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INSTRUMENTATION FOR, AND APPLICATION OF, NOVEL ELECTROCHEMICAL TOOLS FOR *IN VIVO* ANALYSIS OF NEUROTRANSMITTERS IN THE CONTEXT OF PSYCHIATRIC DISEASE

by

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Submitted in Partial Fulfillment of the Requirements

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DEDICATION

I dedicate this to my guardian angel. Gone but never forgotten.

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ACKNOWLEDGEMENTS

To Dr. Parastoo Hashemi, for providing me with numerous eye-opening opportunities and always believing in me.

To the Hashemi lab members, for the everlasting friendships.

To my mom, for loving me for who I am and providing me with undivided support.

To my nieces Estrella and Lilly, for the motivation to continue.

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ABSTRACT

Serotonin's involvement in many physiological processes including anxiety, stress, compulsivity, and mood has been speculated for decades. Insufficient progress in our understanding of serotonin chemistry is due to the lack of effective tools to selectively measure this neurotransmitter on a neurotransmission-relevant time scale *in vivo*. An analytical technique for serotonin measurements, fast-scan cyclic voltammetry (FSCV), was pioneered in the last decade in anesthetized rodents and is beginning to shed light on the complexity of serotonergic activity on a sub-second timescale. Complementary, more recent technological innovations have enabled ambient neurotransmitter levels to be determined every few seconds using a method called fast-scan controlled-adsorption voltammetry (FSCAV). Chapter 1 of this thesis is an introduction to the work. In Chapter 2, the traditional experimental challenges that have thus far limited serotonin measurements are discussed and FSCV is highlighted as a technology that overcomes these difficulties. In Chapter 3, a simplified FSCAV module, which can be easily constructed by non-experts in electronics, is presented. This component is composed of two light-emitting diodes (LEDs). Together, this thesis showcases the design and application of improved electrochemical tools for *in vivo* analysis of neurotransmitters in the context of psychiatric disorders.

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LIST OF SYMBOLS

R1,2 Resistors

L1,2 Light-emitting diode (LED)

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LIST OF ABBREVIATIONS

5-HIAA .....5-hydroxyindoleacetic acid

5-HT .....Serotonin (5-hydroxytryptamine)

AA .....Ascorbic acid

BJT .....Bipolar junction transistor

CFM .....Carbon-fiber microelectrode

CNS ..... Central nervous system

CV .....Cyclic voltammogram

DA .....Dopamine (3-hydroxytyramine)

DAT ..... Dopamine transporter

DBS ..... Deep brain stimulation

DRN ..... Dorsal raphe nucleus

FSCAV ..... Fast-scan controlled-adsorption voltammetry

FSCV .....Fast-scan cyclic voltammetry

HFS.....High frequency stimulation

LED.....Light emitting diode

MFB .....Medial forebrain bundle

mPFC.....Medial prefrontal cortex

NAcc .....Nucleus accumbens core

NET..... Norepinephrine transporter

OCT .....Organic cation transporter

PM .....Premammillary nucleus

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SERT ..... Serotonin transporter

SNR .....Substantia nigra, pars reticulata

SSRI ..... Selective-serotonin reuptake inhibitor

TPH2.....Tryptophan hydroxylase 2

TTL .....Transistor-transistor logic

WINCS..... Wireless instantaneous neurotransmitter concentration system

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CHAPTER 1

INTRODUCTION

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1.1 The Serotonergic System

The neurotransmitter serotonin crucially modulates the activity of many neuronal pathways and is involved in a plethora of physiological processes controlling mood, emotion, and social behavior (Mann 1999; Edwards & Kravitz 1997). Previous studies suggest that deficits in serotonin neurotransmission are a factor in several psychiatric disorders including depression, anxiety, eating disorders, and obsessive-compulsive disorder (Muller & Jacobs, 2009; Lucki 1998). Consequently, selective-serotonin reuptake inhibitors (SSRIs) were developed to increase extracellular serotonin levels by inhibiting serotonin transporters and hindering the reuptake of serotonin back into the cell (Fuller, Perry, Molloy, 1974; Fuller & Wong, 1977). SSRIs are the most commonly prescribed pharmacological treatment for the above-mentioned disorders, however clinical success is inconsistent. The irregularity in therapeutic relief is yet to be understood due to the lack of suitable tools to selectively probe this elusive neurochemical on a neurotransmission-relevant timescale *in vivo.*

Sampling techniques, such as microdialysis, have provided the bulk of our current comprehension of serotonin dynamics (Miyazaki K.W., Miyazaki, K., & Doya, 2011; Fuller 1994), however microdialysis' temporal resolution and large probe size has prevented a deeper understanding of the real-time influence of serotonin on brain circuitry (Jaquins-Gerstl & Michael, 2015). In 2009, Hashemi *et al*. pioneered rapid (10 ms) measurements of *in vivo* stimulated serotonin release and reuptake using fast-scan cyclic voltammetry (FSCV) in the rat substantia nigra pars reticula (SNR). Using FSCV technology, our lab has just

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begun to shed light on *in vivo* serotonin dynamics and the role serotonin plays in psychiatric disorders.

1.2 The Scope of the Thesis

In this thesis, I first describe the traditional challenges of measuring serotonin *in vivo* and introduce FSCV as an electroanalytical technique that can overcome these difficulties. Next, fast-scan controlled-adsorption voltammetry (FSCAV) is underlined as a cutting-edge technology that allows for the rapid measurement of ambient neurotransmitter concentrations (Chapter 2). Finally, the development and characterization of a simplified component for FSCAV instrumentation is reported (Chapter 3). The outline of this thesis is described below:

Chapter 1: Introduction

Chapter 2: This chapter focuses on the historical difficulties of selectively targeting the serotonergic system for measurement. Next, FSCV is highlighted as an emerging method to allow for sub-second detection of phasic serotonin release and reuptake in particular brain regions of rodents. Furthermore, the most recent technological advancement for the measurement of ambient neurotransmitter concentrations using FSCAV are discussed.

Chapter 3: In this chapter, we develop and characterize a simplified component termed the LED circuit for FSCAV measurements. The LED circuit was designed to be able to be constructed by a novice in electronics, enabling ambient neurotransmitter measurements to be performed by a larger community. *In vitro* and *in vivo* analyses were performed and found to be in good agreement

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between the novel LED circuit and the original switch utilized in previous FSCAV reports.

Chapter 4: The final chapter summarizes the conclusions of this work and considers the future directions of this research.

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CHAPTER 2

REAL-TIME RESOLUTION MEASUREMENT OF SEROTONIN IN RODENTS[1]

[1] Robke, R., Parthasarathy, A., Willuhn, I., & Hashemi, P. To be submitted to the

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Investigations of the serotonin system started upon discovery of this messenger in the 1950s. These studies unearthed critical roles for serotonin both in the brain and in the body's periphery. However, much remains to be determined about the precise functions of serotonin, in particular serotonin neurotransmission in the brain. Serotonin is thought to be involved in the neurochemical coding of numerous physiological processes underlying aggression, anxiety, appetite, impulsivity and compulsivity, mood, social behavior, and stress (Muller & Jacobs, 2009). Serotonin's involvement in these disorders and others has been assumed for decades; the cause of the ongoing speculation and insufficient progress in our understanding, however, is primarily the lack of effective tools to selectively probe this elusive neurotransmitter on a neurotransmission-relevant time scale *in vivo*. Sampling techniques, such as microdialysis, have enriched our current comprehension of serotonin dynamics significantly (Miyazaki K.W. et al., 2011; Fuller 1994). However, in general, microdialysis sampling methods lack the temporal resolution to effectively clarify serotonin function *in vivo* (Nandi & Lunte, 2009). Serotonin-relevant behaviors depend on environmental cues and specific actions, therefore high temporal resolution is necessary to unravel the precision function of serotonin in behavior (Anastasio et al., 2013; Burmeister, Lungren, Kirschner, & Neisewander, 2004). Additionally, there are ongoing discussions about the integrity of the chemical data obtained given the high magnitude of brain tissue damage caused by large sampling probes (Borland, Shi, Yang, & Michael, 2005; Jaquins-Gerstl & Michael, 2015). In contrast, a more niche analytical method, fast-scan cyclic voltammetry (FSCV), allows scientists to

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identify and quantify rapid neurotransmission (sometimes referred to as “phasic”) on a sub-second time scale using microelectrodes that cause minimal tissue damage (Phillips, Robinson, Stuber, Carelli, & Wightman, 2003; Clark et al., 2010).

FSCV has been extensively used to study dopamine neurotransmission on an advanced behavioral platform, primarily elucidating dopamine’s roles in learning and motivation (e.g., Hamid et al., 2016; Day, Roitman, Wightman, & Carelli, 2007; Willuhn, Burgeno, Groblewski, Phillips, 2014). More recently, serotonin FSCV was pioneered in anesthetized rodents and is just beginning to shed light on the complexity and impact of serotonergic activity (Hashemi, Dankoski, Petrovic, Keithley, & Wightman, 2009). Complementary, an even more recent cutting-edge technological evolution enables the measurement of ambient (sometimes referred to as “tonic”) extracellular neurotransmitter concentration with FSCV technology using a method called fast-scan controlled-adsorption voltammetry (FSCAV) (Atcherley, Laude, Parent & Heien, 2013). Importantly, FSCV and FSCAV can be combined at a single sensor to measure both “phasic” and “tonic” serotonin concentrations *in vivo*.

In this chapter, the traditional experimental challenges of measuring phasic serotonin will be outlined and FSCV will be highlighted as a method that can overcome these difficulties. The *status quo* of applying FSCV for understanding serotonin function *in vivo* will be highlighted and the existing gaps in our knowledge will be identified. Suggestions for filling these gaps will be

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offered to better shape the fundamental understanding of serotonin's roles in the brain.

2.1 Rapid Measurement of Serotonin *In Vivo*

It is substantially more difficult to make *in vivo* measurements of serotonin, compared to dopamine, a related amine neuromodulator because many regulatory systems work simultaneously to maintain serotonin homeostasis. The result of this strict regulation is a relatively low extracellular concentration of this signaling molecule in comparison to dopamine. The reasons for this strict homeostasis is multi-tiered, but at the most basic tier, it is likely due to the immediately toxic effects of high extracellular serotonin levels that can lead to a fatal disorder termed serotonin syndrome. The profound level of regulation required to maintain serotonin transmission has thus rendered measurements challenging and, therefore, this modulator's involvement in neurological and psychiatric disorders remained elusive.

In the following section, we will discuss the challenges of measuring serotonin dynamics *in vivo* as a function of (1) the contribution of the concentrated anatomical clustering of serotonin cell bodies in the base of the mammalian brain, (2) the dynamics of the extracellular presence of the transmitter, (3) tight regulation at a number of levels, and (4) the detrimental effects of serotonin's metabolites on implanted microelectrodes (not problematic for microdialysis). We speculate that, in the past, these circumstantial factors have hampered the pace of serotonin research as compared to research targeting dopamine neurotransmission. Finally, we highlight exciting innovative

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advancements that have the potential to significantly increase the pace of serotonin research.

2.1a Neuroanatomy of the Serotonin System

The serotonin network in the central nervous system of mammals originates in the raphe nuclei, a cluster of brain nuclei with cell groups distributed along the midline of the midbrain and brainstem (Hornung, 2003). The rostral group of serotonin neurons in the raphe nuclei project widely to forebrain structures like the amygdala, the hippocampus and all regions of the neocortex and striatum. Specifically in the cortex, the fibres innervate the neocortex via the lateral hypothalamus and the median forebrain bundle (MFB). From the MFB, the fibres project to the frontal cortex and then traverse posteriorly to innervate parietal, temporal, and occipital cortices. This widespread innervation of the brain provides an indication for serotonin's global function modulating a variety of fundamentally important processes including sleep and arousal processes, executive function (mediated by the prefrontal cortex), behavioral control of mood and emotion, social behavior, impulsivity, compulsivity, and learning and memory (Muller & Jacobs, 2009). Given this broad presence, in addition to the analytical challenges described above, it has been difficult to identify more precise roles for serotonin.

The dorsal raphe nucleus (DRN) is the principal area of cell bodies containing serotonin that widely innervates the brain. The DRN is embedded in a central location on the mid-line, directly under the lambda fissure of the rodent skull. The DRN is located underneath a dense network of core vasculature and

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the cerebral aqueduct (Paxinos & Watson, 2007). This elusive DRN location means that targeting serotonin's main hub is experimentally extremely challenging due to risk of substantial damage of the surrounding vasculature and diffusion of infused drugs or viruses into the neighboring aqueduct. It is important to directly access serotonergic neurons because the detection of phasic serotonin is still dependent on stimulated release. As of now, there are no known behaviors that reliably evoke serotonin release. Approaches intended to overcome this complication have been to target the DRN at an angle instead of vertically from the top of the skull (**Figure 2.1A**) (Kirby, Rice, & Valentina, 2000; Correia et al., 2017), although this strategy introduces many errors, and thus considerably lowering success rate. Another approach is to target the MFB for the manipulation of serotonin release, which greatly increases success rate (Hashemi, Dankoski, Wood, Ambrose, & Wightman, 2011), however, at the cost of markedly diminished selectivity of target structures by electrical stimulation.

2.1b Heterogeneity and Co-Localization with Other Neurotransmitters in the DRN

The DRN is neuro-chemically not a homogeneous structure and is innervated by neurons containing glutamate, GABA, and dopamine (**Figure 2.1B**) (Charara & Parent, 1998; Qi et al., 2014; Calizo et al., 2011; Challis et al., 2013; Celada et al., 2001; Weissbourd et al., 2014). In the DRN and throughout other areas of the brain, histamine-containing vesicles are often found to be co-localized with serotonin, and is thought to modulate serotonin transmission via inhibitory H_3 hetero-receptors on serotonin-containing neuron terminals (Chase & Murphy,

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1973; Russell, Henry, Phebus, & Clemens, 1990; Brown, Stevens, & Haas, 2001; Haas, Sergeeva, & Selbach, 2008; Threlfell et al. 2004). Due to this high co-localization of numerous neurotransmitters in the DRN, it has been challenging to selectively target serotonergic neurons and to clarify their role in behavior thus far.

In order to target serotonin neurons selectively on a genetic platform, and thereby more selectively stimulate serotonin release for phasic detection, it was attempted to identify and capitalize on molecular markers to circumvent regional neuronal heterogeneity. However, previously identified molecular markers that were considered to be selective for serotonin neurons, ETS domain factor Pet-1 and the serotonin transporter (SERT), have later been shown to be present in glutamate and other neuronal systems (Liu et al., 2014; Hainer et al., 2015). This lack of specificity has restricted the development of reliable transgenic rodent cre lines for optogenetic and chemogenetic targeting of serotonin neurons and represent yet another reason why our understanding of serotonin's influence on neuronal circuits has fallen behind compared to other neurotransmitter systems in which novel genetic-based techniques are reliably employed (Hersman et al., 2017; Tye et al., 2013). Currently, tryptophan hydroxylase 2 (TPH2), the enzyme which catalyzes the first rate-limiting step of serotonin synthesis in the central nervous system, has the potential to become the first valid platform for selective genetic modification of the serotonin system due to its exclusive presence in serotonergic producing cells (Zill et al., 2007; Sakowski et al., 2006; Walther & Bader, 2003).

