Highly Selective and Mechanically Robust Sensors for Electrochemical Measurements of Real-Time Hydrogen Peroxide Dynamics *In Vivo*

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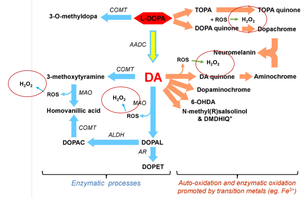
Oxidative Stress in the Brain

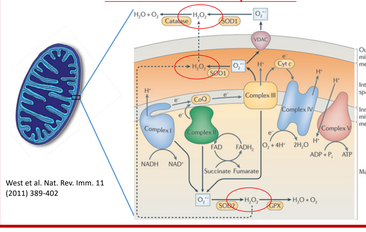
- Oxidative Stress is implicated in various neurodegenerative disorders, such as Parkinson's disease.
- Evidence suggests that H_2O_2 plays an important role as a diffusible neuromodulator in various aspects of brain function.
- **Dopamine (DA) can increase extracellular levels of H_2O_2 due to various enzymatic processes and/or auto-oxidation. Additionally, mitochondrial dysfunction can result in increased formation of O_2 radicals that can combine to form H_2O_2.**

Dopamine Synthesis / Breakdown Pathways

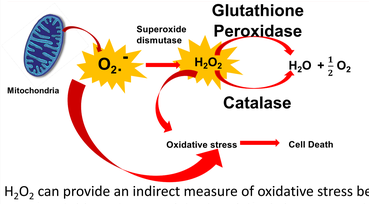


Adapted from: Lipski et al., Progress in neurobiology 94 (2011) 389-407

Mitochondrial Respiration



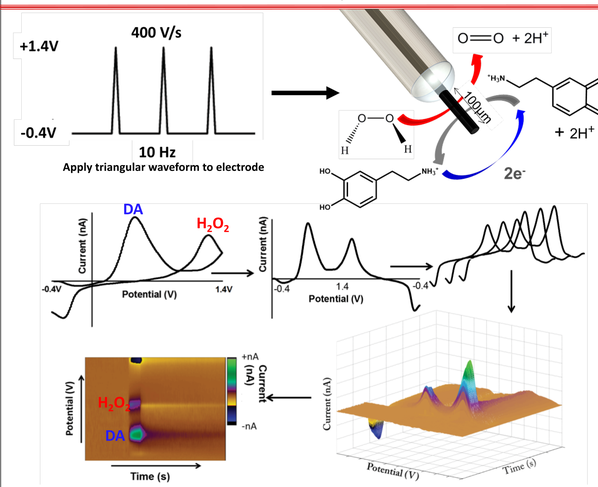
West et al. Nat. Rev. Imm. 11 (2011) 389-402



H_2O_2 can provide an indirect measure of oxidative stress because it remains stable and accumulates to relatively large concentrations.

Goal:
To develop a new tool to selectively monitor endogenous H_2O_2 dynamics.

Fast-Scan Cyclic Voltammetry



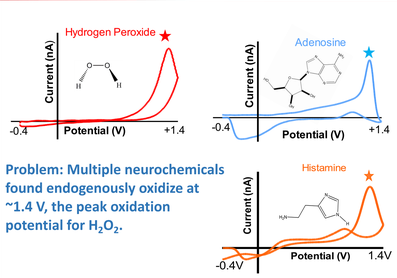
Carbon-fiber microeletrodes:
- **Minimize tissue damage**
- **Reduce surface biofouling**

Background-subtracted cyclic voltammograms (CV); peaks serve to identify analytes.

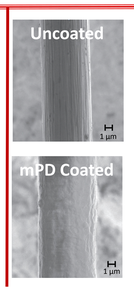
CV's are unfolded at switching potential and arranged in time.

CVs are concatenated into a color plot with current shown in false color.

