ARTICLE
Using *In Vivo* Voltammetry to Demonstrate Drug Action: A Student Laboratory Experience in Neurochemistry

Brian P. Bergstrom
Biology Department, Neuroscience Program, Muskingum University, New Concord, OH 43762

Providing undergraduate neuroscience students an appropriate laboratory experience that demonstrates principles of neurotransmission and drug action is a difficult task. In the following activity, fast-scan cyclic voltammetry was utilized to measure dopamine levels using carbon-fiber microelectrodes in rats *in vivo*. Recordings were made before and after administration of several drugs to assess their ability to alter extracellular dopamine. The following drugs were chosen due to their well established actions: haloperidol, methylphenidate, and alpha-methyl-para-tyrosine. Each demonstrated markedly altered extracellular dopamine dynamics and some basic kinetic analysis enabled students to attribute the alterations to differing modes of action. Dopamine tissue content was also assessed to compare the differences between acute drug action and overall neurotransmitter content.

Any neuroscience laboratory course that desired to demonstrate principles involved in neurotransmission would be well served by the activities involved. This laboratory experience provided: 1) basic principles in experimental design, 2) small animal surgical experiences, 3) exposure to common instrumentation used in collecting neurochemical data, 4) data analysis procedures, and 5) experience in presenting their findings.

Key words: fast-scan cyclic voltammetry; carbon-fiber microelectrode; dopamine; dopamine transporter; haloperidol; alpha-methyl-para-tyrosine; methylphenidate

This laboratory exercise was developed for Neuroscience 386: Neuropharmacology, an upper-level laboratory course that junior and senior Neuroscience and Biology majors have completed at Muskingum University over the past six years. Due to the fact that Muskingum University is a small private liberal arts institution there are limited experiences to expose students to some advanced research techniques especially in neurochemistry. Therefore, as part of their laboratory component for the course they conduct an extensive project that lasts six weeks and is centered on the process of neurotransmission and how drugs can alter signaling in the brain.

Dopamine is arguably one of the most significant neurotransmitter systems in the brain. The dopamine system is involved with cognitive and motivational responses (Aarts et al., 2011) as well as motor functions associated with the basal ganglia (Gerfen and Wilson, 1996). The resulting broad range of clinical conditions such as Schizophrenia, Parkinson's disease, and drug addiction make the dopamine system one that students find highly engaging. Numerous drugs have been developed that target the dopamine synapse in an effort to treat this wide variety of conditions. Haloperidol, acts as a potent D_2 receptor antagonist thereby blocking autoreceptor action and augmenting dopamine release with a lesser effect on uptake inhibition (Sulzer et al., 2010). This results in elevated extracellular dopamine levels and is commonly used in the treatment of Schizophrenia. Methylphenidate is the preferred treatment for attention-deficit/hyperactivity disorder (Taylor et al., 2004). Methyphenidate's ability to increase extracellular dopamine levels is achieved through potent dopamine transporter inhibition (Volkow et al., 1995). Alpha-methyl-para-tyrosine serves as an inhibitor of the tyrosine hydroxylase and shuts down dopamine synthesis. The resulting effect on extracellular dopamine levels is a dramatic decline as dopamine stores are rapidly depleted (Bergstrom et al., 2011).

The following laboratory experience intended to expose students to a cutting edge research technique in the field of neurochemistry. Fast-scan cyclic voltammetry offers unparalleled spatial and temporal resolution for detecting electroactive species such as dopamine in the brain. Recordings were made for all experiments using carbon-fiber microelectrodes to make measurements in "real-time" in the brain. The carbon-fiber microelectrodes serve as an analytical tool capable of rapidly cycling the charge state of its surface such that current changes can be detected from oxidation-reduction reactions. In this regard, current responses collected can be converted to concentration and the analytes can be identified based upon the potential state at which current changes were observed. The voltammetric signals collected consisted of a triangular wave comprised of an "upward" slope that contains components from which dopamine release rates can be determined. This is followed by a "downward" slope that consists purely of uptake. Taken together, the simple shape of the signals was not intimidating to students yet allowed for teasing apart the actions that drugs have at the synaptic level in the dopamine system.