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2.1c The Complexity of Serotonin Neurotransmission

The coordinated effort by many, simultaneous systems to tightly regulate extracellular serotonin levels presents challenges for detection (*vide supra*). The likely consequence of this are endogenous extracellular serotonin concentrations that are much lower than dopamine levels (**Figure 2.1C**) (Shen et al., 2004). To date, 15 serotonergic receptors have been identified that precisely and rapidly control serotonin (Nautiyal & Hen, 2017; Jennings, 2013). In addition, multiple mechanisms influence serotonin reuptake including promiscuous reuptake of serotonin by other monoamine transporters (*e.g*. dopamine transporters (DATs), norepinephrine transporters (NETs) and organic cation transporters (OCTs)), other than SERTs (Baganz et al., 2008; Daws, Toney, Gerhardt, & Frazer, 1998). Further highlighting the sheer intracy of this system, significant control of serotonin reuptake is implemented by serotonin autoreceptors that are G-protein coupled to SERTs. (Montañez, Munn, Owens, Horton, & Daws, 2014; Murphy et al., 2003). To even further obstruct measurements, serotonin metabolites of similar chemical structure can be nearly 500 times more concentrated than serotonin itself (Hashemi et al., 2009), indicating a rapid rate of metabolism. Not only does this fast metabolism reduce the time during which serotonin is detectable, some metabolites have been shown to dramatically degrade the integrity of microelectrode detection probes (Hashemi et al., 2009). Together, these circumstances complicate both the measurement and understanding of

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serotonin *in vivo* and ultimately how serotonergic activity corresponds to behavior.

2.1d Measurement of Serotonin *In Vivo*

Active neurochemical signal delivery relies on sub-second chemical exchange. Thus, to define serotonin neurotransmission, high temporal-resolution measurement is critical. Traditional microdialysis has formed our current apprehension of overall serotonergic activity (for example: Yang, Thompson, McIntosh, Altieri, & Andrews, 2013; Volle et al, 2018). Microdialysis has low detection limits when coupled to efficient analytical techniques for separation, detection, and quantification of analytes (e.g. ultrahigh-performance liquid chromatograph and electrochemical detection) (Liu et al., 2010). However, disadvantages such as temporal resolution ($\geq$ 1 minute) and damage of tissue surrounding the large probe (approximately 200 um) have created a large body of contradictory results (Chefer, Thompson, Zapata, & Shippenberg, 2009; Jaquins-Gerstl & Michael, 2015). Consequently, measurement tools for this analyte that operate at high time resolution with minimal tissue damage are highly desired. Notably, an electrochemical technique called chronoamperometry has been employed previously by Daws and colleagues to explore SERT mechanisms under varying experimental conditions (e.g. genetically-modified animal models and pharmacological manipulations) primarily in the hippocampus (Montanez et al., 2014; France et al., 2009; Daws et al., 2005). These experiments have greatly improved our understanding of the SERTs and their rate of serotonin clearance. However, this technique typically requires exogenous

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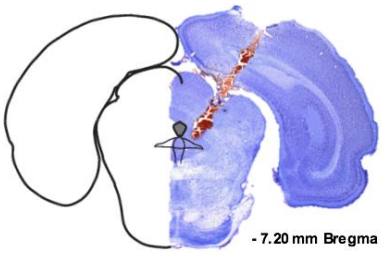
application of serotonin (Daws, Toney, Davis, Gerhardt, & Frazer, 1997) and the external source of serotonin has been thought to cause rapid physiological changes in SERT expression (Daws & Toney, 2007).

FSCV has become one of the most powerful analytical techniques to study neurotransmitter release and reuptake *in vivo* due to its small probe size (6 um) and high selectivity, sensitivity (nM), and temporal resolution (10 Hz). Since the development of FSCV by Ralph Adams in the 1980's, numerous seminal discoveries have been made on dopamine's role in learning and motivation using this technique (e.g. Phillips et al., 2003; Flagel et al., 2011; Cheer et al., 2007). More recently, serotonin FSCV measurements have been established in anesthetized rodents and are just beginning to unearth the dynamics and the sheer complexity of serotonin's *in vivo* actions.

A.



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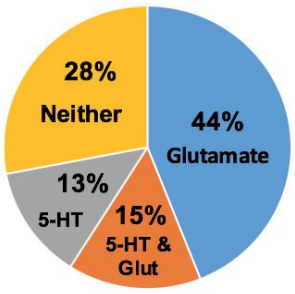

C.

The contents of neurotransmitters in the rat brain

Chemical	Adult whole brain (ng/mg)	mPFC (ng/mg)*	Hypothalamus (ng/mg)*	Striatum (ng/mg)*
Dopamine	0.760 ± 0.023	0.120	0.420	12.000
GABA	445.148 ± 110.767	-	-	-
Glutamate	1408.920 ± 141.825	-	-	-
Serotonin	0.485 ± 0.094	0.510	1.200	0.500
Norepinepherine	0.290 ± 0.034	0.380	1.800	0.100

Figure 2.1. Challenges of measuring serotonin. (A) Histology from the lab showing the experimental targeting of the dorsal raphe nucleus at a 30-degree angle. **(B)** The dorsal raphe nucleus is non-homogenous, shown by the heterogeneous innervation of the ventral tegmental area (VTA). Data adapted from Qi et al, 2014. **(C)** Neurotransmitter content in the whole rat brain (adapted from Zhu et al., 2011) and specific regions. *data obtained from the lab; unpublished results from Botterblom & Feenstra.

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In the next section, general electrochemistry principles governing the dynamics of sub-second FSCV neurotransmitter sampling are described. The contributions of this technique to furthering our understanding of *in vivo* serotonin fluctuations are highlighted and novel frontiers in other electrochemical methodologies are explored.

2.2 FSCV: General Principles

FSCV is an analytical tool that takes advantage of thermodynamic and electrochemical phenomena to govern subsecond measurements. Small, positively charged ions such as dopamine and serotonin are attracted to a negatively charged carbon-fiber microelectrode (CFM) surface during a short holding period (typically 100 ms). Then an analyte specific waveform, which is tailored to induce electron transfer via oxidation and reduction of the ion, is applied to the CFM. The oxidation and reduction occur at discrete potentials and are measured as current vs. potential to obtain a cyclic voltammogram (CV). The CV acts as an electrochemical "stamp" and is used to qualify and quantify the analyte (Phillips & Wightman, 2003).

Besides its advantages in time resolution and small probe size, selectivity can be a concern in any analytical measurement. In FSCV, two primary intrinsic chemical properties can be exploited to provide selectivity; analyte electron transfer kinetics and the charge on the ion. Depending on the ion of interest a combination of approaches can be taken that take into account charge and electron transfer kinetics of the analyte of interest vs. those of interferences. This

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is illustrated below by using dopamine and serotonin detection, respectively, as examples.

2.2a Selectivity of Dopamine Detection

For FSCV dopamine measurements, it is well known that ascorbic acid (AA) is the primary interferent. However, the high ambient levels of ascorbic acid in the brain (McIlwain, Thomas, & Bell, 1956; Schenk, Miller, Gaddis, & Adams, 1982) should not interfere with the signal because FSCV is background subtracted. Nonetheless, AA may reduce the sensitivity of the electrode to dopamine if adsorption sites on the CFM are blocked by AA. More significantly perhaps is that AA is released upon glutamate reuptake (Cammack, Ghasemzadeh, & Adams, 1991) since FSCV stimulations will release both dopamine and glutamate. Thus, AA is likely to be transiently released during FSCV file collection. As discussed above, selectivity can be garnered by utilizing differences in electron transfer kinetics and ionic charge. In solution, ascorbic acid is negatively charged and dopamine is positivity charged, and the electron transfer kinetics of AA oxidation are significantly slower vs. dopamine oxidation (Wightman, May, & Michael, 1988). Therefore, if between scans the electrode is held at a negative potential and a fast enough scan rate is applied, then AA is repelled during scans and does not have an opportunity to transfer electrons to the CFM during the scan. This effect can be potentiated if the CFM surface is 'activated' prior to analysis. Activation refers to oxidation of the surface by heat, electrochemistry or chemical treatments (Heien, Phillips, Stuber, Seipel, & Wightman, 2003; Strand & Venton, 2008). This activation procedure deposits a

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high density of negatively charged oxygen moieties on the electrode surface, thus increases the extent of AA repulsion and actually facilitates dopamine preconcentration. Surface oxidation has a further advantage when performed electrochemically during each scan (i.e. scanning up to 1.3 V). At 1.3V, carbon itself is oxidized and a monolayer of carbon is lost from the CFM surface, essentially providing a fresh electrode for the next measurement (preventing irreversible fouling) (Takmakov et al., 2010; Mitchell, Dunaway, McCarty, & Sombers, 2017).

2.2b Selectivity of Serotonin Detection

The biggest challenge for selective serotonin measurements is interference by dopamine given the mutual presence of both these neuromodulators in many parts of the brain and their similar chemical properties. The transfer kinetics of serotonin electro-oxidation are more rapid vs. dopamine, consequently sensitivity to dopamine can be reduced by increasing scan rate to 1000 V s^{-1} (**Figure 2.2A**). An additional phenomenon that helps here is that the serotonin adsorption equilibrium onto the CFM is more favorable than dopamine's adsorption. This effect means that the resting potential between scans can be kept at a positive potential which, in addition to scanning faster, significantly diminishes the FSCV response to dopamine. The main metabolite of serotonin, 5-Hydroxyindoleacetic acid (5-HIAA) is not thought to be a significant interference since there is no evidence that it is transiently released, however 5-HIAA is problematic for electrode fouling (Hashemi et al., 2009). Electrode fouling is the accumulation of undesired material on the carbon-fiber surface that is detrimental to the

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electrode's function, therefore adjustments in the FSCV detection scheme is necessary to exclude the influence of this metabolite on the CFM. These are discussed in the following section.

2.3 Serotonin FSCV

The first report of serotonin FSCV *in vivo* was an experiment in which dopamine was depleted from dopamine neuron terminals in the striatum via inhibition of L-aromatic acid decarboxylase. These neurons were then loaded with serotonin by a means of tryptophan loading, promoting electrically evoked serotonin release (Stamford, Kruk, & Millar, 1990). This experiment, although elegant, was not indicative of the endogenous function of the serotonin system and the detection scheme utilized did not allow for discrimination between serotonin and dopamine. Additionally, serotonin electro-oxidation produces several intermediary monomers that rapidly polymerize on the electrode to create a 'film' covering the electrode. It is known that such an electrode 'fouling' significantly lowers electrode sensitivity. Later, the development of the 'Jackson Waveform' (Jackson, Dietz, & Wightman, 1995) significantly improved detection selectivity and electrode fouling:

1) to distinguish between dopamine and serotonin 1000 V s^{-1} is utilized and the electrode is held at a positive resting potential
2) to reduce the fouling effects of serotonin electro-polymerization on the CFM, the CFM is held at 0.2 V between scans, which abolishes the production of the the major electro-oxidation product. 1000 V s^{-1} also appears to reduce the production of these side products.

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The Jackson waveform was subsequently utilized in tissue slice preparations to study serotonin dynamics in different brain regions and under SSRI treatment (**Figure 2.2A**) (Bunin & Wightman, 1998; Bunin, Prioleau, Mailman, & Wightman, 1998). The 'Jackson Waveform' was a breakthrough for real-time serotonin measurements, however, the waveform alone did not lead to successful measurements of evoked serotonin *in vivo*. In 2009, the reason for the lack of success for *in vivo* measurements was discovered by Hashemi et al., 2009. One major difference between measurements in slice preparations and *in vivo* is the large ambient concentration of 5-HIAA *in vivo*. As previously mentioned, it is not likely that 5-HIAA would act as an interference, however, it is structurally very similar to serotonin and exerts similar fouling effects on the CFM. Given that it is 100-1000 times more concentrated in the extracellular space *in vivo* (in slice preparations it washes out), it rapidly and profoundly fouls the CFM as it is positioned in the brain *in vivo.* (Hashemi et al., 2009). In solution, 5-HIAA is negatively charged while serotonin is positively charged. Thus, a new protocol was developed to selectively repel 5-HIAA by depositing Nafion onto the carbon fiber surface at an advantageous and controlled thickness (Brazell et al., 1987; Rice & Nicholson, 1989). An unwanted side effect of Nafion application is that applied films that are too thick (1-3 um) lead to a significant decrease in electrode response times, whereas a layer that is too thin will not perform efficiently to repel 5-HIAA and fouling will occur (Nagy et al., 1985). The ideal thickness is about 300 – 350 um, as modeled and described in detail in Hashemi et al., 2009. After coating the CFM with Nafion, the microelectrode is nearly 200

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times more sensitive to serotonin than 5-HIAA because of the pre-concentration of the positively charged serotonin ions trapped in the Nafion membrane, permitting *in vivo* measurements to be performed (Gerhardt, Oke, Nagy, Moghaddam & Adams, 1984; Hashemi et al., 2009). A scanning electron microscope (SEM) image of a Nafion-coated CFM is shown in **Figure 2.2B**. Nafion is not useful with waveforms that apply potentials > 1.0 V (i.e., dopamine waveform) due to the oxidation of the monolayer of the CFM during each scan as previously described which destabilize the Nafion layer. Potential disadvantages of the Nafion technique is the unequal coverage of the CFM and greater electrode variability, however strict adherence to the described electrodepositing procedure has can limit variability.

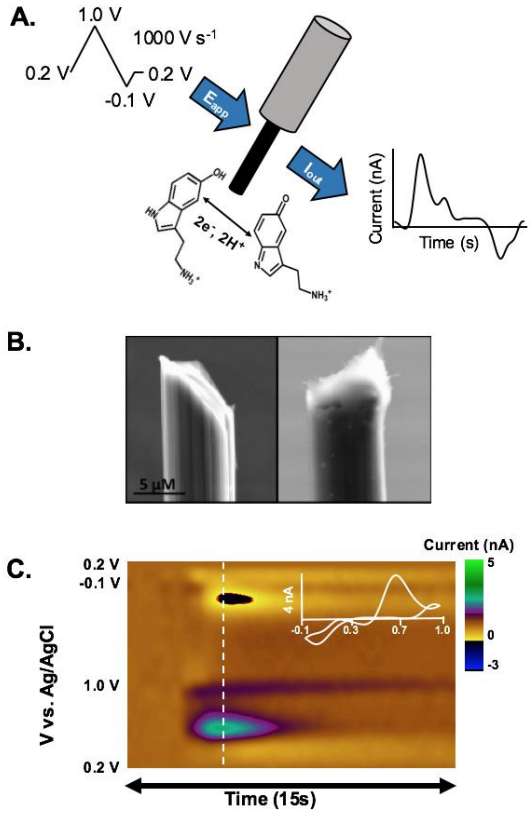

Figure 2.2. Detection of serotonin using fast-scan cyclic voltammetry. (A) A serotonin-selective waveform is applied to the carbon-fiber microelectrode to promote the oxidation and reduction of serotonin, which is measured as current and is proportional to the serotonin molecules near the electrode. **(B)** Nafion-coated carbon-fiber microelectrodes are required to repel interfering metabolites (5-HIAA) and enhance serotonin sensitivity. Adapted with permission from Hashemi et al., 2009. Copyright 2009 American Chemical Society. **(C)** Representative serotonin color plot and identifying cyclic voltammogram.

