IN VITRO STUDY OF A NEW CARBON FIBER MICROELECTRODE FOR REAL-TIME MEASUREMENT OF DOPAMINE WITH DIGITAL WIRELESS TRANSMISSION

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Abstract

Dopamine (DA) is a neurotransmitter that plays a critical role in the central nervous system (CNS). Significant effort has gone into investigating DA's role in the pathophysiology of many CNS diseases such as schizophrenia, drug addiction, and Parkinson's disease. Specifically, real-time in vivo studies of the DA system in conscious, freely moving animals performing behavioral tasks are the foundation for research into the mechanisms behind DA release. This study details the in vitro performance of a new, commercially available, system for dopamine measurements in an untethered, freely moving rat. The system consists of a microelectrode, constructed from stainless steel and fused silica, sheathing the carbon fiber microelectrode, and a wireless potentiostat employing chronoamperometry. The wireless potentiostat, weighing less than 7g, was designed to be mounted on the head of a rat. Amplification and digitization are done on the potentiostat and a digital signal is then transmitted to a basestation connected to a PC. The microelectrode was connected to the wireless potentiostat and tested in vitro for sensitivity and selectivity. The microelectrode showed a DA sensitivity of 2 nA/uM and can be coated with Nafion to improve selectivity. This new type of microelectrode is very robust and displays similar characteristics to other commercially available glass-sealed carbon fiber microelectrodes. Variations on the mechanical design were able to produce fused silica sheathed carbon fiber microelectrodes with an integrated Ag/AgCl reference electrode minus the stainless steel tubing. The microelectrode sheathed in silica is very rigid and durable. Microelectrodes that reach the deepest regions of the brain of large animals can be easily fabricated. Conflict of Interest: Research is conducted, in part, by a company that produces a product related to the work being reported

Introduction

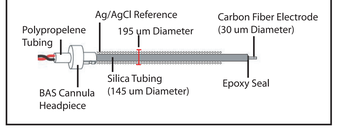
- Finding links between brain chemistry and behavior is critical.
- Carbon fiber microelectrodes allow for real time in vivo monitoring of neurotransmitters such as dopamine, serotonin & norepinephrine
- Traditional, tethered acquisition from implanted electrodes causes stress on the animal as well as measurement noise.
- A wireless potentiostat has been developed that is compatible with high impedance biosensors and carbon fiber microelectrodes
- A novel carbon fiber microelectrode has been developed that can be made to any length for deep implantations.

Materials and Methods

Carbon Fiber Electrodes:

- 30um diameter carbon fibers were secured in a fused silica sheath using epoxy.
- Silica sheathed fibers were secured to a BAS cannula head.
- An electrical connection between the carbon fiber and connector wire was made using graphite epoxy paste.
- Carbon fibers extending from the fused silica sheath were trimmed to 200 - 300 um.
- A 25um diameter Ag/AgCl reference electrode was wrapped concentrically around the silica tubing.
- Selectivity was improved by multiple coatings of Nafion.

Figure 1: Carbon fiber microelectrode diagram



Evalutation:

- All in vitro testing was conducted in 100mM PBS buffer (pH 7.4).
- Carbon fiber electrodes immersed in PBS buffer were subjected to multiple injections of 1uM. Dopamine in 0.1M $HClO_4$ up to a final concentration of 4uM.
- Two wireless systems were tested against a bench top standard, a wireless high-speed chronoamperometry system with 14 bit resolution and a constant potential wireless potentiostat with 24 bit resolution.

Results

Figure 2a: In Vitro Calibration of Carbon Fiber Microelectrodes: Baseline Benchtop Potentiostat

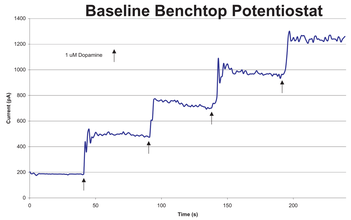


Figure 2b: In Vitro Calibration of Carbon Fiber Microelectrodes: 14-bit Wireless Pulsed Potentiostat

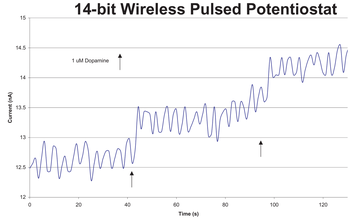


Figure 2c: In Vitro Dopamine Calibration of Carbon Fiber Microelecrodes: 24-bit Wireless Constant Potentiostat

