Multi-site Analysis of Dopamine Uptake in the Somatosensory Cortex.

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***Abstract*— Voltammetry has been used as a method to measure the concentration of monoaminergic neurotransmitters in-vivo. The standard electrode used with voltammetry has been carbon fiber microelectrodes. Despite the advantages of using carbon as a sensing element, carbon fiber microelectrodes have only one site to record the extracellular concentration of neurotransmitters. Studies have shown that the concentration of neurotransmitters, such as dopamine, varies across small regions of the brain (less than 1mm). To study the varying concentration of dopamine, the recording sites of a ceramic-based multi-site electrode was coated with carbon and deployed in the somatosensory cortex of a rat. Known concentrations of dopamine were pressure injected and the diffusion curve, which is the change in concentration over time, was recorded. From the falling phase of the diffusion curve, the initial rate of clearance was measured. The initial rates of clearance from the different recording sites in the somatosensory cortex were compared to a model that used the standard diffusion equation with uptake. The results show that the in-vivo data does not follow the prediction of the model providing an interesting insight to the uptake of monoamines across the different layers of the somatosensory cortex.**

I. Introduction

Monaminergic neurotransmitters, such as dopamine, serotonin and norepinephrine, have generally been thought of as neuromodulators, changing the state of neuronal networks. Small nuclei in the brainstem house the cell bodies of cells that project widely and diffusely throughout the brain. The extracellular concentration of these monamines is controlled by reuptake transporters. It has generally been thought that the maximal rate of clearance (Vmax) of monoamines is constant across different regions of the brain. However, several studies have shown that the clearance of monoamines differs throughout small regions of the brain. Peters and Michael [1] showed that dopamine in the striatum varied across regions as small as 50 microns and Mitchell et al., [2] demonstrated that the Vmax of norepinephrine differed between the upper and lower layers of the anterior cingulate. However, each of these investigations were limited by the fact that it was not possible to record from precisely spaced multiple sites.

Several techniques have been used to measure the concentration of monoaminergic neurotransmitters in the brain. Chronoamperometry takes advantage of the fact monamines can be oxidized by applying a square-wave voltage signal and the resulting oxidation current is proportional to the extracellular concentration of the monoamine at the surface of the microelectrode. Carbon fiber microelectrodes are widely used in conjunction with the voltametric methods including voltammetry. Carbon is used for several reasons. First, it has long been recognized that monoamines, such as dopamine (DA), adsorb to carbon [3]. Second, carbon has a wide window for oxidation without breaking down. This makes carbon an ideal substrate for measuring the extracellular concentration of monoamines.

The drawback to using carbon fiber microelectrodes is that the monoamine concentration can only be measured at a single site. This drawback of carbon fiber microelectrodes does not allow the heterogeneity of monoamine across small regions of the brain, within 50 microns, to be simultaneously recorded. Previous attempts have been made to develop multisite voltammetry probes. These attempts range from multiple carbon fibers to thin film silicon probes with platinum recording. The multiple carbon fibers provided a means to measure monoamine concentration within 50 microns, but the spacing between the multiple fibers could not be maintained [1]. Thin film silicon probes with platinum recording sites allowed precise spacing between recording sites. However, platinum is not a good surface to measure the concentration of monoamines. To overcome these limitations, we designed and built a thin film multisite voltammetry probe with carbon surfaces. The multisite probe utilized technology that was used on our previous ceramic-based multisite electrode (CBMSE). Ion beam assisted deposition was used to coat the recording sites of the CBMSE with carbon. This new carbon coated multisite ceramic-based electrode (CC-CBMSE) was shown to perform as well as standard carbon fiber microelectrodes that are commonly used in voltammetry studies [4].

The goal of this paper was to use the developed CC-CBMSE to study the heterogeneity of clearance in the somatosensory cortex. Using the precise spacing of our microelectrode, we will place the recording sites in the somatosensory cortex and then pressure inject known concentrations of DA, record the change in concentration at each recording site, and compare the clearance of DA across

Manuscript received April 3, 2006.

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the different recording sites.

II. Experimental Methods

A. Carbon coated ceramic array design

The fabrication of ceramic based multi-site electrodes have been previously described [5]. Briefly, thin film technology was used to manufacture a multi-site electrode with independent recording sites for voltammetric recordings. These electrodes were then modified with a rough carbon coating. For this step, a photoresist was deposited leaving only the recording sites exposed. Next, the carbon was e-beam deposited onto the recording sites. The unwanted carbon overlying the photoresist was removed in a final lift-off step. Each CC-CBMSE array was released from the substrate by laser cutting. To complete the assembly, the bonding terminals were mounted onto a micro-connector. The wire was extended from the bonding pad on the electrode to the gold tab on the connector. The leads were covered with a thick layer of non-conducting epoxy to protect them during the implantation procedure.