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2.3a General Findings of Serotonin FSCV

Although serotonin neurons project widely across the mammalian brain, it is not known whether serotonin signaling is uniform throughout the brain or whether regional specificity exists. It is generally postulated that deficits in serotonin neurotransmission can contribute to a number of psychiatric disorders including depression, anxiety, and obsessive-compulsive disorder (OCD). As such, selective-serotonin reuptake inhibitors (SSRIs) were developed in the late 70s (Fuller et al., 1974; Fuller, & Wong, 1977), which aim to increase extracellular serotonin levels by inhibiting SERTs. Clinical success of SSRIs in the above-mentioned disorders (for which SSRI are one of the primary pharmacological treatments) is inconsistent. This irregularity in therapeutic relief may be due to regional differences in serotonin release and reuptake, whereby SSRI-induced global increases in extracellular serotonin availability and signaling may not be useful for therapeutic purposes. Alternatively, SSRI may have different regional effects on serotonin release and reuptake that is otherwise not distinguishable. Therefore, irregularity in therapeutic relief may be better understood by studying *in vivo* regional serotonin dynamics with FSCV. Finally, even in the case of regional uniformity, FSCV measurements may be necessary to uncover SSRI effects on the sub-second level. Although serotonin FSCV is a young field, this section highlights the contributions this method is making to broadening our knowledge of serotonin dynamics in different brain regions.

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Substantia Nigra, Pars Reticulata (SNR)

To date, most FSCV measurements of serotonin have been performed in the SNR where serotonin terminals are the most concentrated of all brain regions (Palkovits, Brownstein, & Saavedra, 1974; Reubi & Emson, 1978). In 2009, the first paper reporting *in vivo* serotonin measurements in the SNR via DRN stimulation was published (Hashemi et al., 2009). As mentioned above, physically targeting the DRN is technically difficult and results in low success rates. In a follow up study, serotonin release in the SNR was observed by stimulating the medial forebrain bundle (MFB), an anatomically easier surgical target located anterior to the terminal release site. Due to the posterior position of the CFM with respect to the stimulating electrode, this suggested serotonin fibers could be retrogradely activated (Hashemi et al., 2011).

The stimulation of MFB yields three distinct serotonin response profiles – fast, slow and hybrid based on their clearance slopes (Wood et al., 2014). As the name suggests, fast and slow response profiles show fast and slow serotonin clearance times (approximately 8s vs. 30s, respectively). The hybrid response profile is a mixture of the two and displays a fast decay to clear serotonin for a couple seconds then switches to a slow decay. Mathematical modelling of these responses revealed that two reuptake mechanisms – one high affinity, low efficiency system, termed Uptake 1 and mediated by SERTs and one low affinity, high efficiency system, facilitated by monoamine transporters other than SERTs, including DATs, NETs and OCTs. It is likely that at low extracellular concentrations, Uptake 1 by SERTs (high affinity, low efficiency) is efficient,

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however at higher concentrations, serotonin may diffuse to other transporters (DATs, NETs, and OCTs) and be reuptaken by Uptake 2 mechanisms (low affinity, high efficiency). A common feature of all stimulated serotonin release is that the serotonin concentration 'dips' below the baseline after concentration levels return to baseline. Thus, an autoreceptor effect (7 seconds after the beginning of the stimulation) was incorporated into the model to account for this dip below baseline. Methiothepin, a non-selective serotonin receptor antagonist with a high affinity for serotonin autoreceptors, (Monachon, Burkhard, Jalfre, & Haefely, 1972) eliminates this dip below baseline, indicating that serotonin autoreceptors may act to reduce serotonin transmission within the time frame of the FSCV collection window (30s) (Barnes & Sharp, 1999; Wood et al., 2014). These findings support prior hypotheses on Uptake 1 and Uptake 2 in synaptosomes (Shaskan & Snyder, 1970; Daws, Koek, & Mitchell, 2013). Multiple reuptake mechanisms and the prolonged autoreceptor control of serotonin support the notion that serotonin a highly regulated system.

SSRIs acutely act on the SERTs to inhibit the reuptake of serotonin, however, therapeutic relief in psychiatric patients may not be achieved until several weeks of chronic administration (Gelenberg & Chesen, 2000). After an acute dose of the SSRI Escitalopram (Lexapro; 10 mg kg^{-1} i.p.), FSCV measurements of serotonin showed immediate dynamic neurochemical changes through the rapidly augmented release of serotonin and gradual increase in clearance time ($t_{1/2}$) over 2 hours (Wood & Hashemi, 2013). The $t_{1/2}$ was positively correlated to an increasing dose of Escitalopram (1, 10, 100 mg kg^{-1})

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while the amplitude was impacted the most by the middle dose (10 mg kg^{-1}), similar to previous observations of DAT inhibition (Song et al., 2012). Alternatively, tissue slice preparations report only a modest increase of serotonin release (~10%), indicating that synaptic processes found *in vivo* additionally modulate SSRI mechanisms (Bunin & Wightman, 1998). Due to the increase in clearance time up to 120 minutes after an acute SSRI administration *in vivo*, it is highly likely that SSRIs cause dynamic physiological changes outside of their accepted mode of action for longer-term therapeutic benefits.

Another important study compared the regulation of serotonin in the SNR to dopamine in the nucleus accumbens. Overall, it was found that serotonin transmission is most sensitive to uptake and metabolic mechanisms, while dopamine is dependent on synthesis and repackaging (Hashemi et al., 2012). The response profile of serotonin was shown to be much broader than dopamine, with measurable release occurring at a MFB stimulation depth of 7.0-10.0 mm below dura, compared to 8.0-9.0 mm for dopamine, supporting the known, wide innervation of the brain by serotonin.

Hypothalamus

The hypothalamus is an important structure, amongst others that comprise the limbic system and contains relatively high levels of serotonin (see **Figure 2.1C**; unpublished data from Feenstra & Botterblom). The premammillary nucleus (PM) is an interesting hypothalamic structure in that it contains high levels of both serotonin and histamine. Histamine had been thought to modulate serotonin transmission due to the presence of inhibitory H_3 heteroreceptors on serotonin

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terminals (Threlfell et al., 2004; Fink, Schlicker, Neise, Gothert, 1990). The effect of histamine on serotonin in the PM was studied in recent work via development of an FSCV method that simultaneously measures serotonin and histamine (Samaranayake et al., 2016). An anterior region of the MFB, containing fewer serotonin axons, (Nieuwenhuys, Geeraedts, & Veening, 1982) was stimulated. This stimulation evoked histamine, but not serotonin in the PM such that modulation of ambient, not evoked, serotonin could be investigated. A potent and rapid inhibition of serotonin was observed in response to histamine release. (Samaranayake et al., 2016). A dose response for thioperamide (2, 20, and 50 mg kg^{-1}), a histamine receptor (H_3) antagonist, was carried out and the corresponding histaminergic and serotonergic responses were mathematically modeled. Overall, it was shown that the potent inhibition of serotonin by histamine in the PM is indeed mediated by H_3 heteroreceptors on serotonergic neurons. This finding supports previous speculations that H_3 receptors on serotonin terminals (Schlicker, Betz, & Gothert, 1988; Esbenshade et al., 2008) inhibit serotonin transmission (Threlfell et al., 2004). Due to the hypothalamus' crucial role of regulating stress hormones, it is important to understand the neurochemical influence this region has on other neuromodulators, such as serotonin, to better understand how to better maintain stress levels and promote healthy individuals.

Cortex

The medial prefrontal cortex (mPFC) has a relatively high serotonin content and has been shown to have broad influence on cognitive control and modulating

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social behavior (see **Figure 2.1C**; unpublished data from Botterblom & Feenstra) (Miller & Cohen, 2001). In 2018, two serotonergic release events corresponding to a single stimulation of the MFB was observed for the first time using FSCV in mice (West et al., 2018). The two responses were related to distinct reuptake profiles encompassing both Uptake 1 and 2 mechanisms, as previously described in the SNR. Through pharmacology, histology, and mathematics, it was found that different subsets of serotonin axons transverse the MFB and terminate in separate domains with distinct reuptake responses in separate cortical layers. This study supports the idea of intricacy of serotonin neurotransmission and presents evidence of a highly elaborate biochemical organization of serotonin in the mPFC.

Serotonin FSCV measurements have also been made in layer 1 of the somatosensory cortex in mice, which is important for processing all sensory information (Jain, Qi, Collins, Kaas, 2008). This study revealed that serotonin neurons have a unique capability to undergo regrowth and gain of function following damage produced by a systemic amphetamine lesion or local stab lesion (Jin et al., 2016). Serotonin release was evoked by MFB stimulation and measured 1 week, 3 months, and 6 months after amphetamine treatment. No evoked serotonin release was recorded 1 week and 3 months post-lesion, however, by 6 months, a recovery in serotonin activity was apparent in comparison to controls (11.9 $\pm$ 2.9 nM vs. 14.5 $\pm$ 8.3 nM, respectively). Behavioral tests displayed similar results, with the recovery of their behavioral measures occurring only 6 months after an amphetamine lesion. Two-photon

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microscopy images showed new serotonergic axon growth after 3 months, however, it was speculated that they may need further maturation to function. Overall, this study showed that a large percentage (>80%) of damaged serotonin axons are susceptible to new growth and recovery of functionality. Two possible explanations for this phenomena were given:

1) since serotonergic axons rely on slow, diffuse volume transmission rather than fast-localized synaptic transmission (Hornung, 2003; DeFilipe & Jones, 1988), their regrowth is more relaxed and allows for an approximate former position to be adequate
2) serotonin axonal growth cones fail to receive stop signals from the tissue matrix.

Understanding the biochemical organization and corresponding serotonergic function of the cortex will lead to invaluable information on cortical plasticity and how to assemble neural connections that promote beneficial decisions and social behavior.

Dorsal Raphe Nucleus (DRN)

High-frequency electrical deep brain stimulation (DBS) is becoming a more common treatment for clinicians to alleviate depression and compulsive symptoms in psychiatric patients (Holtzheimer & Mayberg, 2011; Srejic et al. 2014), however, the mechanisms underlying the effects of this approach is not well understood. A recent study by Srejic *et al.* showed that high frequency stimulation (HFS) of the mPFC resulted in a similar amplitude of serotonin release as a typical short train simulation, however, a more pronounced decay of

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the extracellular serotonin concentration was evident, suggesting an enhancement of reuptake (Srejic, Wood, Zeqja, Hashemi, & Hutchinson, 2016). The enhancement of serotonin reuptake induced by HFS is speculated to be facilitated by an upregulation or modification of the affinity of SERTs (Zhu et al., 2010; Baganz & Blakely, 2012). Additionally, this could be explained by activity-dependent endocytosis, in which rapid endocytosis can be activated by intense stimulation (or exocytosis) when neurotransmitter recycling needs to be accelerated to restore vesicle storage (Klingauf, Kavalali, & Tsien, 1998; Sara, Mozhayeva, Liu, & Kavalali, 2002). These two potential explanations may serve as homeostatic synaptic mechanisms to maintain extracellular serotonin at control levels after HFS, which is in agreement with the previously observed tight regulation of serotonin *in vivo* (Hashemi et al., 2012). Due to presence of serotonergic cell bodies in the DRN, this region is of interest for the treatment of many psychiatric disorders by a means of globally impacting serotonergic circuitry throughout the brain.

It is important to note that these findings would not be possible with a slower sampling technique such as microdialysis because of the required high-temporal and spatial resolution that FSCV provides. Studies are ongoing to understand the biochemical arrangement of serotonin, how it is influenced by other neuromodulators, and why SSRIs are variable and delayed in their efficacy, in a bid to improve these therapies.

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2.3b FSCV Disadvantages

Although FSCV serotonin measurements provide invaluable information about serotonin release and reuptake under different experimental conditions and diseases, there are still substantial limitations for FSCV in investigations of serotonin. First, all of the aforementioned experiments have been conducted in anesthetized rodents. This has prevented a detailed understanding of serotonin activity linked to behaviorally relevant environmental stimuli and corresponding behavior. Second, FSCV is reliant on background subtraction thus electrical, chemical or behavioral stimuli are required to provoke release (Howell, Kuhr, Ensman, & Wightman, 1986). The fast-scan rates employed in FSCV lead to high "background" currents, thereby requiring the removal of the charging current to reveal small, faradaic currents produced by monoamines of low concentration and limiting FSCV measurements to changes in concentration. Third, electrical stimulation is non-selective and affects numerous neurotransmitters and neuronal targets. Furthermore, to evoke serotonin release, a powerful electrical stimulation must be employed (Hashemi et al., 2011; Robinson, Venton, Heien, & Wightman, 2003), which raises questions of physiological relevance and potential tissue damage upon repeated stimulation. Fourth, since FSCV relies on eliciting a change in concentration, ambient measurements could not traditionally be made to understand neurotransmitter basal activity. It is especially important to understand ambient, or tonic serotonin levels on a rapid timescale because serotonin axons are thought to primarily rely on slow, diffuse volume

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transmission rather than fast, localized synaptic transmission (Hornung, 2003; DeFilipe & Jones, 1988).

2.3c Novel Frontiers in Tool Development for the Investigation of Serotonin

Novel technological advancements have been made to promote the understanding of serotonin *in vivo.* Developing optogenetic control of serotonergic neurons has become a major point of interest to combat the problem of non-selective electrical stimulation (McElligott, 2015). The first study that combined serotonergic FSCV measurements to optogenetic stimulation *in vivo* was performed in drosophila and showed that low-frequency stimulations of serotonin induce a steady-state response in comparison to high-frequency stimulation-induced peak responses (Xiao, Priyman, & Venton, 2014). Behavioral studies have also implemented optogenetic control of serotonin, primarily in mice. However, neurochemical readouts by FSCV have not been reported at this point. Some of these studies have indicated serotonin may be a key regulator of patience/waiting, due to the activation of serotonergic neurons corresponding to mice waiting longer for future rewards (Miyazaki K., Miyazaki K.W., & Doya, 2012; Miyazaki K.W., Miyazak K., & Doya, 2012, 2014; Fronseca, Murakami, & Mainen, 2015). With the intention of translation to the clinics, a proof-of-principle study using a system called Wireless Instantaneous Neurotransmitter Concentration System (WINCS) was performed in DRN rat brain slices to measure serotonin with FSCV (Griessenauer et al., 2010). WINCS is a small, sterilizable, wireless data acquisition component designed in compliance with FDA standards for medical electrical device safety. Most recently, steps have

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been taken to mathematically decipher *in vivo* signals, thought to contain a serotonergic component, obtained in Parkinson's patients who received DBS electrodes and performed an investment task (Moran et al., 2018).

Although all of these studies are advancing the methods in which serotonin can be measured and manipulated, there is still fundamentally a lack in our knowledge of the magnitude of ambient extracellular serotonin levels. A new technique, termed fast-scan controlled-adsorption voltammetry, has been recently developed to help fill this gap on a rapid time scale.

