode was implanted around the Ulnar nerve in the contra-lateral forelimb to provide a controlled electrical stimulus input to the sensory-motor system. A 3 mm diameter ischemic infarct was created immediately posterior to the electrode array by light activation of a photosensitive dye (Rose Bengal, 1.3 mg/100 mg body weight) at the cortical surface. M1 activity in response to the peripheral electrical stimulus was recorded before, during and after the cortical ischemic infarct. At 425 min following ischemic infarct the peak peri-stimulus time response had decreased to 30 ± 11 % (electrodes placed 1.5 mm from the infarct core) of the activity before the ischemic onset. The mean response latency increased from 30.1 ± 4.5 ms (before infarct) to 40.6 ± 8.5 ms (at 425 min). This dynamic view of neuroplasticity may eventually assist in optimizing acute stroke therapies and optimize functional recovery further.**

Keywords— **rat, primary motor cortex, recording, acute ischemic infarct**

I. Introduction

STROKE is third leading cause of mortality following heart disease and cancer in the Western industrialized countries. Major advances have been made in the past decade in both acute stroke management (including medication and surgery) and stroke prevention (including treatment of risk factors such as hypotension and diabetes). Despite these excellent achievements approximately 75% of all stroke victims survives with some sort of long-term disability [3].

Cerebral ischemia is caused by occlusion of the blood supply in a part of the brain, and 80-85% of all strokes are ischemic in nature [3]. A blockage of the blood supply will typically result in an ischemic area that are damaged within minutes or hours (i.e. the 'core'), and a surrounding area that are less ischemic (also referred to as the peri-infarct zone or the penumbra). Although the neural basis for motor recovery it not fully elucidated, it likely involves cerebral reorganization or neuroplasticity [1;2], which can include functional compensation within residual or contra-lateral cortical areas [4;5].

Cortical reorganization or neuroplasticity has traditionally been investigated using neurophysiologic neuroanatomic or neuroimaging studies. For example, fMRI imaging provides excellent information on how intact brain tissue compensates for loss of functionality of damaged brain tissue, but it has a relatively low temporal and spatial resolution [6;7].

The use of intra-cortical recordings is a well-established method to study cell functionality, coding and plasticity of the mammalian brain in animals, see e.g. [8;9]. Placement of micro-electrode arrays in the cortical tissue has, however, not traditionally been used to evaluate the direct, intra-cortical response following brain injury such as stroke.

The objective of the present work was to study short-term changes (<8 hrs) in the intra-cortical responses from the immediate surroundings of the infarct core. We recorded from the primary motor cortex area related to the forelimb movement and created an infarct site in the primary motor/primary sensory border area. This work is part of ongoing efforts in our lab to investigate cortical neuroplasticity using intra-cortical recordings in an animal model of stroke. We have previously reported on changes in the sensory responsiveness of the auditory cortex following an ischemic infarct [10], where a single micro-wire was placed in the center of the ischemic infarct zone. Those results showed that auditory evoked responses diminished within an approximate time frame of 20 min.

A better understanding of plasticity using electrophysiological recordings may assist in optimizing acute stroke therapies and optimize functional recovery in the future.

II. Methods

A. Animal preparation

Approval for the procedures was obtained from the Animal Care Committee at University of Illinois at Chicago that follows the AAALAC International accreditation program. Data from one male Sprague Dawley rat was collected. The animal was anaesthetized with intramusuclar injections of Ketamine (100 mg/kg), Xylazine (5 mg/kg) and Acepromazine (2.5 mg/kg). A dose of approximately 0.1 ml / 100 g body weight were given every hour to maintain the anaesthesia. The animal's heart rate and oxygen saturation was monitored. Euthanasia was induced by an over dose of sodium pentobarbital transcardially. A catheter was first placed in the femoral vein in the right hindlimb for administration of the Rose Bengal dye solution. A bipolar nerve cuff electrode (length ~10 mm, diameter ~2 mm) was implanted around the Ulnar nerve branch in the right

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forelimb. Electrical stimulation was provided through the peripheral nerve cuff to evoke antidromic, cortical M1 responses. Direct nerve stimulation was chosen over individual muscle activation to avoid muscle fatigue problems (i.e. repetitive stimulation over several hours would not be possible). A craniectomy was performed over the M1 related to forelimb movement (see Fig 1). A 16-ch tungsten microwire array was implanted into the cerebral cortex after the dura layer had been retracted (wire diameter = 50 µm, spacing ~500 µm, depth 1.7 mm). One, stainless steel bone screw was inserted to work as ground reference point for the cortical recordings. No damage of the cortical tissue was visually observed due to the insertion of the electrode, and it was verified that electrodes registered both spontaneous and driven neural signals before the infarction was introduced.