MATERIALS AND METHODS

Overall Design

Stereotaxic surgery was performed as described below in preparation for the voltammetry procedure and followed by micro-dissection of the brain for tissue analysis. Baseline recordings were collected in each animal and served as a

same animal control. Students were not made aware as to which drugs were being administered to each respective animal. In animals that received haloperidol (0.5 mg/kg i.p.), a stable baseline recording was taken (pre-injection recording) and 10 minutes elapsed before post-injection recordings were made. In animals that received methylphenidate (1.5 mg/kg i.p.) 10 minutes elapsed before post-injection recordings were made. In animals that received alpha-methyl-para-tyrosine (200mg/kg i.p.) a period of 60 minutes elapsed before post-injection recordings were made.

Animals

Adult male Sprague-Dawley rats (125 to 250 g) were purchased from Harlan (Indianapolis, IN). Food and water were provided *ad libitum*. Animals typically weighed between 300-400 g at the time of experimentation. All animal care was in accordance with NIH guidelines (publication 86-23) and approved by the Animal Care and Human Subjects Committee of Muskingum University.

Electrical Stimulation

Bipolar stimulating electrodes were purchased from Plastics One (MS 333/1B, Roanoke, VA). Stimulating electrode tips were separated by hand approximately 1.0 mm apart. Stimulus pulses were computer generated biphasic (±300 µA) and applied at 60 Hz for 2 s. Pulses were passed through a constant-current generator and optical isolator prior to application to stimulating electrodes (NL 80, Neurolog, Medical Systems, Great Neck, NY).

Voltammetry Procedure

Fast-scan cyclic voltammetry using carbon-fiber microelectrodes was performed according to a previously described method (Bergstrom and Garris, 2003). Urethane anesthesia (1.5 mg/kg i.p.) was administered before voltammetric surgery. Once the animal was deeply anaesthetized, a rotary tool was used to drill three holes through the skull, each of which corresponded to placement of the reference, stimulating and recording electrodes. Reference electrodes were positioned in superficial cortex on the left side, whereas recording and stimulating electrodes were positioned on the right side of the brain. The stimulating electrode was lowered over the medial forebrain bundle (-4.6 AP, 1.4 ML, -7.5 to -8.5 DV) to evoke dopamine release. A single carbon-fiber microelectrode was positioned in the caudate putamen (1.2 AP, 1.4 ML). The dorsoventral regions of the caudate putamen sampled varied slightly depending upon signal quality (-4.3 to -4.9). All stereotaxic coordinates were based on Paxinos and Watson (1986). Background subtracted cyclic voltammograms were calculated for all recordings collected and determined to be dopamine (Michael et al., 1998).

Data Analysis

Kinetic analysis used a mathematical model relating the rate of change of extracellular dopamine elicited by electrical stimulation to the opposing actions of dopamine release and uptake (Wightman et al., 1988):

$$d[DA]/dt = [DA]_p * f - V_{max} / (K_m/[DA]) +1)$$

where f is the frequency of the stimulation, $[DA]_p$ is a release term describing the concentration of dopamine evoked by each stimulus pulse, V_{max} is a Michaelis-Menten uptake term related to the dopamine transporter number and their turnover, and K_m is another Michaelis-Menten uptake term inversely related to the affinity of dopamine for its transporter. Curves were fit using non-linear regression with a simplex minimization algorithm (Wu et al., 2001) and were performed by locally written software. K_m was fixed to 0.2 µM, the value obtained previously in the intact striatum (Wu et al., 2001). Fixing K_m assumed a competitive mode of action on uptake inhibition for the drugs being studied and simplified the analysis for students by focusing on alterations of release and uptake alone.

Tissue Dopamine Content

Tissue dopamine content was determined in all animals after the voltammetric experiment (Bergstrom et al., 2001). The brain was removed, chilled in ice-cold saline (150 mM NaCl), and sliced fresh into 1 mm coronal sections using razor blades and a brain block (BS-AL-600C, Braintree Scientific Inc., Braintree, MA) chilled on ice. The appropriate slice was selected, and the striatum was dissected into the approximately 1 mm^3 tissue cube immediately surrounding each approximate recording site. Corresponding tissue was dissected from the contralateral striatum. Tissue samples were frozen at -80 C until assay, which typically was one week later. Dopamine content was determined by high-performance liquid chromatography with electrochemical detection (HPLC-EC) (ESA Coulochem III, Chelmsford, MA) using a millibore, reverse phase column (MD-150X3.2, ESA). The mobile phase is commercially available (MDTM, ESA). Tissue size was estimated by protein content using Bio-Rad Protein Assay (Hercules, CA: Catalog # 500-0006).