2.4 Fast-Scan Controlled-Adsorption Voltammetry (FSCAV)

FSCAV is a cutting-edge voltammetric technique that enables quantitative ambient *in vivo* neurotransmitter measurements (Atcherley, Wood, Parent, Hashemi & Heien, 2015). FSCAV reports basal neurotransmitter concentration over a 10 second period and can be performed in less than 20 seconds (Atcherley et al., 2013; Atcherley et al., 2015; Burrell, Atcherley, Heien, & Lipski, 2015). An advantage of coupling FSCAV to existing FSCV systems is the production of a current versus voltage plot (CV), which, as described above is an essential process to identify analytes of interest (thereby also implicitly testing functionality of the electrode and its placement). Consequently, FSCAV experiments have thus far been initiated with FSCV to optimize the location of the CFM in a brain locale rich in the analyte of interest.

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FSCAV measurements are performed in a three-step procedure. **Step 1**: an analyte-specific waveform is applied to the CFM at a high frequency (100 Hz) and scan rate (1200 V/s) to minimize adsorption to the CFM for 2 seconds, allowing for a response that is not governed by neurotransmitters (i.e. providing a background). **Step 2**: a constant potential (the analyte-specific holding potential) is then applied for 10 seconds to allow the neurotransmitter to pre-concentrate around the electrode and adsorb to the CFM, reaching a coverage density in equilibrium with the surroundings. **Step 3**: the same waveform used in step 1 is reapplied to the CFM and the adsorbed neurotransmitter is measured with FSCV in respect to the first two seconds of the data collection file (background). These steps can be visualized in **Figure 2.3A.** The signal obtained from FSCAV has been shown to be dependent on both mass transfer and adsorption dynamics, which determine the time required to reach equilibrium in step 2 (Atcherley et al., 2013).

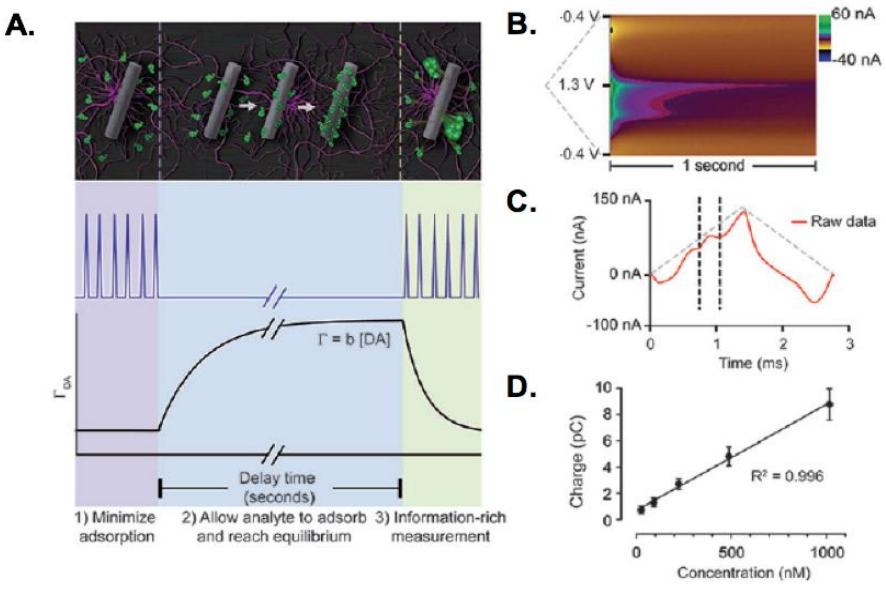

Figure 2.3. Overview of fast-scan controlled-adsorption voltammetry. (A) A visual representation of the three step process: 1) minimized adsorption; 2) equilibrium; 3) adsorption electrochemical measurement. The presence of the neurotransmitter near the carbon-fiber microelectrode, the potential application,

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and surface-accumulated dopamine (rDA) are displayed top to bottom. **(B)** Color plot obtained from the measurement in step 3. **(C)** Representative current trace (red) 10 ms after the start of step 3 and representative integration limits used for data analysis. **(D)** A necessary post-implantation calibration plot. Reproduced from Atcherley et al., 2015 with permission from The Royal Society of Chemistry.

In order to perform FSCAV, an additional piece of equipment, a digital switch, must be fabricated in-house and incorporated into existing FSCV systems. This switch employs an operational amplifier (op amp) coupled to a transistor-transistor logic (TTL), which enables the system to precisely and rapidly alter between applying a FSCV waveform and a constant potential from an external power source to the working electrode (Atcherley et al., 2013). FSCAV can easily be employed through FSCV software (WCCV or Demon Voltammetry, for example) using the stimulation settings. After file collection, data analysis is executed by integrating the first resulting CV of the analyte of interest during step 3. A representative color plot for the ambient measurement of dopamine is shown in **Figure 2.3B**. It is important to note that the first-background subtracted CV becomes visible on the 2nd or 3rd scan after the waveform is re-applied following the constant potential of 10s, due to stray capacitance from the switching component when altering between the constant potential back to the waveform. To remove this large interfering background charge, convolution theory can be utilized (Oldham, 1986; Atcherley et al., 2013, 2014). Due to electrode variability, integration limits need to be set intuitively *in vivo* due to the complex matrix of the brain and additional analytes that are present around the electrode (Cahill et al., 1996; Heien, Johnson, & Wightman, 2004; Takmakov et al., 2010b). Most commonly, using current vs. time traces, the integration limits are set by placing both initial and final limits at the local

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minima and encompassing the entire oxidation peak of the analyte (as seen in **Figure 2.3C**). The resulting area will be calculated as charge (pC) and plotted vs. serotonin concentration. Post-implantation calibration plots are then created and utilized to report *in vivo* concentrations (**Figure 2.3D**). Post-calibration of each electrode is necessary to reliably measure and quantify absolute neurotransmitter concentrations, due to the electrode variability (*vide supra*) and ongoing structural changes to the CFM (due to activating potentials > 1.0 V for instance) that is encountered in traditional FSCV. A disadvantage to this is that lesions cannot be made to histologically identify the CFM placement since the electrode needs to remain intact for post calibrations.

FSCAV was first established for dopamine measurements *in vivo* in 2015 and demonstrated it could become a powerful tool used in parallel with FSCV to disclose both the phasic and tonic changes in neurotransmitters at a single sensor (Atcherley et al., 2015). In 2016, FSCAV was used to record unbound Cu^{2+} in various complex matrices, supporting the ability of FSCAV to and reliably perform selective measurements of analytes of interest in numerous fields (Pathirathna, Siriwardhane, McElmurry, Morgan, & Hashemi, 2016). More recently, ambient measurements of serotonin have been conducted to better understand absolute extracellular concentrations of serotonin (Abdalla et al., 2017; see below).

2.5 Measurement of the Ambient Extracellular Concentration of Serotonin

In 2017 ambient measurements of serotonin were established using FSCAV (Abdalla et al., 2017). The waveform employed is the above-mentioned Jackson

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waveform (0.2V to 1.0V to -0.1V to 0.2V; scan rate of 1000 V/s) with a supplementary controlled-adsorption period (10s) of a 0.2 V constant potential. The third scan after the controlled-adsorption period was extracted for data analysis and the integration limits are set approximately from 0.4V to 0.85V, encompassing the oxidation peak of serotonin.

This study reported mouse CA2 hippocampus basal concentration as 64.9 ± 2.3 nM (**Figure 2.4**). In comparison, previous microdialysis studies have suggested serotonin levels are in a lower nanomolar range (Mathews et al., 2004; Zhang et al., 2013; Lee et al., 2016). However, FSCAV measurements are performed on an entirely different spatial scale due to the fundamentally different dimensions of the two probes. Additionally, in FSCAV, the electrode's position is optimized with FSCV and thus located in a "hot spot", or where there is high serotonin activity, which may account for the higher reported serotonin levels. After determining basal serotonin levels, the study verified the selectivity of the signal *in vivo* via pharmacology and mathematics. Results showed no interference by 5-HIAA, dopamine, or norepinephrine at physiological conditions.

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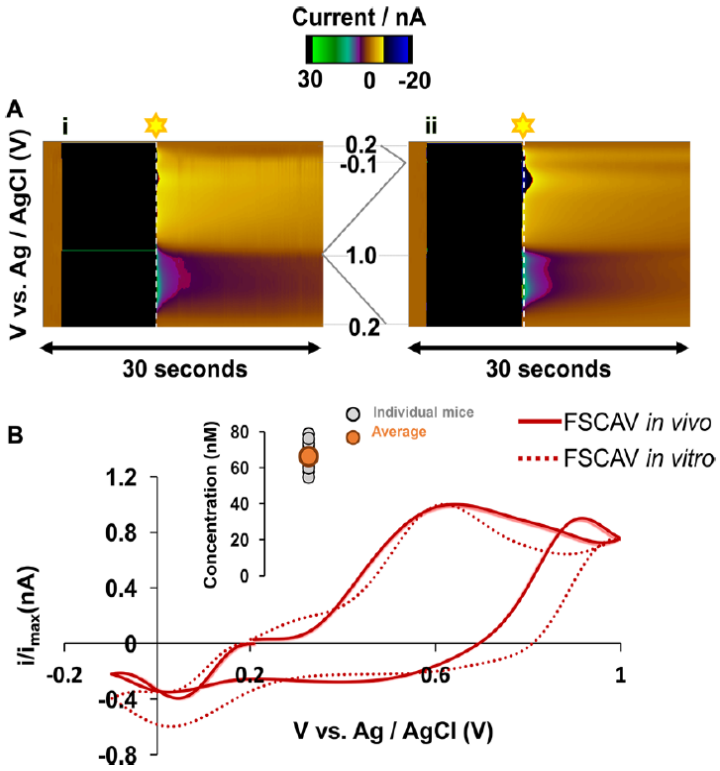

Figure 2.4. Ambient measurement of serotonin using fast-scan controlled-adsorption voltammetry. (A) Representative color plots of serotonin *in vivo* (i) and *in vitro* (ii). **(B)**. Cyclic voltammograms extracted from the 3rd scan during step 3 in (A), shown by dashed, white lines. The inset displays individual (grey) and averaged (orange) ambient serotonin recordings from the mouse CA2 region of the hippocampus. Reprinted with permission from Abdalla et al., 2017. Copyright 2017 American Chemical Society.

The development of FSCAV for serotonin enables the first robust, and high-temporal resolution measurements of serotonin's basal concentrations. Coupling FSCAV to FSCV recordings provides a novel platform to acquire a deeper chemical understanding of serotonin activity at a single sensor.

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CHAPTER 3

A SIMPLIFIED LED-DRIVEN SWITCH FOR FAST-SCAN CONTROLLED-ADSORPTION VOLTAMMETRY INSTRUMENTATION[2]

[2] Robke, R., Hashemi, P., and Ramsson, E. Under Review in *HardwareX,* 05/11/2018.

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Abstract:

Fast-scan cyclic voltammetry (FSCV) is an analytical tool used to probe neurochemical processes in real-time. A major drawback for specialized applications of FSCV is that instrumentation must be constructed, or modified, in-house by those with expertise in electronics. One such specialized application is the newly developed fast-scan controlled-adsorption voltammetry (FSCAV), that measures ambient *in vivo* dopamine and serotonin. FSCAV requires additional equipment, an operational amplifier coupled to a transistor-transistor logic, which was originally termed 'the switch', allowing the system to alter between applying a FSCV waveform and a constant potential from an external power source to the working electrode. To facilitate the integration of FSCAV into existing FSCV instruments by making this method more accessible to the community, in this paper we design a simplified switching component. Specifically, we employ two light emitting diodes (LEDs) to generate the voltage needed to drive a NPN bipolar junction transistor, substantially streamlining the circuitry and fabrication of the switching component. *In vitro* and *in vivo* analyses are performed and compared between the new LED circuit vs. the original switch, showing good agreement. Thus, we present a new, simplified scheme to perform FSCAV that is cheap, simple, and easy to construct by a non-expert.

Keywords: Dopamine, fast-scan cyclic voltammetry, ambient, circuit, instrumentation, component

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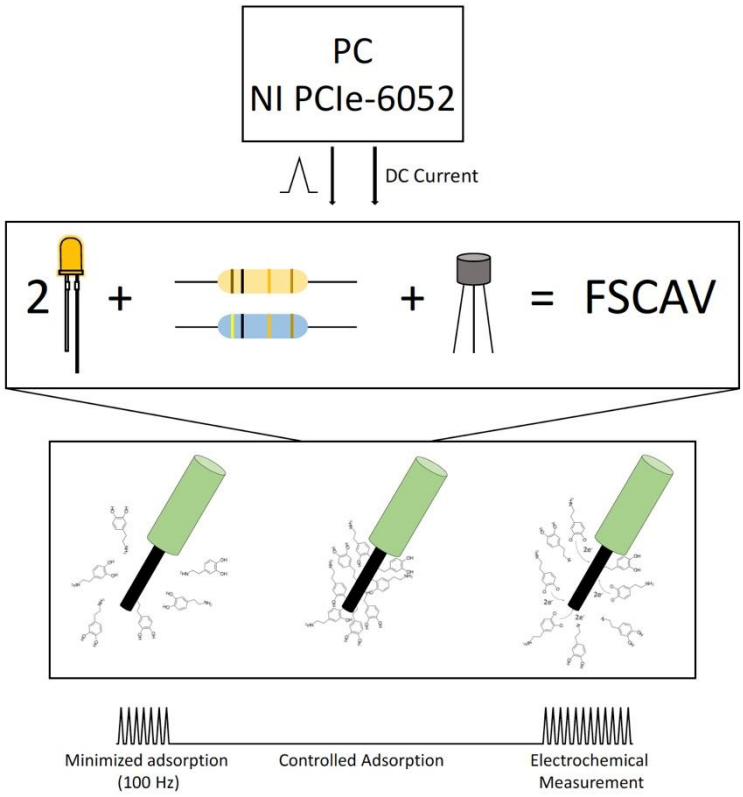

Figure 3.1 LED circuit graphical abstract.

3.1 Hardware in Context

Fast-scan controlled-adsorption voltammetry (FSCAV) is a newly developed tool that is coupled to existing fast-scan cyclic voltammetry (FSCV) systems to measure ambient neurotransmitter concentrations. FSCAV necessitates a custom made component, termed the switch, utilizing an operational amplifier coupled to a transistor-transistor logic, which may be challenging to those without prior experience in electronics. Here, a simplified component, a LED circuit, employs two light emitting diodes (LEDs) to generate the voltage needed to drive a NPN bipolar junction transistor in a straightforward manner. Hardware specifications are shown in **Table 3.1**.

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3.2 Hardware Description

The LED circuit achieves optical isolation of the signal by placing two infrared (890nm; 4V forward voltage) LEDs facing one another. Stimulation outputs are set at 4V. For digital or TTL pulses, a voltage divider can drop the 5V to the necessary 4V, as shown in **Figure 3.2A** (R1 = 10 kOhm; R2 = 40 kOhm, for instance). The light from L1 will illuminate L2 and cause ~0.9V to be generated at L2. This is enough to power the base of the NPN BJT transistor and allow the -0.4V to pass. The output of the circuit is connected to the recording electrode, and when the -0.4V is allowed to pass, it clamps the recording electrode at -0.4V.

