

IMPERIAL

Optimization of *In Vivo* Electrochemical Detection of Neurotransmitters and Application to Neurological Pathologies

Lauren C. Batey

Submitted for the degree of Doctor of Philosophy

Department of Bioengineering
Imperial College London

May 2025

0.1 Declaration of Originality

I, Lauren C. Batey, declare that the original scientific content in this thesis is my own, unless otherwise stated or referenced in the text. Experimental work that was done in collaboration with others is acknowledged within the thesis. The work presented in this thesis was performed at or in affiliation with Imperial College London.

0.2 Copyright Declaration

The copyright of this thesis rests with the author. Unless otherwise indicated, its contents are licensed under a Creative Commons Attribution-Non Commercial 4.0 International Licence (CC BY-NC). Under this licence, you may copy and redistribute the material in any medium or format. You may also create and distribute modified versions of the work. This is on the condition that: you credit the author and do not use it, or any derivative works, for a commercial purpose. When reusing or sharing this work, ensure you make the licence terms clear to others by naming the licence and linking to the licence text. Where a work has been adapted, you should indicate that the work has been changed and describe those changes. Please seek permission from the copyright holder for uses of this work that are not included in this licence or permitted under UK Copyright Law.

ABSTRACT

Depression is one of the leading causes of disability worldwide. Diagnosis and treatment are difficult due to a lack of understanding of the underlying causes and mechanisms of the disorder. Several theories have been proposed over the years, including the monoamine, neuroplasticity, and inflammation theories of depression. The neurotransmitters serotonin and histamine are of particular interest in depression research. Techniques that can accurately capture neurotransmitter dynamics in the brain are constantly being developed and refined, such as fast scan cyclic voltammetry (FSCV) and its offshoot fast scan controlled adsorption voltammetry (FSCAV). The objective of this dissertation is to apply innovative approaches to improve FSCV and FSCAV measurements for histamine and serotonin. To begin this thesis, I introduce neurotransmission, serotonin and histamine signaling, and techniques to measure neurotransmission. Next, I review the three theories of depression and emphasize essential analytical techniques used to study the disorder and showcase how inflammation affects the neurotransmitters dopamine, serotonin, and norepinephrine in the brain. I then detail the general methods used in this research. I then describe an innovative electrode surface modification using amino acids to enhance extracellular serotonin measurements with FSCAV. Subsequently, I introduce the development of FSCAV for histamine detection and describe how anesthetics and an H3R targeting drug alter extracellular histamine concentrations. Additionally, I describe a new approach to replicate in vivo conditions in vitro. I conclude by summarizing the work done in this thesis and my perspectives on future work.

DEDICATION

To my mom and dad.

ACKNOWLEDGEMENTS

First, I would like to thank my advisor and mentor, Dr. Parry Hashemi. You took me in as an undergrad and taught me to believe in myself when I did not. You encouraged me to do a PhD when I thought I would not be able to. You have given me opportunity after opportunity, and my life and career as a scientist would not have been possible without you. I can never thank you enough or repay you for all the support and guidance you have shown me.

Additionally, I owe a great deal to all the Hashemi Lab members, past and present. To Dr. Jordan Holmes, you were the first person I worked with when I joined the lab and taught me everything I know about FSCV and flow cells. To Drs. Shane Berger and Colby Witt, thank you for teaching me everything about brain surgery and for being super fun lab mates! To Drs. Anna Marie Buchanan, Yangguang Ou, and Brenna Parke, thank you for all your help and feedback in the lab. To Eva Borrás Paredes and Maryam Tanvir, thank you for being great undergrad researchers. You helped me learn how to be a better mentor. Drs. Helissa Hexter and Sergio Mena, you both are absolute rock stars! I could not have gotten through the early days of my PhD without your help and support. Bea Baumberger, my lab bestie, your friendship over the last few years has meant so much to me. You are the best! To Dr. Bettina Bohl, Anna Rhodes, Tomas Andriuskevicius, Naima Adan, and Pablo Prieto, thank you all for being great lab mates and your support over the years. It has truly been a blessing to be part of such a wonderful lab and get to work with such amazing people!

I would also like to thank Gilly Kings. Your friendship and support over the last few years has been so wonderful. Also, Zeus, Jupiter, and Fifi for all the emotional support when it was needed.

To Michael Tonhaeuser, you have helped me so much during this time. Your love and support have meant so much to me and I am so grateful to have you in my life.