Statistical Analysis

Data are expressed as individual values and as the mean±SEM. Statistical analysis of averaged effects was performed by Microsoft Excel and used t-test and one-way ANOVA. The significance level was set at $p < 0.05$ for all comparisons.

Drugs and Chemicals

All chemicals and drugs were used as received and purchased from Sigma Chemical Company (St. Louis, MO). Aqueous solutions were prepared in Millipore™ deionized water (Millipore, Billerica, MA). A minimal amount of acetic acid was used in preparing the haloperidol solutions.

RESULTS

The sensitivity of the procedure produced consistent results that are predictable. Administration of haloperidol produced a pronounced increase in the voltammetric recording (Fig.1, Panel A). There was a 55% increase in the extracellular concentration of dopamine. In addition,

the "upward" release containing slope indicates a potential increase in release whereas the "downward" slope indicates slower uptake when compared to the pre-injection recording. Methylphenidate appeared to increase extracellular dopamine by approximately 30% with an obvious decline to uptake rate indicated by the broadening of the signal (Fig. 1, Panel B). The alpha-methyl-para-tyrosine resulted in a 95% decline in extracellular dopamine, indicative of potent synthesis inhibition (Fig. 1, Panel C). In contrast, the saline injected control shows minimal change in response (Fig. 1, Panel D).

Kinetic analysis provided important insights into the mode of action for each drug administered. Haloperidol significantly increased the amount of dopamine released (DA_p) by approximately 30%. A simultaneous and significant 26% decline in dopamine uptake rate (V_{max}) was also determined (Table 1). Methylphenidate showed no real change in dopamine release, however, a significant decline of 80% was observed for uptake rate (Table 1). Alpha-methyl-para-tyrosine produced over a 99% decline in dopamine release that also proved to be significant (Table 1). The saline injected controls showed minimal variation for both dopamine release and uptake (Table 1). Dopamine content showed a significant effect of treatment ($F_{(3,11)}$=7.6; p <0.01) and a clear decline evident in the group that received alpha-methyl-para-tyrosine (Fig. 2).

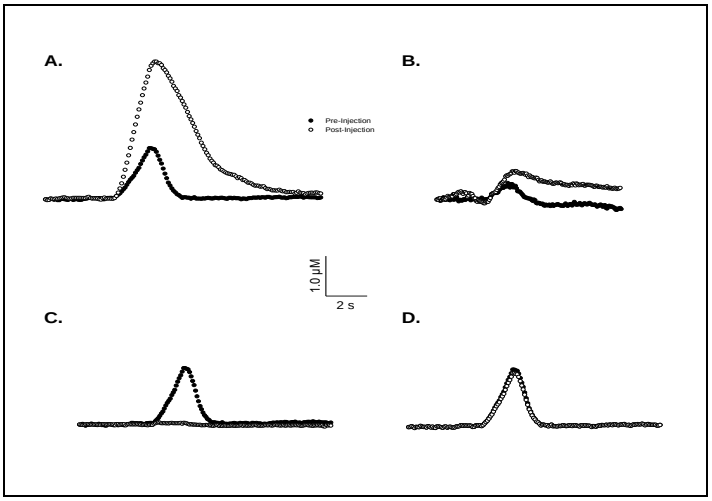

Figure 1. Representative Voltammetric Recordings. Each circle in panels *A-D* represents the concentration of dopamine at 100 ms intervals. Filled circles (●) represent recordings pre-injection and open circles (○) represent post-injection values. Panel *A*: Haloperidol; Panel *B*: Methylphenidate; Panel *C*: Alpha-methyl-para-tyrosine; Panel *D*: Saline.