The original switch used for FSCAV was constructed in-house and connected to existing FSCV systems as an extension of the 'breakout box' with four connections to an external power supply. The switch utilizes an op amp coupled to a TTL, enabling the system to apply the constant potential from the external power source (-0.4 V delay). This allows the user to transition between FSCV and FSCAV, from stimulation to a constant potential, respectively (Atcherley et al., 2013).

Those without prior electronics training my find it challenging to construct the original switch. The new LED circuit described here requires just two infrared LEDs and one NPN transistor, enabling this alternative to be cost-effective and simple to construct with a basic understanding of soldering principles. The LED circuit requires the addition of the external -0.4V power supply through one connection, enabling a direct voltage clamp. Additionally, if older FSCV systems are utilized, this -0.4V can come from 6052 or 6052e National Instruments cards.

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The ability to integrate the LED circuit into older FSCV systems is an additional advantage of this circuit. The bill of materials can be seen in **Table 3.2**.

General points:

- The LED circuit is a simplified, cheaper version of the original switch utilized in FSCAV
- The LED circuit can be easily coupled to older FSCV existing systems
- Using the LED circuit, ambient neurotransmitter concentrations can be measured with high temporal resolution

3.3 Build Instructions

- Wire the components as shown in the schematic in **Figure 3.2A**.
- LED1 (L1) will receive 4V to the anode from a TTL or National Instruments card 6052 (6052E) via a female BNC connector. If receiving 4V from a TTL, a voltage divider is used to drop the voltage. R1 = 10 kOhm; R2 = 40 kOhm. The cathode is connected to ground.
- The anode of L2 is connected to the base of the NPN transistor. The emitter is connected to both the cathode of L2 and an alligator clip or terminal. In **Figure 3.2B**, screws are used as simple terminals, so the -0.4 V input and Working Electrode output are wired to screws. The collector of the NPN transistor will receive the -0.4 V input.
- The LEDs are shrink-wrapped facing one another and further insulated from light by wrapping in electrical tape. This is important as stray light can introduce noise.

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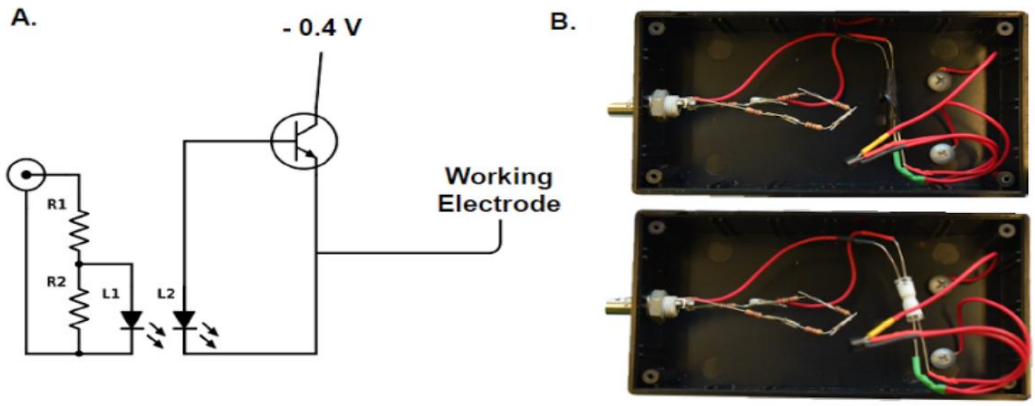

Figure 3.2. Novel LED circuit setup. (A) Simple circuitry scheme of LED circuit. **(B)** Photographic representation of connections. LED lights are masked in electrical tape (top) and revealed (bottom).

3.4 Operation Instructions

The novel LED circuit scheme is shown in **Figure 3.2A**, with an external supply providing the -0.4V to drive the two LEDs to power a NPN BJT transistor, enabling the -0.4V to be applied to the carbon-fiber microelectrode (CFM) through an alligator clip. Alternating from the applied waveform to a constant potential is under the control of a TTL via a BNC cable. Alternatively, for older FSCV existing systems, the -0.4V can come from 6052 or 6052e National Instruments cards. The -0.4V power supply, when coming from the NI boards, originates from the second analog output (ao1). The first analog output (ao0) is utilized for the triangle wave output. A DC task for the -0.4V output can be created in National Instruments Automation Explorer. We do not foresee any potential safety hazards while setting up or utilizing our described circuit.

Through the use of FSCV software (WCCV or Demon Voltammetry, for example), FSCAV can be employed using the stimulation settings. The pre-event

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time is set at 2s, the event time at 10s, and the entire file length at 30s. During the 10s event time, the LED circuit will act as a voltage clamp that will hold the working electrode at a constant potential of -0.4V (or the resting potential of the analyte of interest), allowing the analyte to adsorb onto the carbon fiber electrode and reach equilibrium with its surroundings (Atcherley et al., 2015; Abdalla et al., 2017). After file collection, analysis can be done by looking at the first-background subtracted dopamine cyclic voltammogram (CV) with in-house LabVIEW 2012 software. It is important to note that the first-background subtracted dopamine CV becomes visible on the 2nd or 3rd scan after the waveform is applied after the constant potential of 10s. Due to electrode variability, integration limits are set intuitively (Abdalla et al., 2017; Burrell et al., 2015; Pathirathna et al., 2016).

3.5 Validation and Characterization

In Vitro FSCAV

To test agreement between FSCAV signals obtained with the switch and the LED circuit, a calibration was performed *in vitro* (n=6 electrodes). Standard solutions of dopamine concentrations were prepared by dissolving dopamine hydrochloride (Sigma-Aldrich, CO., MO, USA) in Tris-buffer (15 mM H_2NC-$(CH_2)(OH)_3$·HCl, 140 mM NaCl, 3.25 mM KCl, 1.2 mM $CaCl_2$, 1.25 mM NaH_2PO_4·H_2O, 1.2 mM $MgCl_2$, and 2.0 mM Na_2SO_4 at pH = 7.4 in deionized water). A calibration was performed using 50, 100, 200, and 500 nM dopamine concentrations using the dopamine FSCAV wavefrom (-0.4 V to 1.3 V at 1200 V s^{-1}). These concentrations were

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previously established to be within the linear calibration range for dopamine FSCAV (Atcherley et al. 2015). Either the switch or the LED circuit was first utilized to complete a full calibration. The electrode was then cycled in Tris Buffer for 10 minutes before the same calibration was performed using the other component. The initiating component was altered between separate electrodes to reduce biases.

In **Figure 3.3A**, representative FSCAV color plots obtained with the LED circuit and switch are shown. The color plots contain the voltage (vs. Ag/AgCl) on the y-axis, time (30s) on the x-axis, and current (nA) is shown in false color. The three steps of FSCAV can be seen. **Step 1**: the waveform is applied to the carbon-fiber microelectrode at high frequency (100 Hz) to minimize adsorption for 2 seconds (yellow beginning). **Step 2**: a constant potential being applied for 10 seconds to allow the neurotransmitter to adsorb, reaching a coverage density in equilibrium with the surroundings (black area). **Step 3**: the waveform is reapplied and the adsorbed neurotransmitter is measured in respect to the first step (blue event area, noted by the starred, dashed white line).

To compare measurements, the 3rd scan was utilized in each system when a clear, Faradaic dopamine peak was visible. **Figure 3.3B** shows the respective current trace of the 3rd scan for the LED circuit (orange) and switch (blue) with current on the y-axis and time in milliseconds on the x-axis. The dashed lines note integration limits, or where the area under the curve was calculated, for the quantification of dopamine for each system. **Figure 3.3C** is the resulting calibration plot of the LED circuit and switch with charge (pC) on the y-

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axis and concentration (nM) on the x-axis. Both systems show good agreement in their measurements with no statistical difference from 50 to 500 nM of dopamine (n=6; $p > 0.05$ for all concentrations), consistent with expected *in vivo* concentrations (Atcherley et al. 2013).

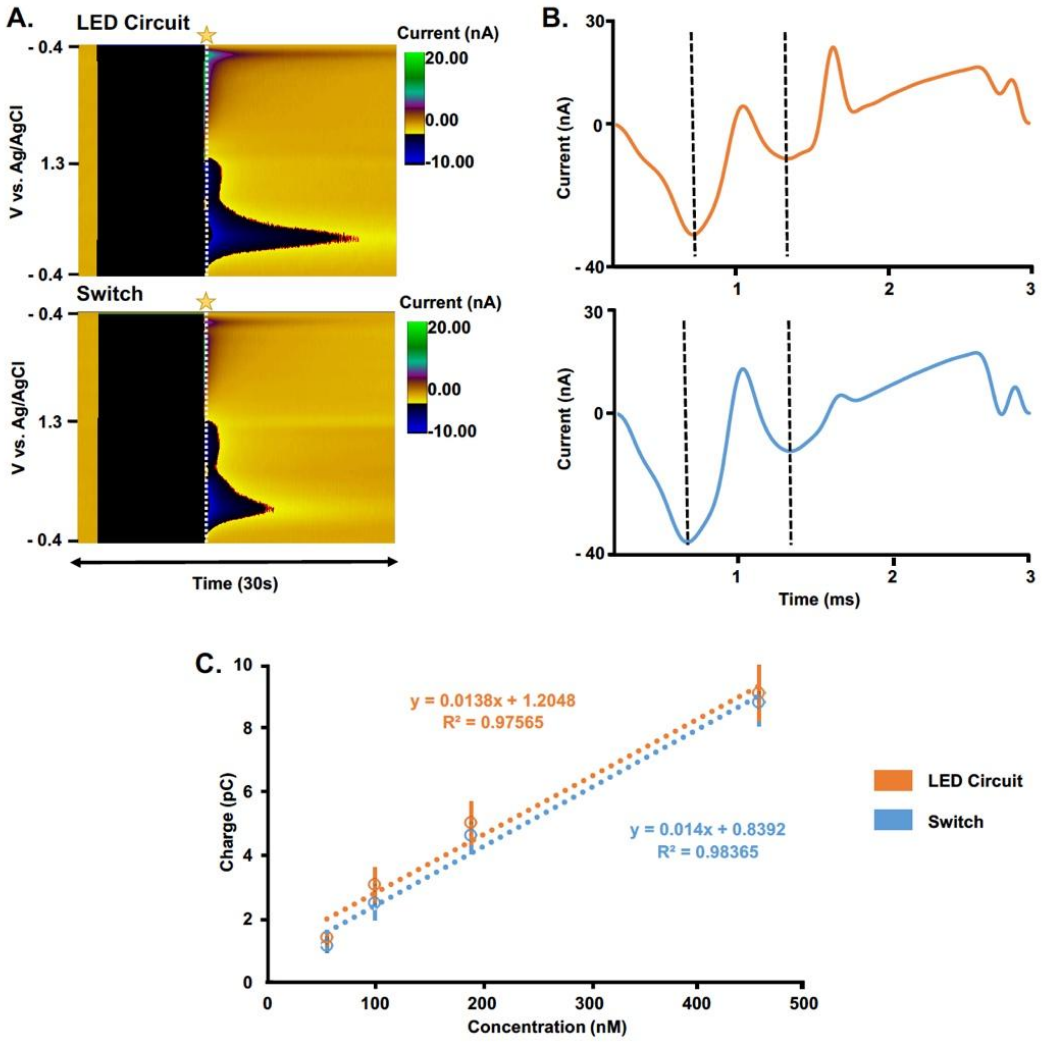

Figure 3.3. LED Circuit vs. Switch *in vitro* measurement comparison. (A) Representative color plots of the LED circuit (top) and switch (bottom) *in vitro.* **(B)** Extracted cyclic voltammograms for the LED circuit (orange) and switch (blue) from dashed, starred line in A. **(C)** Calibration plot comparison of the LED circuit and switch, showing good agreement in their measurements (n=6) ± SEM.

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In Vivo FSCAV

After validating that the switch and the LED circuit enable statistically identical FSCAV measurements *in vitro*, we moved to comparisons of *in vivo* responses. *In vivo* dopamine measurements were performed in the nucleus accumbens core (NAcc), a brain locality rich in dopamine terminals (Nirenberg et al., 1997; Meredith et al., 1992). First, to verify that the electrode was correctly placed in the NAcc, stimulation of the medial forebrain bundle (MFB) was used to confirm the release of dopamine with FSCV using a triangular waveform (-0.4V to 1.3V at 400 V s^{-1}). Handling and surgery of 3 male C57BL/6J mice weighing 20-25 g (Jackson Laboratory, Bar Harbor, ME) were in agreement with The Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care Use Committee. Urethane (25% dissolved in 0.9% NaCl solution, Hospira, Lake Forest, IL) was administered via intraperitoneal (*i.p.*) injection and stereotaxic surgery (David Kopf Instruments, Tujunga, CA) was performed. A heating pad sustained mouse body temperature around 37°C (Braintree Scientific, Braintree, MA). Stereotaxic coordinates were taken in reference to bregma. A Nafion modified CFM was inserted into the nucleus accumbens core (NAcc) (AP: +1.09, ML: -1.25, DV: -4.30). 120 Biphasic pulses were applied through a linear constant current stimulus isolator (NL800A, Neurolog, Medical Systems Corp., Great Neck, NY). An Ag/AgCl reference electrode was implanted into the brain's opposite hemisphere.

Figure 3.4A (top left) displays the experimental scheme, noting the working electrode in the NAcc and the stimulating electrode in the MFB. **Figure**

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3.4B shows the *in vivo* FSCV color plot, in which the green event denotes the release of dopamine by stimulation (grey bar below – 2 second duration). Dopamine is confirmed by its distinctive cyclic voltammogram; oxidation at 0.6 V and reduction at -0.2 V (white inset taken from the vertical, dashed white line).

Next, the dopamine FSCAV waveform was applied to the working electrode and the two FSCAV components compared. The components used to obtain initial *in vivo* measurements were altered between animals. **Figure 3.4C** shows the LED circuit *in vivo* color plot and corresponding current trace for the 3rd scan, shown by the star and dashed, white line. The integration limits are set by first placing the final limit where a stable minimum can be found after the oxidative peak, and then lining up the cross hairs of the initial limit to the final (**Figure 3.4C**). It is important to note that the 2nd scan could not be taken for the LED circuit, as the background, governed by capacitative current, was large enough (~600 nA) to mask the Faradaic peaks, because of the presence of additional adsorbing analytes *in vivo* or more likely stray capacitance from the BJT transistor. In **Figure 3.4D**, a color plot and current trace are shown for the original switch. For the switch's integration limits, we utilize the 2nd scan, as previously published (Atcherley et al., 2015), instead of the 3rd scan, which is shown by the star and white dashed line. While this scan has a large, non-Faradaic background peak (~350 nA), most importantly, the 2nd scan has the first clearly distinguishable Faradaic dopamine peak.