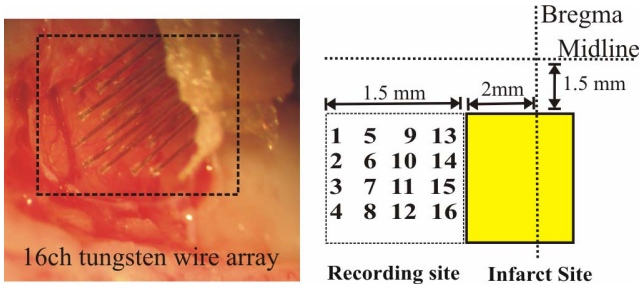

Figure 1. Left: 16-ch tungsten wire array in place over the exposed cortex. Right: Location of the 16-ch tungsten wire array used for recording of the evoked responses, and the light source used to create an ischemic infarct core.

B. Focal infarction procedure

In this study, we chose to implement an animal model of localized ischemia, since a majority of clinical stroke incidents are ischemic in nature [3]. Further, a controlled, localized ischemic event allowed us to accurately position electrode and stroke area with respect to each other, which is not possible with a more clinical relevant animal stroke model such as MCA occlusion. A localized, ischemic infarct was created by activation of a photosensitive dye in the animal's blood stream [11]. A Rose Bengal dye solution (Aldrich Chemicals, 7.5 mg/ml saline solution, 1.3 mg/100 mg body weight) was administered intravenously through the femoral vein catheter. A fiber optic light source (3 mm diameter, wavelength 560 ± 50 nm) was simultaneously placed directly over the exposed pia surface immediately posterior to the implanted micro-wire array to create the ischemic infarct core. The target location for the ischemic infarct was chosen to be the border area between the primary somatosensory cortex (S1) and primary motor cortex (M1). The brain tissue was exposed to light for 20 minutes. The efficacy of the infarction procedure has previously been verified in similar experiments [10].

C. Peripheral nerve stimulation

Electrical stimulation of the ulnar nerve was provided by a Grass stimulator (Grass Telefactor SD9/PSIU6, monophasic stimulation, pulse width = 100µs, frequency ~ 2 Hz). To determine the stimulation current level, we evaluated the motor evoked threshold of selected forearm muscles. An incision was made in the skin over the right forearm, and two needle electrodes were placed 4-5 mm apart in the M. Flexor Carpi Ulnaris and M. Flexor Digitorum Profundus. The EMG response was recorded (Standford research systems SR560, gain = 500, filter frequency 1 kHz -3 kHz) and displayed on an oscilloscope in real-time, while stimulating the Ulnar nerve. The stimulation amplitude was increased until a consistent EMG response was observed, and this level was defined as the motor threshold current (6.2 mA in the present case).

D. Measurements and data-analysis

A head-stage was connected to the micro-wire array for recording of intra-cortical signals (Tucker Davis Technologies). The analog data were filtered (800 Hz - 8 kHz) before sampling at 24 kHz. On-line spike detection was performed using a lower threshold fixed at 1.5 times the RMS value. The threshold was determined based on spontaneous motor cortex firing activity without peripheral nerve stimulation before inducing the ischemic infarct. This threshold level was maintained throughout the experiment. All spike and electrical stimulation timestamps were saved to file for off-line analysis. Neural data were colleted several hours after induction of the ischemic infarct. Peri-stimulus time histograms (PSTH) were generated and synchronized to the onset of the peripheral nerve stimulation. The peak PSTH response (overall maximum) and corresponding peak latency (i.e. the time from onset of the stimulation to the peak response) was identified for comparison. The relative change in the peak response over time was calculated by normalizing the data to the peak response of the control data obtained before induction of the ischemic infarct.

III. RESULTS

The raw PSTH responses obtained before the ischemic onset, at 205 min and 425 min after ischemic onset are plotted in Fig. 2. The responses are grouped according to the electrode distance from the ischemic core (3.0 mm, 2.5 mm, 2.0 mm and 1.5 mm). We observed a modulation of both the peak activity and onset latency in all 16 channels over time.