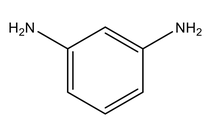
Chemically Selective Coating



Problem: Multiple neurochemicals found endogenously oxidize at ~1.4 V, the peak oxidation potential for H_2O_2.

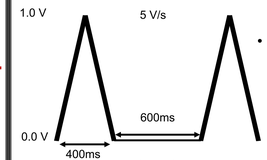


Solution: Electrodeposition of 1,3-phenylenediamine (mPD) onto the carbon fiber creates a size exclusion polymer to exclude many larger interferents.

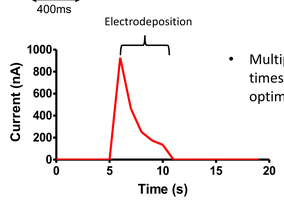


DMED Performance *In Vitro*

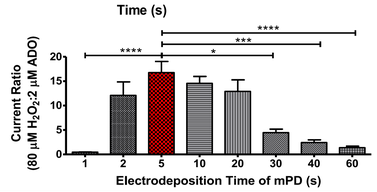
Optimization of mPD

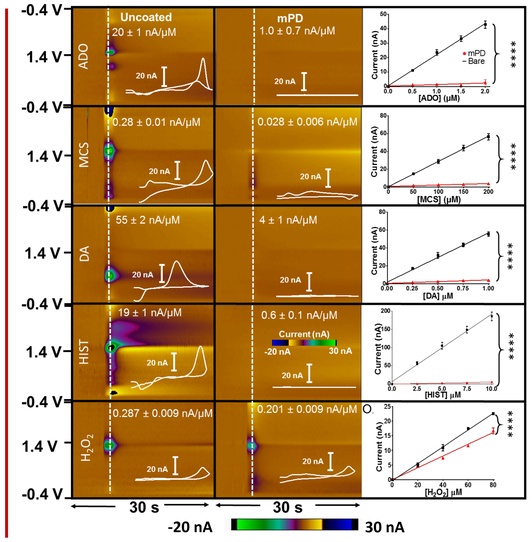


- mPD was electrodeposited using a triangular waveform from 0-1 V for 400 ms with 600 ms between waveforms.



- Multiple electrodeposition times were tested and was optimized at 5 seconds



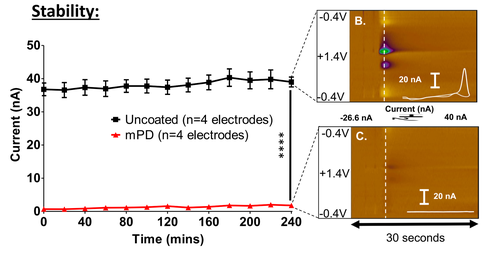


In Vitro Characterization

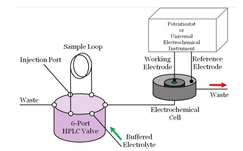
Selectivity:

- Dual Micro Electrode Devices (**DMEDs**) allows for a side-by-side comparison of the mPD membrane's performance compared to an uncoated electrode.
- A DMED was used to test the selectivity of the mPD coated electrode *in vitro*.
- The mPD coated electrode successfully excluded interferents such as adenosine (ADO) , MCS, and DA.
- H_2O_2 was selectively detected on the mPD electrode.

Stability:

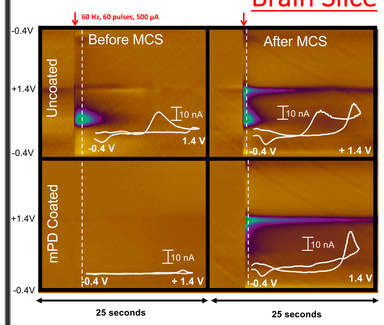


- The coating was shown to be stable over a 4 hour time period.
- All of these data were collected using a flow injection apparatus.