The original work on FSCAV with the switch performed convolution prior to analysis (Atcherley et al., 2013; Atcherley et al., 2014), however, it has since

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been shown that convolution is not always required (Abdalla et al. 2017; Burrell et al. 2015; Pathirathna et al., 2016). We did not employ convolution data analysis to our results. The switch's integration limits are set by placing both initial and final limits at the local minima, and encompassing the entire peak. It is important to note that the integration limits *in vivo* were altered from the previous *in vitro* comparison of both systems to account for the complex matrix of the brain and additional analytes that are present (Takmakov et al., 2010b; Cahill et al., 1996; Heien et al., 2004). A post-calibration was performed on the experimental electrodes using 50, 100, 200, and 500 nM dopamine concentrations.

Using the same calibration concentrations and procedure as the *in vitro* experiment, the resulting *in vivo* dopamine concentration for the switch = 95.0 $\pm$ 11.0 nM and the novel LED circuit = 94.4 $\pm$ 7.0 nM. These values are statistically identical and in good agreement with values that we previously published (Atcherley et al., 2015) (n=3; p = 0.96).

As a final characterization, the root-mean-square of the signal and noise in the *in vivo* recordings was calculated. For a direct comparison, using the 3rd scan for both the LED circuit and switch, we found a signal to noise ratio (S/N) of 281.7 and 199.5, respectively. Furthermore, to compare the scans we used for the *in vivo* analysis above, we performed the same RMS calculation for the 3rd scan of the LED circuit vs. the 2nd scan for the switch. The resulting S/N was 281.7 for the LED circuit and 562.6 for the switch. It is important to note that during the 2nd scan for the switch, there is a large background peak which contributes to the signal, as shown in the IT curve in **Figure 3.4D**. However, as

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previously mentioned, since the first, clear dopamine peak is also obtained during the 2nd scan, it is feasible to obtain *in vivo* concentrations of dopamine. The BJT transistor, utilized by the LED circuit, takes longer to not only shut off, but may add stray capacitance that appears as additional background in scan 2. The CMOS precision switch has a much faster switching capability, enabling the dopamine peak to already appear by the 2nd scan. Nonetheless, it is necessary to point out that the direct comparison of the 3rd scan shows a higher S/N for the LED circuit, indicating it has more favorable recording capabilities *in vitro.* Additionally, the LED circuit provides high enough S/N for *in vivo* measures with accurate results.

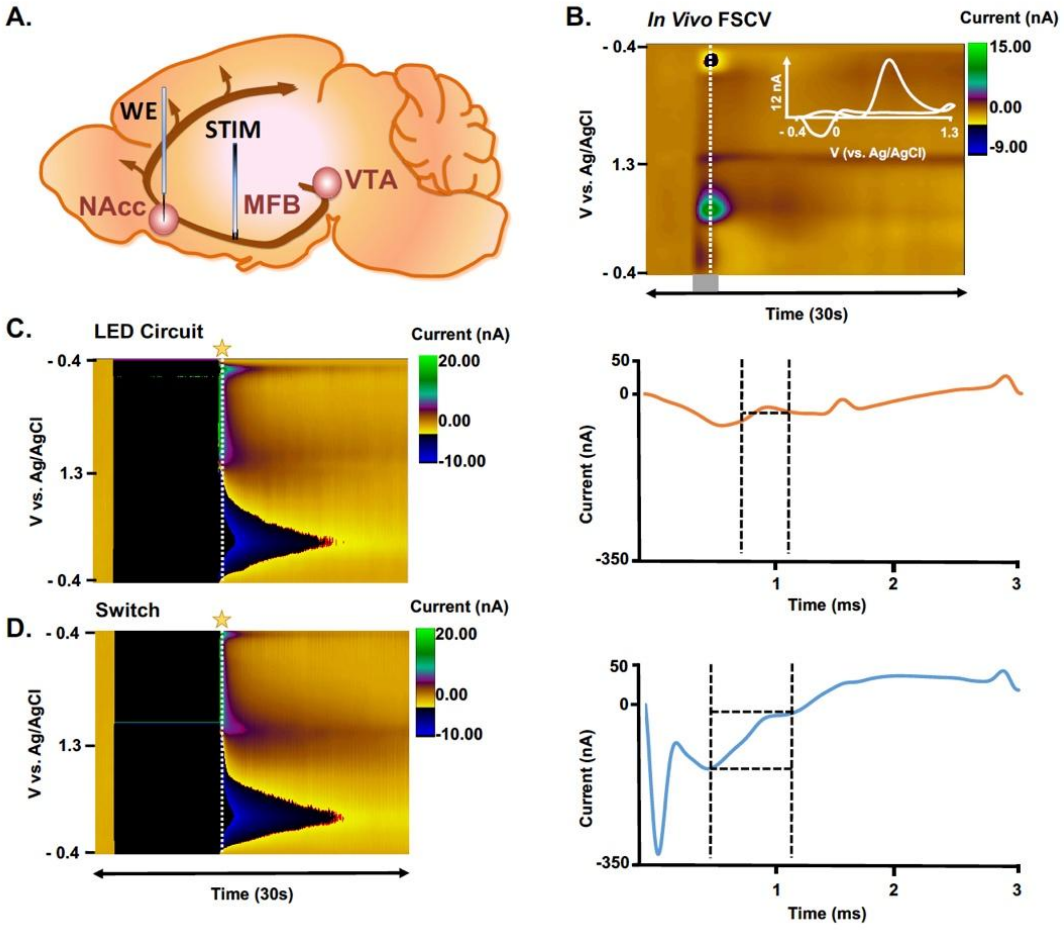

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Figure 3.4. LED Circuit vs. Switch *in vivo* measurement comparison. (A) Experimental scheme of *in vivo* experiments in the mouse brain. **(B)** FSCV verification recording of dopamine release in the nucleus accumbens core after electrical stimulation of the medial forebrain bundle. **(C)** LED circuit color plot and extracted cyclic voltammogram from the dashed, starred line (3^{rd} scan). **(D)** Switch color plot and extracted cyclic voltammogram from the dashed, starred line (2^{nd} scan). Integration limits are shown in black, dashed lines.

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Table 3.1 LED Circuit Hardware Specifications

Hardware name	*LED Circuit*
Subject area	• Chemistry and Biochemistry • Neuroscience • Biological Sciences (e.g. Microbiology and Biochemistry)
Hardware type	• Field measurements and sensors • Voltammetry
Open Source License	*Creative Commons Attribution-ShareAlike 3.0*
Cost of Hardware	~$5.00
Source File Repository	*N/A – design included in body of manuscript*

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Table 3.2 LED Circuit Bill of Materials

Designator	Component	#	Cost per unit -currency	Total cost - currency	Source of materials	Material type
LED	OP295A Infrared 890nm LED	2	$0.75	$1.50	Mouser Electronics	N/A
NPN Bipolar Transistor	BC548CTA NPN 30V 100mA	1	$0.20	$0.20	Mouser Electronics	N/A
BNC connector	565-6741 BNC female	1	$3.49	$3.49	Mouser Electronics	N/A

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CHAPTER 4

CONCLUSIONS AND PROSPECTS

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Serotonin is an important biomolecule and key regulator in many neurochemical pathways. However, a precise definition of normal and irregular serotonin neurotransmission has yet to be determined due to the lack of tools that can detect serotonin on a relevant timescale *in vivo*. This thesis has described the most recent work done to characterize serotonin's synaptic activity and elucidate this transmitter's distinct function using novel electrochemical techniques.

This thesis addressed the historical challenges of selectively targeting and measuring serotonin and highlighted FSCV as a powerful electroanalytical technique that able to overcome these obstacles in order to better understand phasic-release serotonin dynamics. Furthermore, FSCAV technology has allowed for the first rapid measurement of ambient neurotransmitter concentrations (Chapter 2). In Chapter 3, simplified instrumentation for fast-voltammetric analysis was developed and characterized to allow non-experts in electronics and electrochemistry to utilize FSCAV.

To better understand *in vivo* serotonin dynamics, an innovative platform is needed to measure serotonin dynamics during real-time environmental stimuli and behavior. FSCV measurement of serotonin in freely-moving animals would provide critical information on:

a. the underlying processes that serotonin encodes

b. which environmental stimuli induce serotonin release

c. how serotonin events are time-locked to behavioral events on a physiologically relevant time scale (10 Hz)

d. regional differences in the brain for a) - c)

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These topics are routinely studied via dopamine FSCV (Klanker, Feenstra, Willuhn, & Denys, 2017; Howe, Tierney, Sandberg, Phillips, & Graybiel, 2013). Therefore, given the development of suitable tools, it is feasible to address these questions for serotonin as well. It is foreseeable that the concentrations of evoked serotonin would differ in freely moving vs. anesthetized animals due to serotonin's role in circadian rhythms and heightened activity during wakefulness (Penalva et al., 2003). As a result, even though evoked serotonin levels in anesthetized animals are low, it is expected that these concentrations may be higher in freely moving animals.

Currently, all serotonin FSCV experiments utilize acute, glass electrodes which are lowered the day of electrochemical recording. Due to damage and stress of implanting the electrode for the animal, it is not recommended to do this procedure more than once in an individual animal. Thus, chronically implanted electrodes for serotonin would greatly aide behavior studies so that serotonin measurements could be conducted for longer periods of time during the evolution of behavior such as addiction, depression or compulsivity (Clark et al., 2010; Rodeberg, Sandberg, Johnson, Phillips, & Wightman, 2017).

It will also be of critical importance to record tonic extracellular serotonin in different disease states, such as depression, to explore whether or not ambient serotonin levels influence the development, maintenance, or reversal of neuropsychiatric disorders. The LED circuit was constructed for novices in electronics and electrochemistry, thus skilled neuroscientists can now utilize this new technology to measure ambient serotonin in any respective animal model.

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Furthermore, with respect to the rise of novel genetics-based tools for specific manipulation (i.e. optogenetics and chemogenetics) and execution in mice, FSCV would act as a neurochemical readout to verify the selectivity of the manipulation. The coupling of genetic manipulation and serotonin FSCV would also greatly improve our knowledge of serotonin activity and influences on behavior, mood, and other neuronal systems. Although these methods are beginning to be executed in mice, it is crucial to obtain a neurochemical readout via FSCV to verify the selectivity of the manipulation. Overall, there are still many questions about serotonergic mechanisms governing healthy brain function that remain unanswered. Exciting new developments in fast-voltammetric techniques mean that the scientific community is one step closer to unraveling the function of this elusive neurotransmitter.

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REFERENCES

Abdalla, A. et al. (2017). In Vivo Ambient Serotonin Measurements at Carbon-Fiber Microelectrodes. *Analytical Chemistry,* 89(18), pp. 9703-9711.

Anastasio, et al. (2013). Functional Status of the Serotonin 5-HT_{2C} Receptor (5-HT_{2C}R) Drives Interlocked Phenotypes that Precipitate Relapse-Like Behaviors in Cocaine Dependence. *Neuropsychopharmacology,* 39(2), pp. 370-382.

Atcherley, C.W., Laude, N.D., Parent, K.L., & Heien, M.L. (2013). Fast-scan controlled-adsorption voltammetry for the quantification of absolute concentrations and adsorption dynamics. *Langmuir,* 29(48), pp. 14885-14892.

Atcherley, C.W., Laude, N.D., Monroe, E.B., Wood, K.M., Hashemi, P., & Heien, M.L. (2014). Improved Calibration of Voltammetric Sensors for Studying Pharmacological Effects on Dopamine Transporter Kinetics in Vivo. *ACS Chemical Neuroscience,* 6, pp. 1509-1516.

Atcherley, C.W., Wood, K.M., Parent, K.L., Hashemi, P., & Heien, M.L. (2015). The coaction of tonic and phasic dopamine dynamics. *Chemical Communications*, 51(12), pp. 2235-2238.

Baganz, N.L., et al. (2008). Organic cation transporter 3: Keeping the brake on extracellular serotonin in serotonin-transporter-deficient mice. *Proceedings of the National Academy of Sciences,* 105(48), pp. 18976-18981.

Baganz, N.L. & Blakely, R.D. (2012). A Dialogue between the Immune System and Brain, Spoken in the Language of Serotonin. *ACS Publications*, 4(1), pp. 48–63.

Barnes, N.M. & Sharp, T. (1999). A review of central 5-HT receptors and their function. *Neuropharmacology,* 38, pp. 1083-1152.

Borland, L.M., Shi, G., Yang, H., & Michael, A.C. (2005). Voltammetric study of extracellular dopamine near microdialysis probes acutely implanted in the striatum of the anesthetized rat. *Journal of neuroscience methods,* 146(2), pp. 149-158.

Brazell, M.P., Kasser, R.J., Renner, K.J., Feng, J., Moghaddam, B., & Adams, R.N. (1987). Electrocoating carbon fiber microelectrodes with Nafion

www.manaraa.com

improves selectivity for electroactive neurotransmitters. *Journal of neuroscience methods,* 22(2), pp. 167-172.

Brown, R.E., Stevens, D.R. & Haas, H.L. (2001). The physiology of brain histamine. *Progress in Neurobiology*, 63(6), pp. 637–672.

Bunin, M.A., Prioleau, C., Mailman, R.B., & Wightman, R.M. (1998). Release and uptake rates of 5-hydroxytryptamine in the dorsal raphe and substantia nigra reticulate of the rat brain. *Journal of neurochemistry,* 70(3), pp. 1077-1087.

Bunin, M.A. & Wightman R.M. (1998). Quantitative evaluation of 5-hydroxytryptamine (serotonin) neuronal release and uptake: an investigation of extrasynaptic transmission. *Journal of Neuroscience,* 18, pp. 4854-4860.

Burmeister, J.J., Lungren, E.M., Kirschner, K.F., & Neisewander, J.L. (2004). Differential roles of 5-HT receptor subtypes in cue and cocaine reinstatement of cocaine-seeking behavior in rats. *Neuropsychopharmacology,* 29(4), pp. 660.

Burrell, M.H., Atcherley, C.W., Heien, M.L., & Lipski, J. (2015). A novel electrochemical approach for prolonged measurement of absolute levels of extracellular dopamine in brain slices. *ACS chemical neuroscience*, 6(11), pp. 1802-1812.

Cahill, P.S., Walker, Q.D., Finnegan, J.M., Mickelson, G.E., Travis, E.R., & Wightman, R.M. (1996). Microelectrodes for the measurement of catecholamines in biological systems. *Analytical Chemistry,* 68(18), pp. 3180-3186.

Calizo, L.H., et al. (2011). Raphe serotonin neurons are not homogenous: electrophysiological, morphological, and neurochemical evidence. *Neuropharmacology,* 61(3), pp. 524-543.

Cammack, J., Ghasemzadeh, B., & Adams, R.N. (1991). The pharmacological profile of glutamate-evoked ascorbic acid efflux measured by in vivo electrochemistry. *Brain Research,* 565, pp. 17-22.

Celada, P., Puig, M.V., Casanovas, J.M., Guillazo, G., & Artigas, F. (2001). Control of Dorsal Raphe Serotonergic Neurons by the Medial Prefrontal Cortex: Involvement of Serotonin-1A, GABA, and Glutamate Receptors. *Journal of Neuroscience*, 21(24), pp. 9917–9929.

Challis, C., et al. (2013). Raphe GABAergic neurons mediate the acquisition of avoidance after social defeat. *Journal of Neuroscience,* 33(35), pp. 13978-13988.

www.manaraa.com

Charara, A. & Parent, A. (1998). Chemoarchitecture of the primate dorsal raphe nucleus. *Journal of chemical neuroanatomy,* 15(2), pp. 111-127.