B. Electrode Calibration

Calibration curves were generated using the IVEC-10: In-Vivo Electrochemistry Computer system. Electrodes were mounted on a frame and connected to the IVEC-10 headstage. The CC-CBMSE array and a reference electrode (Ag/AgCl) were lowered into a beaker with 40 mL of 0.1 M phosphate buffered saline (PBS), pH 7.4. A step potential of +0.50V for DA versus Ag/AgCl was used. Baseline currents were recorded and a gain parameter was set to normalize the background current. Known concentrations of DA were added to the solution and the resulting oxidation current was recorded. After the last addition, the software automatically performed the linear regression of the oxidation and reduction curves. The resulting calibration curve's slope was representative of the sensitivity of the electrode to DA. The values were stored and automatically transferred to an acquisition module of the program for measuring monoamine concentrations in vivo with this electrode.

C. Animal preparation

Adult rats were anesthetized with an intraperitoneal injection of urethane (1300mg/kg) and placed in a stereotaxic frame with the top of the skull in the horizontal plane. The head was shaved and an incision was made above the skull. The skin and fascia were removed and two burr holes were made. The first hole was made at 2.0 mm posterior to bregma and 5.2 mm lateral over the primary somatosensory cortex, where the working electrode would be lowered. The second hole was made posterior to lamda where the reference (Ag/AgCl) electrode would be inserted and held in place by Durelon dental cement.

D. Electrode Preparation

Dopamine was utilized as the model monoamine because it is not endogenous to the somatosensory cortex but is taken up by the norepinephrine (NE) transporter [7]. Therefore, we do not expect any interference from endogenous DA in our signal. To measure the reuptake and diffusion of DA in the brain, a micropipette was attached to the electrode, acting as a delivery system. The micropipette was pulled from a small capillary tube with an inner diameter of .86mm (A-M System, Inc., Calsborg, WA) to a final inner diameter of 25 microns. The tip of the micropipette was placed 120 microns from recording site B, making it 180 microns from recording site C, 256 microns from recording site D and 365 microns from recording site A. The micropipette was filled with .2 uM of DA. The electrode pipette combination was lowered 1mm through the craniotomy in the skull made above the SI (2.0 mm posterior to bregma and 5.2 mm lateral). A Ag/AgCl reference electrode that was used in calibrating the elctrode was placed into the brain posterior to lamda.

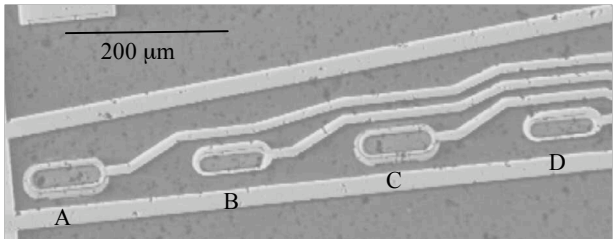

Fig.1. Picture of the tip of a CC-CBMSE. Recording sites were labeled A through D for clarity.

E. Monitoring Diffusion and Uptake of DA In-vivo

The IVEC-10 In-Vivo Electrochemistry Computer system was used to monitor the diffusion and uptake of DA in-vivo. Similar to calibration, the electrode and attached micropipette were mounted on a frame and connected to the IVEC-10 headstage. After baseline currents were collected, known volumes of DA were pressure injected from the micropipette into the extracellular space. The resulting diffusion curve, which is the change in DA concentration over time, was recorded. Pressure injecting DA flooded the system with DA and created an overflow condition at all sites. From the initial slope of the falling phase of the diffusion curves, the rate of clearance, $C_{20\text{-}60}$, can be calculated.

F. Modeling Diffusion and Uptake of DA

A mathematical model was derived and integrated using methods described by Nicolson to model the change in DA concentration over time and space [6]. An uptake term that characterized the maximal rate of clearance, Vmax, and a Michaelis-Menton constant Km was added to the standard form of the diffusion equation to model the uptake of DA in the SI after pressure injecting known concentrations of DA. The Vmax used in the model was calculated from the in-vivo slope of the falling phase of the diffusion curve from the site closest to the injection source. The binding affinity of the norepinephrine transporter to DA (Km) was used in all calculations [7]. Dopamine is not endogenous to SI, but