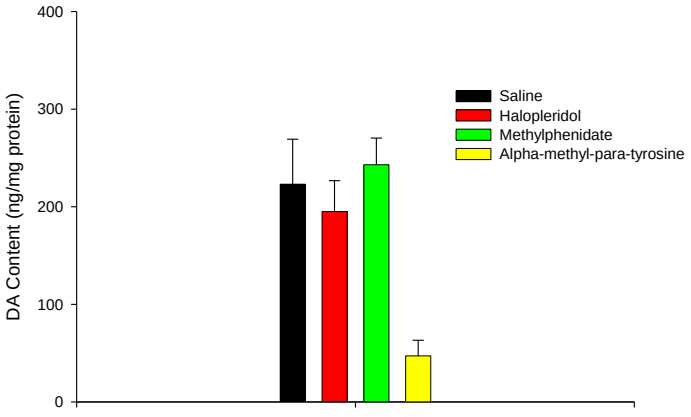

Figure 2. Dopamine Tissue Content. All groups had an n = 3 and represent the dopamine content from the approximate location of the recording site. Dopamine content values showed a significant effect of treatment ($F_{(3,11)}$=7.6; p <0.01).

DISCUSSION

This laboratory exercise required a significant amount of time and demanded close supervision. Students were informed early in the semester that animals will be used to demonstrate several principles throughout the laboratory portion of the course. I explained some of the benefits to animal research and also gave students the option of not attending specific surgical procedures. The exercise was split into several phases with overall instructor preparation time and student laboratory time listed in brackets: 1) preparation of carbon-fiber microelectrodes and background discussion, [Prep: 2 hrs, Lab: 3 hrs] 2) fast-scan cyclic voltammetry surgical procedure and data collection, [Prep: 2 hrs, Lab: 3-6 hrs] 3) brain micro-dissection and tissue analysis via HPLC-EC, [Prep: 1 hr, Lab: 3 hrs] 4) data analysis, [Prep: 1 hr, Lab: 3 hrs] and 5) preparation of a short scientific talk to discuss their findings. The total cost of consumables was approximately $300; however, total equipment costs are considerably more expensive. One of the critical aspects of the exercise was having the students blinded to which drugs the animals received. I have found this greatly increased student engagement because they must rely solely on their data to determine the drug administered. My own observation was that they seemed to be invested in the project to a greater extent using this design as compared to one in which they "know" what to expect.

The preparatory procedure for the fast-scan cyclic voltammetry provided an excellent experience in small animal surgery. I typically worked in small groups (four or less) and allowed each student to take a turn recording stereotaxic positions and moving micro-manipulators into appropriate locations. We also worked on understanding how to use the rat brain atlas. Upon completion of the fast-scan cyclic voltammetry data collection we performed the brain micro-dissection. At that point, I informed the students that if they would like to leave during the decapitation they were welcome to do so. Over the years, I have estimated less than 3% of students who have taken the course have left. Performing the tissue analysis via HPLC-EC was a very important comparison to make for the students. Indeed, as seen in Fig. 2 only the alpha-methyl-para-tyrosine showed a major decline in dopamine tissue content yet major alterations in extracellular dopamine were evident for all drugs administered using the voltammetry. This finding specifically demonstrated how vastly different conclusions can be drawn from data

Table 1. Kinetic Analysis of Voltammetric Traces

Parameter	Haloperidol		Methylphenidate		AMPT		Saline	
	Pre-Inj.	Post-Inj.	Pre-Inj.	Post-Inj.	Pre-Inj.	Post-Inj.	Pre-Inj.	Post-Inj.
DA_p (μM)	0.022 ±0.001	*0.031 ±0.011	0.016 ±0.002	0.014 ±0.002	0.025 ±0.004	*0.002 ±0.0002	0.032 ±0.001	0.033 ±0.002
V_{max} (μM/s)	4.6 ±0.3	*3.3 ±0.4	5.7 ±0.9	*1.1 ±0.02	6.2 ±1.2	5.1 ±0.5	6.4 ±0.2	6.1 ±0.4

Table 1. Kinetic Analysis of Voltammetric traces. K_m was fixed at 0.2 μM for all curve-fitting. All groups possessed an n = 3. An * represents a significant difference with $p < 0.05$.

collected at the tissue level versus the "real-time" voltammetry data collected as an acute drug action observed at the cellular level *in vivo*.

Using fast-scan cyclic voltammetry in my own research enabled me the opportunity to develop such an exercise for my students. Ultimately, feasibility of implementing this type of technique is dependent upon available equipment and instructor ability to understand principles in monitoring brain neurochemistry. Although the system I accumulated was before any were commercially available, there are several companies that offer voltammetry systems for purchase. The data acquisition and analysis software was locally written using LabView for the experiments described but alternatives to this do exist. The Demon Voltammetry and Analysis software suite is a recently developed option available to non-profit and academic institutions at no charge (Yorgason et al., 2011). This software provides a free source for voltammetry acquisition software as well as an assortment of analysis tools to evaluate voltammetric responses. The recent development of this software eliminates one of the most significant hurdles to this type of experimentation.