Chase, T.N. & Murphy, D.L. (1973). Serotonin and Central Nervous System Function. *Annual Review of Pharmacology*, 13(1), pp.181–197.

Cheer, J.F., Aragona, B.J., Heien, M.L., Seipel, A.T., Carelli, R.M., & Wightman, R.M. (2007). Coordinated accumbal dopamine release and neural activity drive goal-directed behavior. *Neuron,* 54(2), pp. 237-244.

Chefer V.I., Thompson, A.C., Zapata, A., & Shippenberg, T.S. (2009). Overview of brain microdialysis. *Current protocols in neuroscience,* 47(1), pp. 7-11.

Clark, J.J., et al., (2010). Chronic microsensors for longitudinal, subsecond dopamine detection in behaving animals. *Nature methods*, 7(2), pp. 126.

Correia, P.A., Lottem, E., Banerjee, D., Machado, A.S., Carey, M.R., & Mainen, Z.F. (2017). Transient inhibition and long-term facilitation of locomotion by phasic optogenetic activation of serotonin neurons. *eLife*, 6.

Daws, L.C., Toney, G.M., Davis, D.J., Gerhardt, G.A., & Frazer, A. (1997). In vivo chronoamperometric measurements of the clearance of exogenously applied serotonin in the rat dentate gyrus. *Journal of neuroscience methods,* 78(1-2), pp. 139-150.

Daws, L.C., Toney, G.M., Gerhardt, G.A., & Frazer, A. (1998). In vivo chronoamperometric measures of extracellular serotonin clearance in rat dorsal hippocampus: contribution of serotonin and norepinephrine transporters. *The Journal of pharmacology and experimental therapeutics*, 286(2), pp. 967–976.

Daws, L.C., et al. (2005). Transport mechanisms governing serotonin clearance in vivo revealed by high-speed chronoamperometry. *Journal of neuroscience methods,* 143(1), pp. 49-62.

Daws, L.C. & Toney G.M. (2007). High-speed chronoamperometry to study kinetics and mechanisms for serotonin clearance in vivo. In Hua, Y. & Michael, A.C. (Eds), *Electrochemical Methods for Neuroscience.* Boca Raton (FL): CRC Press/Taylor & Francis.

Daws, L.C., Koek, W., & Mitchell, N.C. (2013). Revisiting Serotonin Reuptake Inhibitors and the Therapeutic Potential of “Uptake-2” in Psychiatric Disorders. *ACS Chemical Neuroscience,* 4(1), pp. 16-21.

Day, J.J., Roitman, M.F., Wightman, R.M., & Carelli, R.M. (2007). Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nature neuroscience,* 10(8), pp. 1020.

www.manaraa.com

DeFelipe, J. & Jones, E.G. (1988). A light and electron microscopic study of serotonin-immunoreactive fibers and terminals in the monkey sensory-motor cortex, *Experimental Brain Research,* 71, pp. 171-182.

Ellenroek, B. & Youn, J. (2016). Rodent models in neuroscience research: is it a rat race? *Disease models & mechanisms,* 9(10), pp. 1079-1087.

Esbenshade, T.A., Browman, K.E., Bitner, R.S., Strakhova, M., Cowart, M.D., & Brioni, J.D. (2008). The histamine H3 receptor: an attractive target for the treatment of cognitive disorders. *British Journal of Pharmacology,* 154, pp. 1166-1181.

Fink, K., Schlicker, E., Neise, A., & Gothert, M. (1990). Involvement of presynaptic H3 receptors in the inhibitory effect of histamine on serotonin release in the rat brain cortex. *Naunyn-Schmiedeberg's archives of pharmacology,* 342(5), pp. 513-519.

Flagel, S.B. et al. (2011). A selective role for dopamine in stimulus-reward learning. *Nature,* 469(7328), pp. 53.

France, C.P., Li, J.X., Owens, W.A., Koek, W., Toney, G.M., & Daws, L.C. (2009). Reduced effectiveness of escitalopram in the forced swimming test is associated with increased serotonin clearance rate in food-restricted rats. *International Journal of Neuropsychopharmacology,* 12(6), pp. 731-736.

Fronseca, M.S., Murakami, M., & Mainen, Z.F. (2015). Activation of dorsal raphe serotonergic neurons promotes waiting but is not reinforcing. *Current Biology,* 25(3), pp. 306-315.

Fuller, R.W., Perry, K.W, & Molloy, B.B. (1974). Effect of an uptake inhibitor on serotonin metabolism in rat brain: studies with 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine (Lilly 110140). *Life sciences,* 15(6), pp. 1161-1171.

Fuller, R.W. & Wong, D.T. (1977). Inhibition of serotonin reuptake. *Federation proceedings,* 36(8), pp. 2154-2158.

Fuller, R.W. (1994). Uptake inhibitors increase extracellular serotonin concentration measured by brain microdialysis. *Life sciences,* 55(3), pp. 163-167.

Gelenberg, A.J. & Chesen, C.L. (2000). How fast are antidepressants? *Journal of Clinical Psychiatry,* 61, pp. 712-721.

Gerhardt, G.A., Oke, A.F., Nagy, G., Moghaddam, B., & Adams, R.N. (1984). Nafion-coated electrodes with high selectivity for CNS electrochemistry. *Brain research,* 290(2), pp. 390-395.

www.manaraa.com

Griessenauer, C.J., et al. (2010). WINCS-based wireless electrochemical monitoring of serotonin (5-HT) using fast-scan cyclic voltammetry: proof of principle. *Journal of Neurosurgery,* 113(3), pp. 656-665.

Haas, H.L., Sergeeva, O.A. & Selbach, O. (2008). Histamine in the Nervous System. *Physiological Reviews*, 88(3), pp. 1183–1241.

Hainer, C., et al. (2015). Beyond Gene Inactivation: Evolution of Tools for Analysis of Serotonergic Circuitry. *ACS Chemical Neuroscience,* 6(7), pp. 1116-1129.

Hamid, A.A., et al. (2016). Mesolimbic dopamine signals the value of work. *Nature neuroscience,* 19(1), pp. 117.

Hashemi, P., Dankoski, E.C., Petrovic, J., Keithley, R.B., & Wightman, R.M. (2009). Voltammetric detection of 5-hydroxytryptamine release in the rat brain. *Analytical chemistry*, 81(22), pp. 9462–9471.

Hashemi, P., Dankoski, E.C., Wood, K.M., Ambrose, R.E., & Wightman, R.M. (2011). In vivo electrochemical evidence for simultaneous 5-HT and histamine release in the rat substantia nigra pars reticulata following medial forebrain bundle stimulation. *Journal of Neurochemistry*, 118(5), pp. 749–759.

Hashemi, P., Dankoski, E.C., Lama, R., Wood, K.M., Takmakov, P., & Wightman, M.R. (2012). Brain dopamine and serotonin differ in regulation and its consequences. *Proceedings of the National Academy of Science of the USA,* 109(29), pp. 11510-11515.

Heien, M.L., Phillips, P.E., Stuber, G.D., Seipel, A.T., & Wightman, R.M. (2003). Overoxidation of carbon-fiber microelectrodes enhances dopamine adsorption and increase sensitivity. *Analyst,* 128(12), pp. 1413-1419.

Heien, M.L., Johnson, M.A., & Wightman, R.M. (2004). Resolving neurotransmitters detected by fast-scan cyclic voltammetry. *Analytical Chemistry,* 76(19), pp. 5697-5704.

Hersman, S., Cushman, J., Lemelson, N., Wassum, K., Lotfipour, S., & Fanselow, M.S. (2017). Optogenetic excitation of cholinergic inputs to hippocampus primes future contextual fear associations. *Scientific Reports,* 7(1), pp. 2333.

Holtzheimer, P.E. & Mayberg, H.S. (2011). Deep brain stimulation for psychiatric disorders. *Annual Review of Neuroscience,* 34, pp. 289-307.

Hornung, J.P. (2003). The human raphe nuclei and the serotonergic system. *Journal of Chemical Neuroanatomy*, 26(4), pp. 331–343.

www.manaraa.com

Howe, M.W., Tierney, P.L., Sandberg, S.G., Phillips, P.E., & Graybiel, A.M. (2013). Prolonged dopamine signaling in striatum signals proximity and value of distant rewards. *Nature*, 500(7464), pp. 575.

Howell, J.O., Kuhr, W.G., Ensman, R.E., & Wightman, R.M. (1986). Background subtraction for rapid scan voltammetry. *Journal of Electroanalytical Chemistry,* 209, pp. 77-90.

Jackson, B.P., Dietz, S.M., & Wightman, R.M. (1995). Fast-scan cyclic voltammetry of 5-hydroxytryptamine. *Analytical Chemistry,* 67(6), pp. 1115-1120.

Jain, N., Qi., H.X., Collins, C.E., & Kaas, J.H. (2008). Large-scale reorganization in the somatosensory cortex and thalamus after sensory loss in macaque monkeys. *Journal of Neuroscience,* 28(43), pp. 11042-11060.

Jin, Y. et al., (2016). Regrowth of serotonin axons in the adult mouse brain following injury. *Neuron,* 91(4), pp. 748-762.

Jaquins-Gerstl, A. & Michael, A.C. (2015). A review of the effects of FSCV and microdialysis measurements on dopamine release in the surrounding tissue. *Analyst*, 70(2), pp. 584-593.

Jennings, K.A. (2013). A comparison of the subsecond dynamics of neurotransmission of dopamine and serotonin. *ACS Chemical Neuroscience,* 4, pp. 704-714.

Kirby, L.G., Rice, K.C., & Valentina, R.J. (2000). Effects of Corticotropin-Releasing Factor on Neuronal Activity in the Serotonergic Dorsal Raphe Nucleus. *Neuropsychopharmacology,* 22(2), pp. 148-162.

Klanker, M., Feenstra, M., Willuhn, I., and Denys, D. (2017). Deep brain stimulation of the medial forebrain bundle elevates striatal dopamine concentration without affecting spontaneous or reward-induced phasic release. *Neuroscience,* 19(364), pp. 82-92.

Klingauf, J., Kavalali, E.T. & Tsien, R.W. (1998). Kinetics and regulation of fast endocytosis at hippocampal synapses. *Nature*, 394(6693), pp. 581–585.

Lee, W.H. et al. (2016). Microfabrication and in vivo performance of a microdialysis probe with embedded membrane. *Analytical chemistry,* 88(2), pp. 1230-1237.

Liu, Y. et al. (2010). Capillary ultrahigh performance liquid chromatography with elevated temperature for sub-one minute separations of basal serotonin in submicroliter brain microdialysate samples. *Analytical chemistry,* 82(23), pp. 9611-9616.

www.manaraa.com

Liu, Z. et al. (2014). Dorsal Raphe Neurons Signal Rewards through 5-HT and Glutamate. *Neuron,* 81(6), pp. 1360-1374.

Mathews, T.A., Fedele, D.E., Coppelli, F.M., Avila, A.M., Murphy, D.L., & Andrews, A.M. (2004). Gene dose-dependent alterations in extraneuronal serotonin but not dopamine in mice with reduced serotonin transporter expression. *Journal of neuroscience methods,* 140(1-2), pp. 169-181.

McElligot, Z. (2015). Optogenetic and Chemogenetic Approaches to Advance Monitoring Molecules. *ACS Chemical Neuroscience,* 6, pp. 944-947.

McIlwain, H.J., Thomas, J., & Bell, J.L. (1956). The composition of isolated cerebral tissues: ascorbic acid and cozymase. *Biochemical Journal,* 64(2), pp. 332.

Meredith, G.E, Agolia, R., Arts, M.P.M., Groenewegen, H.J., & Zahm, D.S. (1992). Morphological differences between projection neurons of the core and shell in the nucleus-accumbens of the rat. *Neuroscience* 50(1), pp. 149-162.

Miller, E.K., & Cohen, J.D. (2001). An integrative theory of prefrontal cortex function. *Annual review of neuroscience,* 24(4), pp. 167-202.

Mitchell, E.C., Dunaway, L.E., McCarty, G.S., & Sombers, L.A. (2017). Spectrochemical Characterization of the Dynamic Carbon-Fiber Surface in Response to Electrochemical Conditioning. *Langmuir,* 33(32), pp. 7838-7846.

Miyazaki, K.W., Miyazaki, K., & Doya, K. (2011). Activation of the central serotonergic system in response to delayed but not omitted rewards. *European Journal of Neuroscience,* 33(1), pp. 153-160.

Miyazaki, K., Miyazaki, K.W., & Doya, K. (2012). The role of serotonin in the regulation of patience and impulsivity. *Molecular Neurobiology,* 45(2), pp. 213-224.

Miyazaki, K.W., Miyazaki, K., & Doya, K. (2012). Activation of dorsal raphe serotonin neurons is necessary for waiting for delayed rewards. *Journal of Neuroscience,* 32(31), pp. 10451-10457.

Miyazaki, K.W., et al. (2014). Optogenetic activation of dorsal raphe serotonin neurons enhances patience for future rewards. *Current Biology,* 24(17), pp. 2023-2040.

Monachon, M.A., Burkhard, W.P., Jalfre, M., & Haefely, W. (1972). Blockade of central 5-hydroxytryptamine receptors by methiothepin. *Naunyn-Schmiedeberg's Archives of Pharmacology,* 274, pp. 192-197.

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Montañez, S., Munn, J.L., Owens, W.A., Horton, R.E., & Daws, L.C. (2014). 5-HT1B receptor modulation of the serotonin transporter in vivo: studies using KO mice. *Neurochemistry international*, 73, pp. 127–131.

Moran, R.J. et al., (2018). The protective action encoding of serotonin transients in the human brain. *Neuropsychopharmacology,* pp. 1-11.

Muller, C.P. & Jacobs, B. (2009). *Handbook of the behavioral neurobiology of serotonin*, 21. Academic Press.

Murphy, D.L. et al. (2003). Experimental gene interaction studies with SERT mutant mice as models for human polygenic and epistatic traits and disorders. *Genes, Brain and Behavior*, 2(6), pp. 350–364.

Nagy, G. et al. (1985). Ion exchange and transport of neurotransmitters in nation films on conventional and microelectrode surfaces. *Journal of electroanalytical chemistry and interfacial electrochemistry,* 188(1-2), pp. 85-94.

Nandi, P. & Lunte, S.M. (2009). Recent trends in microdialysis sampling integrated with conventional and microanalytical systems for monitoring biological events: a review. *Analytica chimica acta,* 651(1), pp. 1-14.

Nautiyal, K.M. & Hen, R. (2017). Serotonin receptors in depression: from A to B. *F1000Research,* 6.

Nieuwenhuys, R., Geeraedts, L.M., & Veening, J.G. (1982). The medial forebrain bundle of the rat. I. General introduction. *The Journal of Comparative Neurology,* 206, pp. 48-81.

Nirenberg, M., et al. (1997). The dopamine transporter: Comparative ultrastructure of dopaminergic axons in limbic and motor compartments of the nucleus accumbens. *Journal of Neuroscience* 17(18), pp. 6899-6907.

