Electroanalytical Tools and Molecular-Based Assays to Measure the Impact of Noise on Dopamine Neurotransmission in the Central Auditory Pathway

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Introduction

- In the United States, the effects of hearing loss affect approximately 48.1 million people.[1]
- According to the Department of Veteran's Affairs, hearing loss and tinnitus are the top two disability claims, estimating $1.8 billion in compensation.[2]
- Patients with Parkinson's disease, which is associated with dopamine depletion, exhibit deficits with auditory processing.[3]
- Following acoustic trauma, changes in tyrosine hydroxylase (TH) gene expression have been demonstrated within the central auditory brain regions (cochlear nucleus and inferior colliculus).[4]
- Electrophysiological experiments have demonstrated that dopamine within the inferior colliculus responds to auditory responses in a heterogeneous manner.[5]
- However, exactly how dopamine neurotransmission modulates hearing processes and how the neurotransmitter system is compromised through hearing loss is not well understood.
- The present research seeks to evaluate the connection between damaging noise and dopamine neurotransmission through electrochemical and molecular-based techniques.

Experimental Design and Methods

Sound Exposures

- Sound booth dimensions: 36" wide x 30" long x 25.5" tall.
- The booth was calibrated by measuring the extent of the speaker's sound cone within 3 dB from a variety of angles to control for any possible contributions from random noise.
- Animal subjects (Adult Sprague Dawley Rats) were exposed to 10 kHz at 118 dB SPL at 1/3 octave band for four hours.

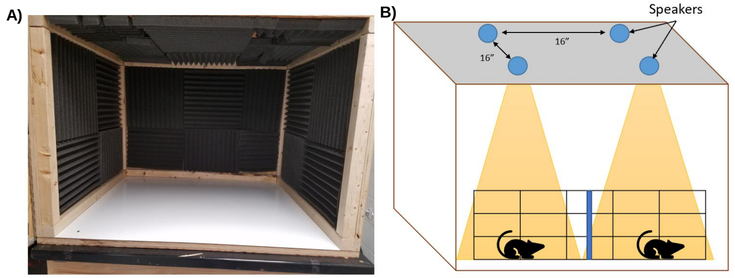

Figure 1. Sound Both. (A) Box interior lined with acoustic dampening foam. (B) Speakers arranged 16" apart in a square, animal subjects housed in wire cage beneath speakers.

Fast Scan Cyclic Voltammetry (FSCV)

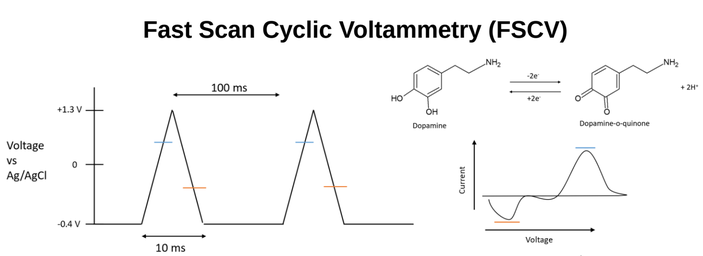

Figure 2. FSCV Characterizing of dopamine. Electrical potential in a triangular waveform is applied at a carbon fiber microelectrode surface from -0.4 V to 1.3 V and back to -0.4 V at a scan rate of 400 V/s. In the forward scan, dopamine is oxidized to dopamine-o-quinone while in the reversed scan, dopamine-o-quinone is reduced to dopamine. The current generated as a result of the redox reaction is related to the concentration of dopamine. Neurochemicals demonstrate a specific cyclic voltammogram associated with the identity of the chemical.

References

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(3) Lai, S.-W.; Liao, K.-F.; Lin, C.-L.; Lin, C.-C.; Sung, F.-C. Hearing Loss May Be a Non-Motor Feature of Parkinson's Disease in Older People in Taiwan. *European Journal of Neurology* **2014**, *21* (5), 752–757. https://doi.org/10.1111/ene.12378.

(4) Fyk-Kolodziej, B. E.; Shimano, T.; Gafoor, D.; Mirza, N.; Griffith, R. D.; Gong, T.-W.; Holt, A. G. Dopamine in the Auditory Brainstem and Midbrain: Co-Localization with Amino Acid Neurotransmitters and Gene Expression Following Cochlear Trauma. *Front. Neuroanat.* **2015**, *9*. https://doi.org/10.3389/fnana.2015.00088.