And of course, none of this would have been possible without my family. Chloe, Eddie, and Wesley, I love you all so much, my sweet babies. And most importantly, my mom and dad. I owe you both absolutely everything. This truly would not have been possible without you both and everything you have done and sacrificed for me over the years. I love you both so much!

TABLE OF CONTENTS

ABSTRACT	3
DEDICATION	4
ACKNOWLEDGEMENTS	5
TABLE OF CONTENTS.....	7
LIST OF ABBREVIATIONS	11
LIST OF FIGURES	13
CHAPTER 1 - INTRODUCTION	14
Mental Health.....	15
Neuroinflammation	17
Neurotransmission.....	21
Serotonergic system	23
Histaminergic system	26
Serotonin and Histamine Co-Modulation.....	29
Neurotransmitter Measurement Techniques	30
Microdialysis.....	31
Fast scan cyclic voltammetry.....	32
Fast scan controlled adsorption voltammetry.....	36
Electrode Modifications	38
Scope of Dissertation	39
References.....	41
CHAPTER 2 - LIPOPOLYSACCHARIDE EFFECTS ON NEUROTRANSMISSION: IMPLICATIONS FOR DEPRESSION	49
Abstract	50
Introduction	51
Dopamine	55
Norepinephrine	59
Serotonin	62
Conclusion.....	65
References.....	66

CHAPTER 3 – THREE PIECES OF THE SAME PUZZLE: HOW DEPRESSION THEORIES CONVERGE AT INFLAMMATION.....	74
Abstract	75
Introduction	76
Theories of Depression	76
Monoamine Theory of Depression	77
Neuroplasticity and Neurogenesis	79
Cytokine/Inflammation Theory of Depression	85
Three Theories: One Story	89
References.....	91
CHAPTER 4 – HOW ELECTROCHEMICAL MEASUREMENTS IN THE BRAIN HAVE SHAPED OUR UNDERSTANDING OF DEPRESSION	98
Abstract	99
Introduction	100
Diagnosis and Treatment of Depression	101
Studying the Brain Requires Exquisite Technology.....	102
The Three Theories of Depression.....	103
Monoamine Hypothesis of Depression	103
Neuroplasticity Theory of Depression.....	103
Inflammation Theory of Depression.....	104
Analytical Methods for Brain Chemistry	105
Chemical Underpinnings of Depression	107
Chronoamperometry	107
Voltammetry	110
Biosensors	115
Conclusion and Future Perspectives	116
References.....	117
CHAPTER 5 - GENERAL METHODS.....	124
Solutions.....	125
Electrode Fabrication	126
Electrode Modifications	128
Data Collection and Analysis	130
Flow Injection Analysis (FIA)	131

Animal Surgery	134
References.....	138
CHAPTER 6 – NOVEL EXPERIMENTAL STRATEGIES FOR FAST VOLTAMMETRY: LEVERAGING AMBIENT IN VIVO AMINO ACIDS TO AMELIORATE FSCAV STABILITY TOWARD SEROTONIN.....	
	139
Abstract	140
Introduction	141
Methods.....	143
Results and Discussion	148
In Vitro Responses to Serotonin Decay while In Vivo Responses Grow	148
Several Brain Amino Acids Improve FSCV Response Amplitude to Serotonin	151
Amino Acids Improve Electrode Equilibration Time	155
Amino Acid Pretreatment Improves In Vivo Equilibration Time	158
Conclusions	160
References.....	161
CHAPTER 7 - FAST SCAN CONTROLLED ADSORPTION VOLTAMMETRY: A NEW METHOD FOR AMBIENT MEASUREMENTS OF HISTAMINE	
	165
Abstract	166
Introduction	167
Methods.....	170
Results and Discussion	172
Histamine FSCV and FSCAV display significant alignment	172
Investigating FSCAV Parameters	176
Lubricin Ideally Maintains Sensitivity and Equilibration Time	179
Histamine FSCAV is Selective	185
In vivo Histamine FSCAV.....	187
Conclusion.....	190
References.....	191
CHAPTER 8 – OPTIMIZING MEDIA AND ELECTRODE COATINGS FOR FAST SCAN CYCLIC VOLTAMMETRY CALIBRATIONS	
	195
Abstract	196
Introduction	197
Methods.....	200
Results and Discussion	204

A Comparison of FSCV Histamine Signals Collected In vivo vs. In vitro in Different Media.....	204
Modifying CFMs with Nafion and ECM better Models In Vivo System In Vitro	208
The Effect of Lubricin on CFM Response to Histamine and Serotonin	214
Conclusion.....	219
References.....	221
CHAPTER 9 – CONCLUSION AND FUTURE WORK	225
APPENDICES	228
Appendix A – Rights and Permissions to Reprint	229