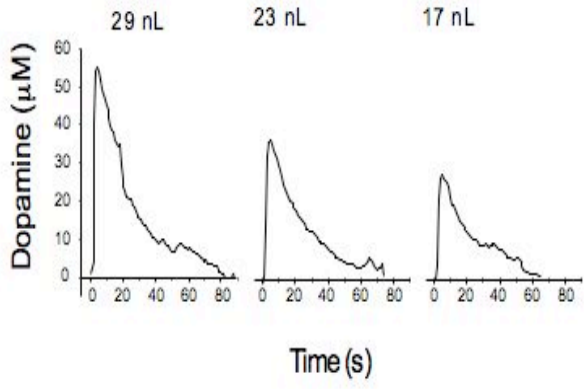

Fig. 2. Diffusion curves for different volumes of injected DA at the same recording site. At time zero, DA was pressure injected into the extra cellular space. The maximal rate of clearance is calculated from the falling phase of the diffusion curve.

is taken up by the NE transporter. Therefore, the concentration of DA that is seen in-vivo in the SI is due only to what is pressure injected and is not effected by natural release. The output of the model gave the change in DA concentration over time as distance increased from the source of the injection.

III. Data Analysis

The CC-CBMSE was attached to a micropipette that was filled with DA. Known concentrations of DA was pressure injected into the extracellular space. Pressure injecting DA caused an overflow condition at all of the recording sites. From the diffusion curves, the initial slope of the falling

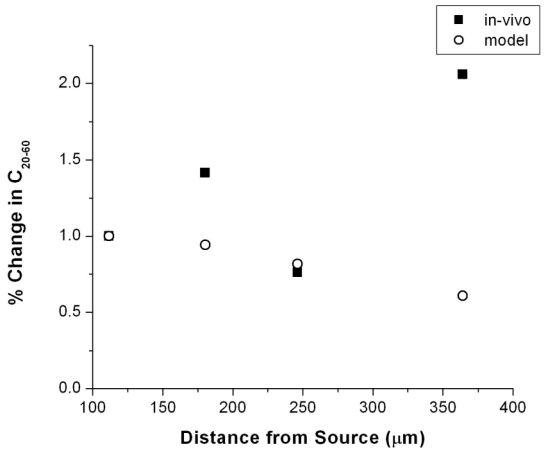

Fig. 3. In-vivo and model relative change in $C_{20\text{-}60}$. Data was normalized to the site closest to the injection source. The model used the slope of the falling phase of the diffusion curve as a value for Vmax.

phase of the diffusion curve was calculated. The initial slope is defined as the decay of the signal from 20% to 60% of the maximum concentration of the signal. This rate of clearance, $C_{20\text{-}60}$, is used to describe the clearance of DA from the SI. The $C_{20\text{-}60}$ values were also calculated from the diffusion curves generated by the model and compared to the values calculated in-vivo.

IV. Results

The goal of this paper was to use the developed CC-CBMSE to study the heterogeneity of clearance across the SI. Known concentrations of DA were pressure injected into the SI of the rat and the change in concentration at each recording site was recorded. The change in concentration over time can be seen in the diffusion curve. At time zero, a known concentration of DA was pressure injected into the extracellular space. The resulting diffusion curve is the concentration of DA that diffused past the recording site. Each recording site was in overflow condition. Overflow condition exists when the concentration of DA in the extracellular space is not at steady state. From the diffusion curve, the initial slope of the falling phase of the diffusion curve, $C_{20\text{-}60}$, can be calculated. The $C_{20\text{-}60}$ values were calculated from both the in-vivo and model data and normalized to the site closest to the injection source. The data was normalized to the site closest to the injection source because the model used the slope of the falling phase of the diffusion curve as a value for Vmax.

The model showed a minimal change in the $C_{20\text{-}60}$ values relative to the site closest to the injection source with each site decreasing slightly as distance increased. However, the in-vivo $C_{20\text{-}60}$ changed drastically from site to site relative to the site closest to the injection source. While the model showed a slight decrease in the relative $C_{20\text{-}60}$, the in-vivo data showed only one site decreased relative the site closest to the source.

V. Discussion

A. Multi-site electrode for electrochemical recordings

Thin film technology has allowed us to make a multisite electrode that has precise spacing in between recording sites [5]. We have further demonstrated that after the recording sites of the multisite electrode are carbon coated, the electrode can be used for electrochemical recordings [4]. Using a multisite electrode for electrochemical studies can greatly enhance the understanding of the reuptake of neurotransmitters across small regions of the brain.

B. Significance of in-vivo data

The results suggest that Vmax, which is mainly a reflection of clearance, may be different across different layers of the cortex. With Vmax held constant, the model showed a slight decline in the $C_{20\text{-}60}$ values as distance increased from the site closest to the injection source. Since the in-vivo data did not follow the prediction of the model, this could suggest that the Vmax across the SI differs. Since NE fibers are uniformly distributed, by changing the parameters of the uptake of NE, the extracellular concentration can be controlled independently.

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