(5) Gittelman, J. X.; Perkel, D. J.; Portfors, C. V. Dopamine Modulates Auditory Responses in the Inferior Colliculus in a Heterogeneous Manner. *J Assoc Res Otolaryngol* **2013**, *14* (5), 719–729. https://doi.org/10.1007/s10162-013-0405-0.

Experimental Design and Methods

Electrode Fabrication

- Carbon fiber was aspirated through a glass capillary, then pulled using a puller.
- The fiber was cut to 50 µm under a microscope.
- A silver wire was fed through the other end of the capillary tube and a pin was soldered to it.
- Electrode is reinforced and sealed with heat shrink.

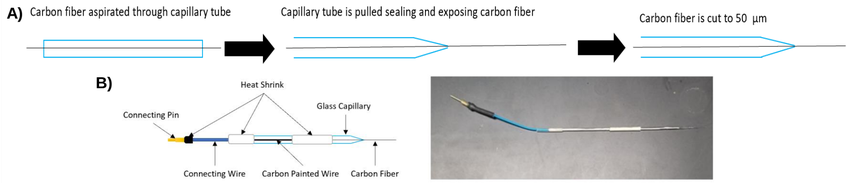


Figure 3. Carbon fiber microelectrode fabrication. (A) Process of glass capillary preparation, carbon fiber sealed by heating and pulling the glass capillary, fiber cut to optimal neurochemical detection length. (B) Schematic of completed electrode, heat shrink reinforces integrity of the electrode, connecting pin soldiered to interface with FSCV instrument.

FSCV Sensitivity & Selectivity Calibration

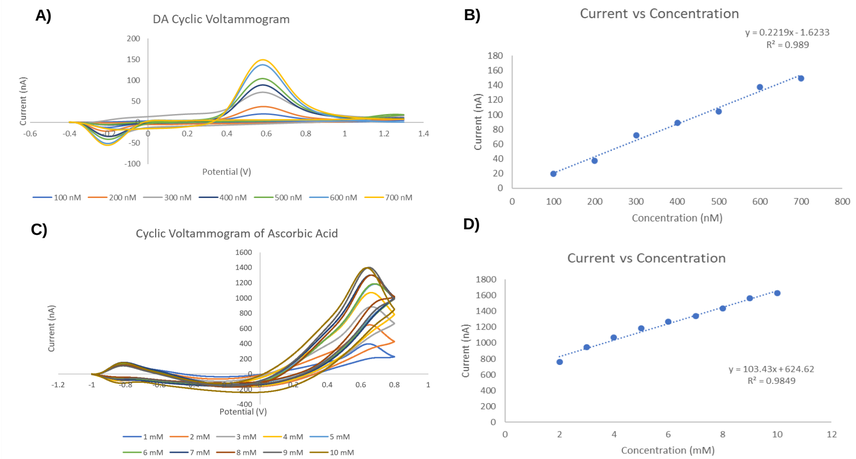
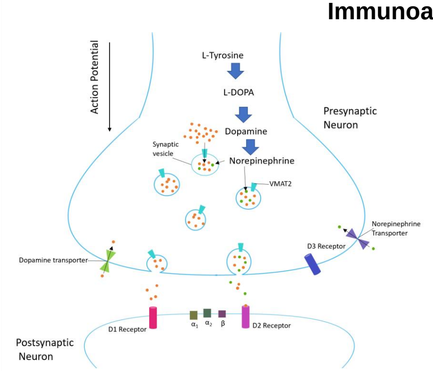


Figure 4. Optimization of sensitivity/selectivity of an electroanalytical assay (A) Cyclic voltammograms of dopamine overlay at varying concentrations. (B) Calibration curve plotted from the peak current of the cyclic voltammogram. (C) Overlay of ascorbic acid cyclic voltammograms indicating selectivity of the method. (D) Calibration curve of ascorbic acid indicating sensitivity of the electroanalytical method.

Results

Immunoassays



Hypothesis:
Deafness-related changes in the monoamine neurotransmission are a result of modulation of the auto receptor function.

On-going Experiment:
Evaluating the impact of noise-induced deafness monoamine:
- Receptor function using FSCV and pharmacology.
- Receptor levels using Western blot.
- Receptor localization/distribution using immunocytochemistry.