LIST OF ABBREVIATIONS

5-HIAA	5-hydroxyindoleacetic acid
5-HTP	5-hydroxytryptophan
AA	Amino acid
AADC	amino acid decarboxylase
AP-1	Activator Protein-1
BBB	Blood brain barrier
BDNF	Brain-derived neurotrophic factor
CFM	Carbon fiber microelectrode
CNT	Carbon nanotubes
CV	Cyclic voltammogram
OCT	Organic cation transporter
DA	Dopamine
DAMP	Damage-associated molecular patterns
DAT	Dopamine transporter
DC	Dendritic cell
EC	Enterochromaffin cell
ECM	Extracellular matrix
ECT	Electroconvulsive therapy
FIA	Flow injection analysis
FMH	α -fluoromethylhistidine
FSCAV	Fast scan controlled adsorption voltammetry
FSCV	Fast scan cyclic voltammetry
H1R	Histamine H1 receptor
H2R	Histamine H2 receptor
H3R	Histamine H3 receptor
H4R	Histamine H4 receptor
H ₂ O ₂	Hydrogen peroxide
HA	Histamine
HDC	Histidine decarboxylase
HNMT	Histamine N-methyltransferase
HPLC	High-performance liquid chromatography
IBS	Irritable bowel syndrome
IL-1B	Interleukin-1B
IL-6	Interleukin-6
KO	Knockout
LPS	Lipopolysaccharide
LSD	Lysergic acid diethylamide
Lub	Lubricin
MAO	Monoamine oxidase
MAOI	Monoamine oxidase inhibitor
NE	Norepinephrine
NET	Norepinephrine transporter
NF- κ B	Nuclear Factor kappa B

NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NRI	Norepinephrine reuptake inhibitor
NSAID	Non-steroidal anti-inflammatory drug
O_2^-	Superoxide anion
OH $\cdot$	Hydroxyl radical
PAMP	Pathogen-associated molecular patterns
PBS	Phosphate buffered saline
PEDOT	poly-(3,4-ethylenedioxythiophene)
PMAT	Plasma membrane monoamine transporter
PRR	Pattern recognition receptors
ROS	Reactive oxygen species
SERT	Serotonin transporter
SSRI	Selective serotonin reuptake inhibitor
TCA	Tricyclic antidepressant
TLR-4	Toll-like receptor-4
TPH	Tryptophan hydroxylase
TNF- α	Tumor Necrosis Factor-alpha
VMAT	Vesicular monoamine transporter
VTA	Ventral tegmental area

LIST OF FIGURES

Figure 1.1 Steps of Neurotransmission.....	22
Figure 1.2 Serotonin synthesis and metabolism in the nervous system.....	25
Figure 1.3 Histamine synthesis and metabolism in the nervous system.....	27
Figure 1.4 Microdialysis schematic.....	32
Figure 1.5 FSCV Schematic.....	35
Figure 1.6 FSCAV Schematic	37
Figure 2.1 Representation of LPS induced microglial activation.....	53
Figure 2.2 Effects of microdialysis probe.....	58
Figure 2.3 Acute NE exposure can decrease release of cytokines by microglia.....	61
Figure 2.4 A detailed representation of the co-modulation of serotonin, histamine, and glia.....	64
Figure 3.1 Mechanism of ketamine action on plasticity.....	82
Figure 3.2 Evolutionary hypothesis of inflammation and depression.....	87
Figure 3.3 Peripheral LPS effects on brain serotonin.....	88
Figure 4.1 Overview of three analytic techniques used in neurotransmitter research.....	106
Figure 4.2 Chronoamperometry measurements of serotonin clearance in the CA3 region of a mouse hippocampus.....	110
Figure 4.3 Modeling of oral chronic dosing escitalopram effects on brain.....	114
Figure 5.1 CFM under light microscope.....	127
Figure 5.2 Picture of assembled CFM.....	128
Figure 5.3 Lubricin deposition onto CFMs.....	129
Figure 5.4 FIA setup.....	132
Figure 5.5 Schematic of animal experiment procedure.....	136
Figure 6.1 In vitro and in vivo representative examples of FSCAV acquisition for 120 min.....	149
Figure 6.2 Surface modifications change FSCV response to serotonin.....	153
Figure 6.3 Surface treatments of carbon fibers with polymerized amino acids.....	156
Figure 6.4 Amino acid-modified CFM equilibration times in vivo.....	159
Figure 7.1 Histamine FSCV and FSCAV show good agreement.....	175
Figure 7.2 Bar graphs of histamine FSCAV parameters.....	177
Figure 7.3 Histamine FSCAV Calibrations.....	180
Figure 7.4 Lubricin Schematic.....	182
Figure 7.5 Histamine FSCAV coatings and stability.....	183
Figure 7.6 Histamine FSCAV interferences.....	186
Figure 7.7 Extracellular histamine before and after drug administration.....	188
Figure 8.1 Flow cell apparatus setup.....	198
Figure 8.2 Histamine signals in vivo versus in vitro.....	205
Figure 8.3 Dopamine and histamine oxidation mechanisms.....	209
Figure 8.4 Histamine amperometry.....	210
Figure 8.5 ECM coating.....	213
Figure 8.6 SEM imaging of ECM coated CFM.....	216
Figure 8.7 Sensitivity and stability of Lubricin and Nafion-modified CFMs.....	218