The PSTH activity showed a non-linear modulation over time. An increased activity (i.e. hyperexcitability) was first observed after onset of the ischemic infarct in the majority of the channels. This period of hyperexcitability was followed by a gradual decrease of the cortical activity over time. The PSTH activity decreased more rapidly and to a larger extent in the recording channels closest to the ischemic core (ch 13-16).

We calculated the relative change in peak PSTH response over time, i.e. the peak PSTH response before the ischemic onset was normalized to 1 (see Table 1). The onset and degree of the hyperexcitability was dependent on the distance of the electrode from the ischemic core. As such, the onset of the hyperexcitability first occurred a distance of 1.5 mm and then at electrodes at 2.0 mm, whereas the modulation of the PSTH activity at electrodes at 2.5 mm and

3.0 mm was not nearly as pronounced.

We also examined the peak latency over time. Opposite to the peak response, the latency appeared to increase in an almost linear fashion over time. The mean latency across all channels before infarct was 30.1 ± 4.5 ms and this increased to 40.6 ± 8.5 ms at 425 min after ischemic onset.

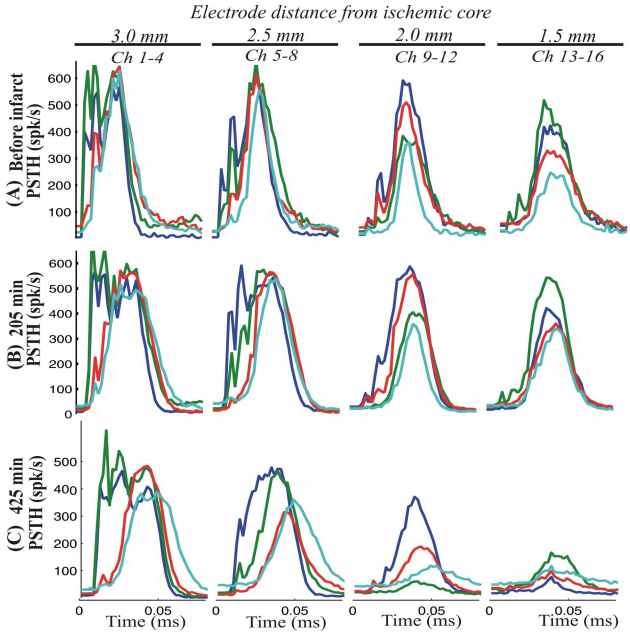

Figure 2. Peri-stimulus time histograms (PSTH) from all 16 electrodes plotted as a function of the distance from the ischemic core. A decrease in the evoked, cortical responses was found to be dependent on the distance to the ischemic core. A) Control data before infarct. B) Responses at 205 min after onset of ischemic infarct. C) Responses at 425 min.

TABLE 1. Relative change of the peak PSTH responses (mean ± std) over time as a function of the electrode distance from the ischemic core. The responses were normalized to the responses before onset of the ischemic infarct (0 min).

DIST TIME	3.0 mm	2.5 mm	2.0 mm	1.5 mm
0 min	100 %	100 %	100 %	100 %
109 min	90 ± 12 %	90 ± 3 %	102 ± 7 %	121 ± 21 %
205 min	97 ± 13 %	93 ± 5 %	104 ±8 %	117 ± 14 %
307 min	101 ± 26 %	86 ± 2 %	83 ± 15 %	64 ± 9 %
425 min	82 ± 14 %	75 ± 6 %	53 ±14 %	30 ± 11 %

IV. Discussion and Conclusions

The objective of the present work was to investigate if short-term neuroplasticity could be monitored in the motor cortex in an animal model of ischemic stroke. We found that the cortical responses in the motor cortex do in fact exhibit marked changes, including a period of hyperexitability. Extinction effects of electrophysiological results are directly proportional to distance from the lesion. Cerebral ischemia causes a series of events that can lead to neuron damage and/or cell death. The reduced supply of oxygen and glucose following the ischemia can cause e.g. release of neurotransmitters, altered ionic flux and peri-infarct depolarization. These effects may explain the observed period of hyperexcitability in the electrodes closest to the ischemic core (2.0 mm and 1.5 mm). The same hyperexitability may have been observed in the electrodes further away from the ischemic core (3.0 mm and 2.5mm) if the recording session had continued beyond the 425 min in the present data set. We are currently in the process of collecting more data to quantify the variability of the cortical responses following stroke within the acute time frame. The extent of the effect of the ischemic infarct will be examined further in future experiments using both electrophysiolgical and histological techniques.

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