Figure 5. Neuron Schematic. Combination of dopamine and norepinephrine synthesis and neurotransmission. Immunoassay targets will provide mechanistic details of neurotransmission modulations from sound exposures. Potential mechanisms include feedback inhibition or reactive oxygen species.

Results

Control Slice FSCV

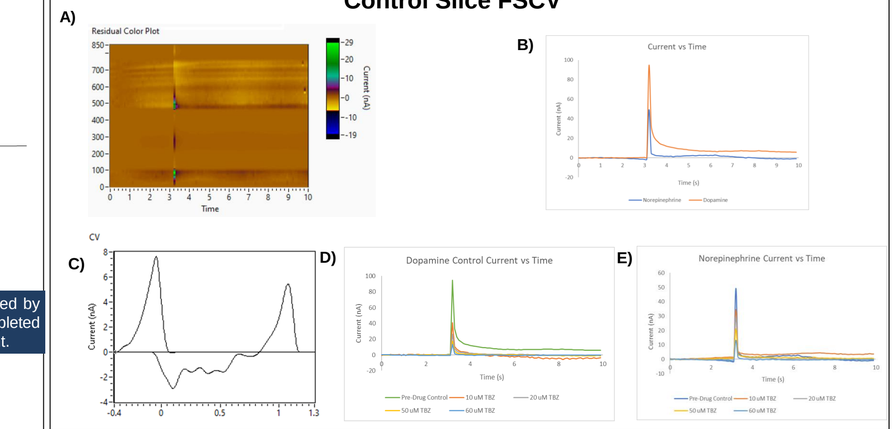


Figure 6. FSCV control data in rat central inferior colliculus (CIC). (A) Color plot denoting two oxidation peaks: dopamine (DA) top, and norepinephrine (NE) bottom. The first time two neurochemicals have been detected simultaneously (B) Current vs. time overlay demonstrating NE/DA neurotransmission. (C) Cyclic voltammogram of DA/NE combination. (D) Tetrabenazine (TBZ) treatment inhibiting dopamine release. (E) TBZ treatment inhibiting concentration of NE release. Both drug treatments confirm monoamines as the source of FSCV recorded signals.

Sound Exposed Slice FSCV

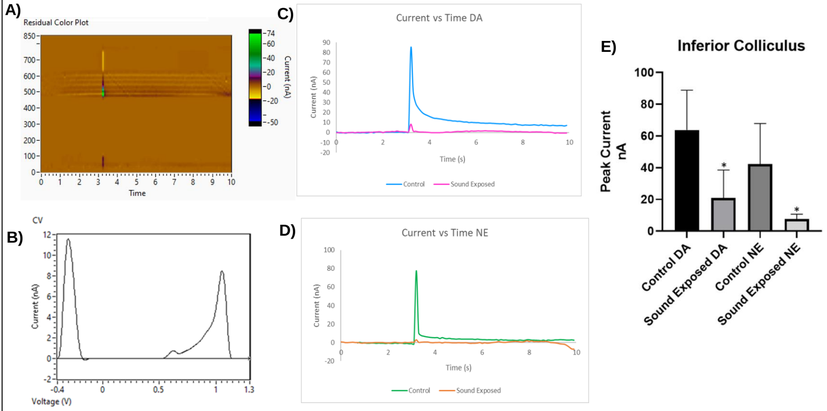


Figure 7. Effects of noise on dopamine neurotransmission in the inferior colliculus. (A) Color plot from sound-exposed subject indicating decline of second peak. (B) Cyclic voltammogram from sound-exposed subject, both peaks remain present (C) Overlay of dopamine neurotransmission of control vs. sound-exposed subjects. (D) Overlay of norepinephrine neurotransmission of control vs. sound-exposed subjects. (E) Quantitative comparison of the effects of sound exposure on dopamine and norepinephrine neurotransmission between experimental groups, both sound-exposed groups were significant at 95% confidence interval, p<0.05.

Conclusions

- Demonstrate the utility of electroanalytical tools in brain measurement.
- Both dopamine and norepinephrine linked with auditory processes.
- Decrease in dopamine and norepinephrine neurotransmission following noise-induced deafness implicates the monoamines as possible therapeutic targets.

Acknowledgement

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