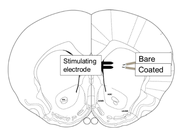


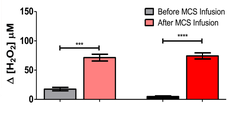
DMED Performance *In Vivo*

Brain Slice

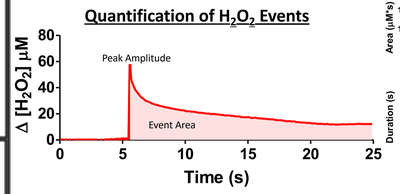


- **MCS:** Glutathione peroxidase inhibitor that increase concentrations of endogenous H_2O_2 (see graphic; top left panel).





Quantification of H_2O_2 Events



Results indicate the DMED electrode can be used to effectively quantify H_2O_2 transients and eliminate interferents in brain slice.

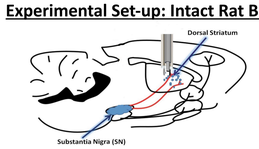
In Vivo

Device Fabrication

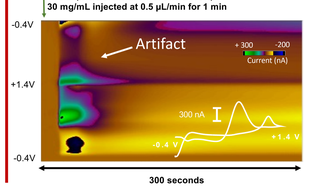
DMEDs attached to microinfusion needles, termed "injectrodes" allow for local infusion of pharmacological agents in close proximity to the working electrodes.

Micro-Infusion Needle

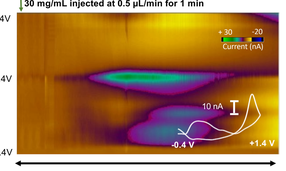
Experimental Set-up: Intact Rat Brain

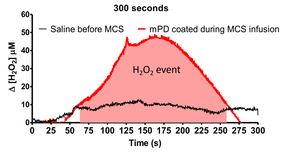


Uncoated Electrode



mPD Coated Electrode



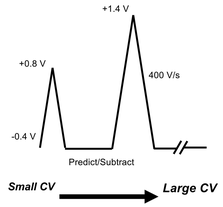


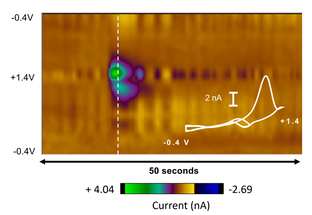
	Amplitude (µM)	Area (µM*s)
Saline (control)	4.9 ± 0.4	357 ± 111
MCS	48 ± 3	8608 ± 1606
t-statistic	t(3)=20.00	t(3)=4.820
p-value	***p<0.001	*p<0.05

- MCS increased the amplitude and area of H2O2 events recorded on the mPD-Coated microelectrode
- H_2O_2 events are difficult to distinguish on uncoated as MCS itself is electroactive, producing a large signal.
- MCS provided pharmacological validation of the endogenous H_2O_2 signal on the mPD electrode.

Applications

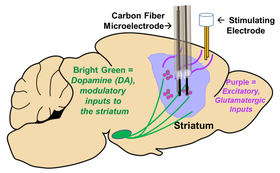
H_2O_2 Generation in the Dorsal Striatum Evoked by Electrical Stimulation





Data provided by Karen E. Butler

- The double waveform essentially scans up to 0.8 V and back down before the larger waveform (-0.4 V to 1.4 V) to quantify the pH contribution to the signal. This is subtracted from the original signal to produce a more accurate measurement of the signal.
- H_2O_2 signals will be determined in animals post stimulation using a double waveform applied to a DMED to selectively quantify H_2O_2 signals from adenosine.



Stimulating electrodes will be placed in the prefrontal cortex (PFC) and biphasic electrical stimulation parameters will be varied, including frequency and duration.

Conclusions

- Carbon-fiber microelectrodes coated with mPD are a powerful new tool which can be used to selectively monitor H_2O_2 in the brain.
- Pharmacological manipulation with MCS confirm selective measurements of endogenous H_2O_2 in the intact brain.

Acknowledgements

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