This laboratory experience provided a unique opportunity for students. Students that enrolled in this course planned to go to graduate school or were in a pre-professional program. The experiences they obtained were comparable to a summer internship and provided an invaluable experience for their future. Although I don't collect specific evaluative materials from students for the laboratories I teach, the average evaluation score I received for this course/laboratory is the highest out of all the courses that I have taught (4.73/5) and I have regularly heard students and alumni comment about how much they enjoyed the laboratory portion of the course.

REFERENCES

Aarts E, van Holtstein M, Cools R (2011) Striatal dopamine and the interface between motivation and cognition. Front Psychol 163: 1-11.

Bergstrom BP, Sandberg SG, Andersson M, Mithyantha J, Carroll FI, Garris PA (2011) Functional reorganization of the presynaptic dopaminergic terminal in parkinsonism. Neuroscience 193: 310-322.

Bergstrom BP, Garris PA (2003) "Passive stabilization" of striatal extracellular dopamine across the lesion spectrum encompassing the presymptomatic phase of Parkinson's disease: a voltammetric study in the 6-OHDA-lesioned rat. J Neurochem 87: 1224-1236.

Bergstrom BP, Schertz KE, Weirick T, Nafziger B, Takacs SA, Lopes KO, Massa KJ, Walker QD, Garris PA (2001) Partial, graded losses of dopamine terminals in the rat caudate-putamen: an animal model for the study of compensatory adaptation in preclinical parkinsonism. J Neurosci Methods 106:15–28.

Gerfen CR, Wilson CJ (1996) The basal ganglia. In: Handbook of chemical neuroanatomy, Vol. 12: Integrated systems of the CNS, part III (Swanson LW et al., eds), pp 371–468. Amsterdam: Elsevier.

Michael D, Travis ER, Wightman RM (1998) Color images for fast-scan CV measurements in biological systems. Anal Chem 70:586A–592A.

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. New York: Academic Press.

Sulzer D, Zhang H, Benoit-Marand M, Gonon F (2010) Regulation of extracellular dopamine: release and reuptake. In Handbook of basal ganglia structure and function: a decade of progress (Steiner H and Tseng KY, eds), pp 297–319. New York: Academic Press.

Taylor E, Dopfner M, Sergeant J, Asherson P, Banaschewski T, Buitelaar J, Coghill D, Danckaerts M, Rothenberger A, Sonuga-Barke E, Steinhausen HC, Zuddas A (2004) European clinical guidelines for hyperkinetic disorder—first upgrade. Eur Child Adolesc Psychiatry 13(Suppl 1):I7–I30. Maryland Heights, MO, USA: Elsevier Science.

Volkow ND, Ding YS, Fowler JS, Wang GJ, Logan J, Gatley JS, Dewey S, Ashby C, Liebermann J, Hitzemann R (1995) Is methylphenidate like cocaine? Studies on their pharmacokinetics and distributionin the human brain. Arch Gen Psychiatry 52:456–463.

Wightman RM, Amatore C, Engstrom RC, Hale PD, Kristensen EW, Kuhr WG, May LJ (1988) Real-time characterization of dopamine overflow and uptake in the rat striatum. Neuroscience 25:513–523.

Wu Q, Reith ME, Wightman RM, Kawagoe KT, Garris PA (2001) Determination of release and uptake parameters from electrically evoked dopamine dynamics measured by real-time voltammetry. J Neurosci Methods 112:119-133.

Yorgason JT, Espana R, Jones SR (2011) Demon voltammetry and analysis software: analysis of cocaine-induced alterations in dopamine signaling using multiple kinetic measures. J Neurosci Methods 202: 158-164.

Received December 12, 2011, revised January 12, 2012, accepted February 06, 2012.

Address correspondence to: Dr. Brian P Bergstrom, Biology Department, Neuroscience Program, Muskingum University, 163 Stormont St., New Concord, OH, 43762. Email: brianb@muskingum.edu

Acknowledgement: I would like to thank all the students that have taken NRSC 386 for their assistance in gathering and analyzing the data.