Oldham, K. (1986). Convolution of voltammograms as a method of chemical analysis. *Journal of the Chemical Society, Faraday Transactions,* 82, pp. 1099-1104.

Palkovits, M., Brownstein, M., & Saavedra, J.M. (1974). Serotonin content of the brain stem nuclei in the rat. *Brain research,* 80(2), pp. 237-249.

Pathirathna, P., Siriwardhane, T., McElmurry, S.P., Morgan, S.L., & Hashemi, P. (2016). Fast voltammetry of metals at carbon-fiber microelectrodes: towards an online speciation sensor. *Analyst,* 141, pp. 6432-6437.

Paxinos, G. & Watson, C. (2007). The Rat Brain in Stereotaxic Coordinates. London: Academic Press.

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Penalva, R.G., Lancel, M., Flachskamm, C., Reul, J.M., Holsboer, F., Linthorst, A.C. (2003). Effect of sleep and sleep deprivation on serotonergic neurotransmission in the hippocampus: a combined in vivo microdialysis/EEG study in rats. *European Journal of Neuroscience,* 17(9), pp. 1896-1906.

Phillips, P.E.M., Robinson, D.L., Stuber, G.D., Carelli, R.M., & Wightman, R.M. (2003). Real-time measurements of phasic changes in extracellular dopamine concentration in freely moving rats by fast-scan cyclic voltammetry. In *Drugs of Abuse* (pp. 443-464). Humana Press.

Phillips, P.E.M. & Wightman, M.R. (2003). Critical guidelines for validation of the selectivity of in-vivo chemical microsensors. *Trends in Analytical Chemistry,* 22(9), pp. 509-514.

Qi, J., et al. (2014). A glutamatergic reward input from the dorsal raphe to ventral tegmental area dopamine neurons. *Nature communication,* 12(5), pp. 5390.

Reubi, J.C. & Emson, P.C. (1978). Release and distribution of endogenous 5-HT in rat substantia nigra. *Brain research,* 139(1), pp. 164-168.

Rice, M.E. & Nicholson, C. (1989). Measurement of nanomolar dopamine diffusion using low-noise perfluorinated ionomer coated carbon fiber microelectrodes and high-speed cyclic voltammetry. *Analytical Chemistry,* 61(17), pp. 1805-1810.

Robinson, D.L., Venton, B.J., Heien, M.L., & Wightman, R.M. (2003). Detecting subsecond dopamine release with fast-scan cyclic voltammetry in vivo. *Clinical Chemistry,* 49(10), pp. 1763-1773.

Rodeberg, N.T., Sandberg, S.G., Johnson, J.A., Phillips, P.E., & Wightman, R.M. (2017). Hitchhiker's guide to voltammetry: acute and chronic electrodes for in vivo fast-scan cyclic voltammetry. *ACS chemical neuroscience,* 8(2), pp. 221-234.

Russell, W.L., Henry, D.P., Phebus, L.A., & Clemens, J.A. (1990). Release of histamine in rat hypothalamus and corpus striatum in vivo. *Brain research*, 512(1), pp. 95–101.

Sakowski, S.A., Geddes, T.J., Thomas, D.M., Levi, E., Hatfield, J.S., & Kuhn, D.M. (2006). Differential tissue distribution of tryptophan hydroxylase isoforms 1 and 2 as revealed with monospecific antibodies. *Brain Research,* 1085(1), pp. 104-114.

Samaranayake, S. et al., (2016). A voltammetric and mathematical analysis of histaminergic modulation of serotonin in the mouse hypothalamus. *Journal of Neurochemistry*, 138(3), pp. 374–383.

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Sara, Y., Mozhayeva, M.G., Liu, X., & Kavalali, E.T. (2002). Fast Vesicle Recycling Supports Neurotransmission during Sustained Stimulation at Hippocampal Synapses. *Journal of Neuroscience*, 22(5), pp.1608–1617.

Schenk, J.O., Miller, E., Gaddis, R., & Adams, R.N. (1982). Homeostatic control of ascorbate concentration in CNS extracellular fluid. *Brain research,* 253(1-2), pp. 353-356.

Schlicker, E., Betz, R., & Gothert, M. (1988). Histamine H3 receptor-mediated inhibition of serotonin release in the rat brain cortex. *Naunyn Schmiedeberg's Archives of Pharmacology,* 337, pp. 588-590.

Shaskan, E.G. & Snyder, S.H. (1970). Kinetics of serotonin accumulation into slices from rat brain: relationship to catecholamine uptake. *The Journal of pharmacology and experimental therapeutics,* 175, pp. 404-418.

Shen, H.W., et al. (2004). Regional differences in extracellular dopamine and serotonin assessed by in vivo microdialysis in mice lacking dopamine and/or serotonin transporters. *Neuropsychopharmacology,* 29(10), pp. 1790.

Song, R. et al., (2012). Increase vulnerability to cocaine in mice lacking dopamine D3 receptors. *Proceedings of the National Academy of Sciences of the USA,* 109, pp. 17675-17680.

Srejic., L.R. et al., (2014). Paired pulse depression in the subcallosal cingulate region of depression patients. *Biological Psychiatry,* 78(1), pp. e3-e4.

Srejic, L.R., Wood, K.M., Zeqja, A., Hashemi, P., & Hutchinson, W.D. (2016). Modulation of serotonin dynamics in the dorsal raphe nucleus via high frequency medial prefrontal cortex stimulation. *Neurobiology of disease*, 94, pp.129–138.

Stamford, J.A., Kruk, Z.L., Millar, J. (1990). Striatal dopamine terminals release serotonin after 5-HTP pretreatment: in vivo voltammetric data. *Brain research,* 515(1-2), pp. 173-180.

Strand, A.M. & Venton, B.J. (2008). Flame etching enhances the sensitivity of carbon-fiber microelectrodes. *Analytical chemistry,* 80(10), pp. 3708-3715.

Takmakov, P. et al. (2010). Carbon microelectrodes with a renewable surface. *Analytical chemistry*, 82(5), pp. 2020-2028.

Takmakov, P., Zachek, M.K., Keithley, R.B., Bucher, E.S., McCarty, G.S., & Wightman, R.M. (2010b). Characterization of Local pH Changes in Brain Using Fast-Scan Cyclic Voltammetry with Carbon Microelectrodes. *Analytical Chemistry*, 82(23), pp. 9892-9900.

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Threlfell, S. et al. (2004). Histamine H3 receptors inhibit serotonin release in substantia nigra pars reticulate. *The Journal of Neuroscience,* 24, pp. 8704-8710.

Tye, K.M., et al. (2013). Dopamine neurons modulate neural encoding and expression of depression-related behavior. *Nature,* 493(7433), pp. 537.

Volle, J., et al. (2018). Deep brain stimulation and fluoxetine exert different long-term changes in the serotonergic system. *Neuropharmacology,* 135, pp. 63-72.

Walther, D.J. & Bader, M. (2003). A unique central tryptophan hydroxylase isoform. *Biochemical Pharmacology,* 66(9), pp. 1673-1680.

Weissbourd, B., Ren, J., DeLoach, K.E., Guenthner, C.J., Miyamichi, K., & Luo, L. (2014). Presynaptic partners of dorsal raphe serotonergic and GABAergic neurons. *Neuron*, 83(3), pp. 645–662.

West, A.W., Best, J., Abdalla, A., Nijhout, F., Reed, M., Hashemi, P. (2018). Voltammetric Evidence for Discrete Serotonin Circuits, Linked to Specific Reuptake Domains, in the Mouse Medial Prefrontal Cortex. *Neurochemistry International.*

Wightman, R.M., May, L.J., & Michael, A.C. (1988). Detection of dopamine dynamics in the brain. *Analytical chemistry*, 60(13), pp. 769A-793A.

Willuhn, I., Burgeno, L.M., Groblewski, P.A., Phillips, P.E. (2014). Excessive cocaine use results from decreased phasic dopamine signaling in the striatum. *Nature neuroscience,* 17(5), pp. 704.

Wood, K. M. & Hashemi, P. (2013). Fast-Scan Cyclic Voltammetry Analysis of Dynamic Serotonin Responses to Acute Escitalopram. *ACS Chemical Neuroscience,* 4, pp. 715-720.

Wood, K.M., Zeqja, A., Nijhout, H.F., Reed, M.C., Best, J., & Hashemi, P. (2014). Voltammetric and mathematical evidence for dual transport mediation of serotonin clearance in vivo. *Journal of Neurochemistry*, 130(3), pp. 351–359.

Xiao, N., Privman, E., & Venton, J.B. (2014). Optogenetic Control of Serotonin and Dopamine Release in Drosophila Larvae. *ACS Chemical Neuroscience,* 5, pp. 666-673.

Yang, H., Thompson, A.B., McIntosh, B.J., Altieri, S.C., and Andrews, A.M. (2013). Physiologically relevant changes in serotonin resolved by fast microdialysis. *ACS Chem Neuroscience,* 4(5), pp. 790-798.

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Zhang, J., Jaquins-Gerstl, A., Nesbitt, K.M., Rutan, S.C., Michael, A.C., & Weber, S.G. (2013). In vivo monitoring of serotonin in the striatum of freely moving rats with one minute temporal resolution by online microdialysis-capillary high-performance liquid chromatography at elevated temperature and pressure. *Analytical chemistry,* 85(20), pp. 9889-9897.

Zhu, C.B., Linder, K.M., Owens, A.W., Daws, L.C., Blakely, R.D., & Hewlett, W.A. (2010). Interleukin-1 Receptor Activation by Systemic Lipopolysaccharide Induces Behavioral Despair Linked to MAPK Regulation of CNS Serotonin Transporters. *Neuropsychopharmacology*, 35(13), pp. 2510–2520.

Zhu, K.Y., et al. (2011). The establishment of a sensitive method in determining different neurotransmitters simultaneously in rat brains by using liquid chromatography-electrospray tandem mass spectrometry. *Journal of Chromatography B,* 879(11-12), pp. 727-742.

Zill, P., Buttner, A., Eisenmenger, W., Moller, H.J., Ackenheil, M., & Bondy, B. (2007). Analysis of tryptophan hydroxylase I and II mRNA expression in the human brain: a post-mortem study. *Journal of Psychiatric Research,* 41(1-2), pp. 168-173.

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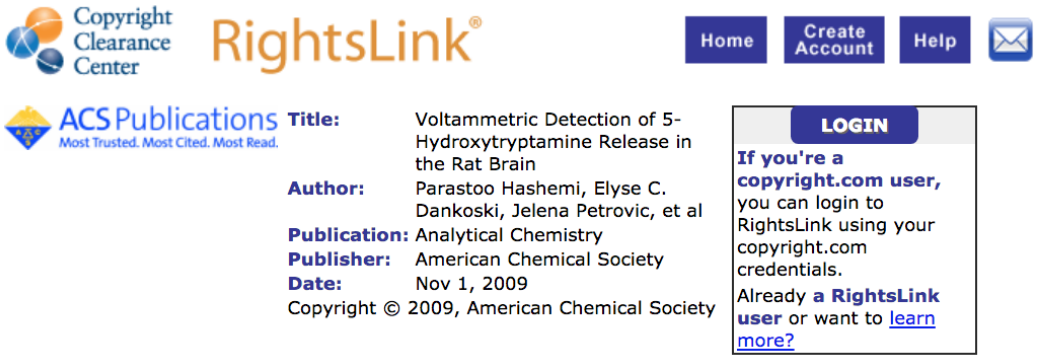

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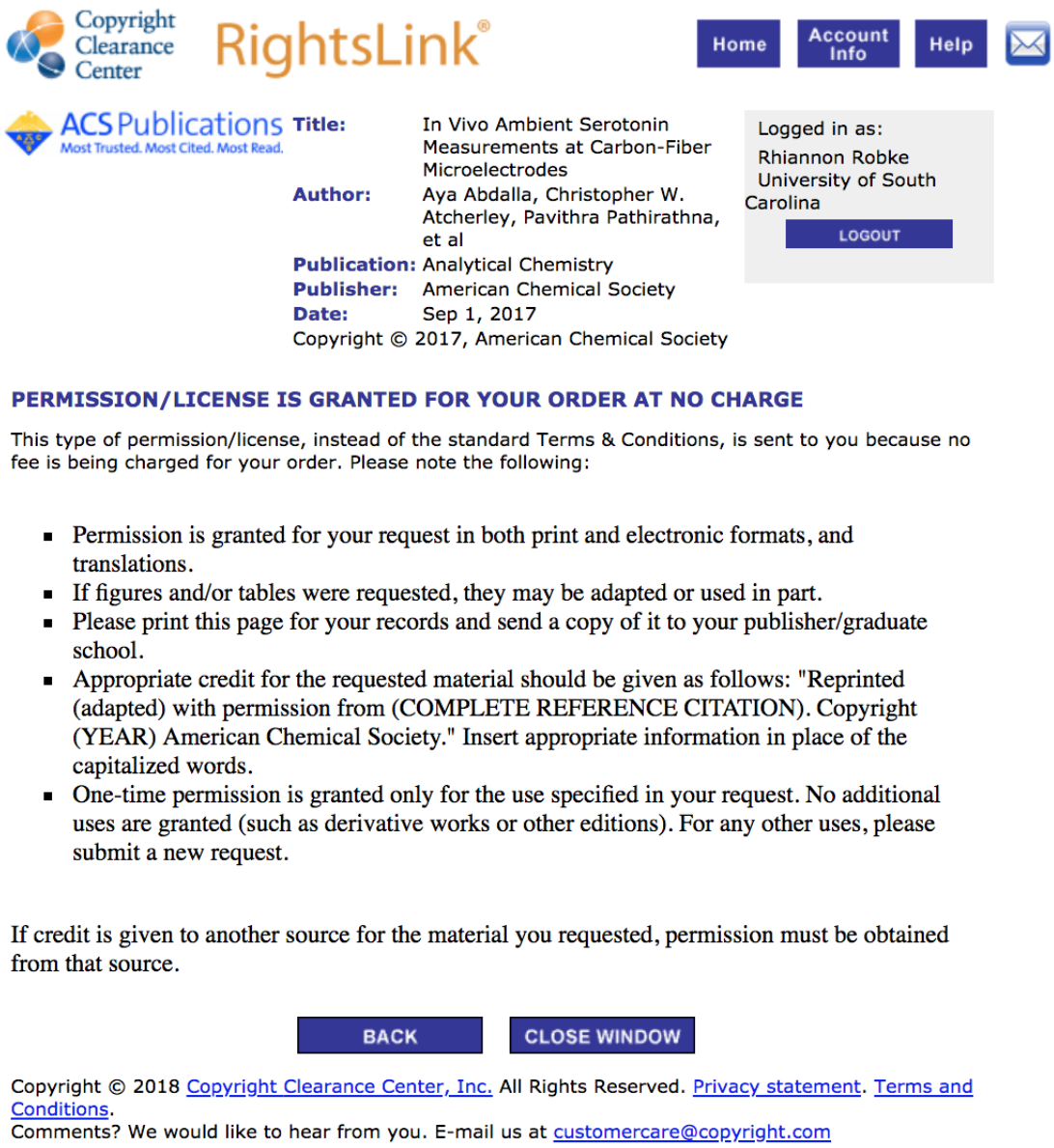

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