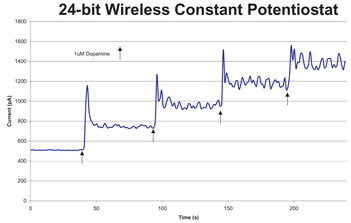
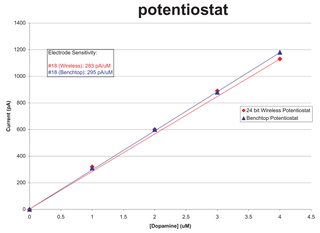


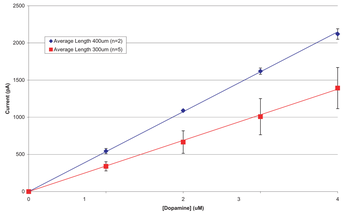
Figure 2a, 2b & 2c shows the same electrode with an integrated Ag/AgCl reference electrode calibrated using the benchtop, 14 bit pulsed wireless and 24 bit constant potential wireless potentiostats. The benchtop system showed excellent signal resolution (Figure 2a). The pulsed wireless potentiostat effectively amplified the signal at the cost of signal resolution (Figure 2b). The constant potential wireless potentiostat showed signal resolution comparable to the benchtop potentiostat (Figure 2c).

Figure 3: Carbon Fiber Calibration Curve: Electrode tested with both the 24 bit constant potential wireless potentiostat and benchtop potentiostat



Measurements of a single electrode using the bench top potentiostat and 24 bit constant potential wireless potentiostat show very low noise The difference in measured sensitivity is +/- 1 % for the electrode used in this study (representative of all electrodes). The pulsed wireless system signal to noise ratio is very low and therefore, it was not included in this analysis. (Figure 3)

Figure 4: Carbon Fiber Calibration Curve: Variation of Sensitivity due to Length



Variation of electrode sensitivity is largely due to differences in the length of the active carbon fiber surface. (Figure 4)

Conclusion

- High-speed chronoamperometry offers many advantages in bench top applications; however, for small, low-power, wireless designs fixed potential systems are a better choice.
- Silica sheathed carbon fiber microelectrodes have very similar characteristics to commercially available glass encased carbon fiber electrodes.
- Unlike glass enclosed carbon fibers, Silica sheathed carbon fibers can be made to any length and can incorporate an integrated Ag/AgCl reference electrode
- A high resolution, wireless, head mounted, potentiostat has been developed and tested.

Discussion

Carbon fibers sheathed in fused silica tubing share many characteristics similar to more commercially available carbon fiber microelectrodes sealed in glass. Fused silica sheathed carbon fibers do offer several advantages over carbon fibers encased in glass capillaries. The fused silica sheath provides a very rigid yet flexible vehicle for the delicate carbon fibers, consistently along the shaft of the electrode. This makes the fused silica sheath more durable than other electrodes sheathed in tapered glass capillaries. Also, thanks to the fused silica tubing, electrodes can be made to nearly any length to accommodate the deepest of implantations. However, because of the tapered design, glass encased carbon fibers will cause less damage to the area of interest during implantation.

The goal of high-speed chronoamperometry is to increase the signal to noise ratio by increasing the magnitude of the response seen for a particular concentration of dopamine. The general approach is to cycle the bias between two values. In a normal situation, square pulses of 60 mS duration and 0-0.65 V in amplitude vs. Ag/AgCl reference electrode are applied to the working electrode. The bias is then returned to 0V and the process is repeated every 250 ms the sensor current is measured a short time after applying the bias and then again before removing the bias. It is important to note that although this technique increases the available signal level, sensor selectivity is not improved. For wireless systems our results have shown that the increase is signal is achievable, but there are numerous problems with the general approach. The primary issue is significant amount of digital traffic is required to create the pulse streams required. This digital traffic increases the general noise in the system. If the working potential is always returned to zero than a simple switch could replace the digital to analog converter, but this reduces system flexibility. Secondary issues include battery life, analog to digital resolution and custom pulse requirements. In our prototypes battery life was decreased by approximately 75% due to the increased activity of the signal converters. Also, given the size, power and temporal constraints inherent in this design, the maximum resolution obtainable with of the shelf components is 14 bits. In addition, the technique requires that the pulse and measurement timing must be adjusted for different experimental set-ups. While this is possible, a significant amount of experimental "tweaking" may be required. Telemetry power requirements are not affected since several measurements can be averaged. That is, the telemetry requirements are effectively the same as our standard model 3102 wireless potentiostat.

Citation:

Swiergiel, A.H., Palamarchouk, V.S. and Dunn, A.J. (1997)A new design of carbon fiber microelectrode for in vivo voltammetry using fused silica, J. Neurosci. Methods, 73: 29-33.

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