CHAPTER 1 - INTRODUCTION

Mental Health

Mental health disorders affect millions of people worldwide. The World Health Organization estimates that around 280 million people suffer from depression, making it one of the leading causes of disability worldwide (Organization, 2023). Diagnoses and treatments for depression are flawed and inconsistent. Diagnoses are based on patient responses to questionnaires that are open to interpretation (Eack et al., 2006, Wittkamp et al., 2009). Treatments are given on a trial-and-error basis and can often take months to years to become effective, if ever.

Despite its high prevalence, the causes of depression remain unclear. Over the years, numerous theories have been proposed to explain the underlying mechanisms of depression.

The first theory proposed as to the underlying cause of depression was the Monoamine Hypothesis of Depression in the 1960s. This theory states that extracellular serotonin, norepinephrine and dopamine levels are dysregulated during depression. Initially, several studies found that serotonin levels were lower in the brain of depressed suicide patients (Shaw et al., 1967), and norepinephrine levels were lower in the cerebral spinal fluid and urine of patients with depression (Bunney and Davis, 1965, Schildkraut, 1965). During that time, it was also discovered that drugs targeting monoamines, such as monoamine oxidase inhibitors and tricyclic antidepressants, could help relieve depression (O'Connor, 1953, Lehmann et al., 1958, KUHN, 1958, Schatzberg, 1998). Eventually, antidepressants that specifically target the serotonin system were developed

(Wong et al., 1974, Wong et al., 2005, Fuller et al., 1974). These selective serotonin reuptake inhibitors (SSRIs) act by blocking serotonin transporters (SERTs) and preventing serotonin reuptake (Fuller et al., 1974, Wong et al., 1974, Wong et al., 2005). While these medications were widely popular for some time, the low success rate, adverse side effects and delayed onset (as patients have to take SSRIs for weeks to notice a response, if any) have motivated the medical community to pursue alternative theories of depression.

Another major theory of the basis of depression is the neuroplasticity theory of depression. Neuroplasticity is the process in which the brain is able to reorganize itself by forming new neural connections. The idea behind the neuroplasticity theory of depression is that depression is caused by impairments in the brain's ability to adapt and generate these new neuronal connections. In the 1990s, scientists discovered that patients with recurrent depression suffered a loss in hippocampal volume and, moreover, they discovered a link between hippocampal volume and the length of depression (Sheline et al., 2003, Sheline et al., 1999, Sheline et al., 1996). Additionally, studies found that acute stress suppressed the rate of dentate gyrus (part of the hippocampus) neurogenesis in adults of a number of species (Sapolsky, 1996). This work was reinforced when Jacobs and colleagues showed that chronic fluoxetine (an antidepressant) increased hippocampal neurogenesis in rats (70% increase in the number of cells) (Jacobs and Fornal, 1999). This theory has spurred the use of ketamine and lysergic acid diethylamide (LSD) as potential therapeutics for the treatment of depression (Nutt et al., 2023, Smith-Apeldoorn et al., 2022).

The final major theory for the catalyst of depression is the inflammation theory of depression which states that depression is caused by activation of the immune system. At the same time scientists were discovering a link between neuronal loss and depression, they were also finding a link between immune cells and depression. It was shown that patients treated with inflammatory cytokines developed depressive-like behaviors (Smith, 1991). This concept gained quick support when it was proposed that sickness behavior and depression share similar pathways through immune activation (Maes et al., 1995a, Maes et al., 1995b). Soon after, non-steroidal anti-inflammatory drugs (NSAIDs) and cytokine inhibitors emerged as potential treatment options for depression (Hang et al., 2021, Du et al., 2024).

While all three theories show promise as to revealing the causes of depression, no single theory stands alone. The challenges that accompany studying the brain indicate more work is needed to uncover the underlying cause of this illness.

Neuroinflammation

Inflammation is a term used to describe the body's immune response to a harmful or foreign stimulus.

Acute inflammation is a short-term tightly regulated process. Inflammation occurs when pattern recognition receptors (PRRs) located on the surface of immune cells recognize pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). These PRRs trigger the secretion of cytokines (small signaling proteins produced by cells that regulate immune responses by facilitating communication

between cells, modulating inflammation, and influencing various physiological processes). Cytokines include tumor necrosis factor- α (TNF- α), interleukin-1B (IL-1B), and interleukin-6 (IL-6). Chemokines are also secreted; these are a subset of cytokines that specifically direct the movement of immune cells to sites of infection, injury, or inflammation through a process known as chemotaxis and include CCL2 (Monocyte Chemoattractant Protein-1, MCP-1) and CX3CL1. Finally, transcription factors, such as Nuclear Factor kappa B (NF- κ B) and Activator Protein-1 (AP-1), are also involved in acute inflammation. Transcription factors are proteins that regulate the expression of genes involved in inflammation by binding to specific DNA sequences, thereby controlling the activation or suppression of inflammatory pathways and govern the response of immune cells (Ahmed, 2011, Turner et al., 2014, Akira et al., 2006).

Chronic inflammation is an ongoing and abnormal immune response where the immune system remains activated beyond the resolution phase of acute inflammation. This sustained activation is typically due to unresolved pathogen clearance or tissue damage, or an autoimmune response. Reactive oxygen species (ROS), such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH\cdot$), play a significant role in the onset and progression of chronic inflammation. ROS can intensify inflammatory signaling by activating several intracellular pathways, including the NF- κ B pathway, a key regulator of inflammation. As ROS accumulate, they increase the production of pro-inflammatory cytokines, such as TNF- α , IL-1 β , and IL-6, along with chemokines, which exacerbate the inflammatory response. This creates a continuous cycle where inflammation persists, leading to ongoing tissue damage and dysfunction. ROS can directly activate immune cells, such as macrophages and neutrophils, by triggering the

18

release of inflammatory mediators like TNF- α , IL-1 β , and IL-6. This activation maintains the immune response, causing immune cells to remain in the tissue and continue producing cytokines and ROS. As a result, the ongoing release of these molecules contributes to the persistence of chronic inflammation and ongoing tissue damage (Mittal et al., 2014). This ongoing immune dysfunction has been linked to several diseases, including depression (Troubat et al., 2021).

Neuroinflammation specifically refers to inflammation of the brain and spinal cord and is mediated by the brain's glial cells, particularly microglia and astrocytes.

Microglia are the resident immune cells of the central nervous system (CNS). When triggered by a foreign substance, microglia are activated and undergo microgliosis, where they release various pro-inflammatory cytokines (such as TNF- α (Hide et al., 2000), IL-1 β , and IL-6 (Olson and Miller, 2004)) and chemokines (e.g., CCL2, CXCL1) that recruit other immune cells to the site of damage (Häusler et al., 2002, Lee et al., 2002). Activated microglia also release ROS and nitric oxide (NO), which can exacerbate neuroinflammation (Simpson and Oliver, 2020).

Astrocytes are the most abundant glial cells in the brain and play a vital role in maintaining the blood-brain barrier, supporting neurons, and regulating the extracellular environment (Heithoff et al., 2021, Liddelow and Barres, 2017). In response to neuroinflammation, astrocytes become activated and undergo astrogliosis and can release pro-inflammatory cytokines, such as TNF- α , IL-1 β , and IL-6, chemokines like CCL2-5 and CXCL12, and ROS such as O₂⁻ and H₂O₂, which contribute to the recruitment

and activation of other immune cells (Lau and Yu, 2001, Ambrosini et al., 2005, Sheng et al., 2013). Additionally, astrocytes can release and reuptake glutamate, an excitatory neurotransmitter, in response to inflammation (Martinez-Hernandez et al., 1977). Excess glutamate in the extracellular space can lead to neurotoxicity by excessive stimulation of NMDA (N-methyl-D-aspartate) receptors on neurons (Choi et al., 1988).

Neuroinflammation is not only caused by direct exposure of the brain and/or spinal cord to a stimulus, but can also be induced by peripheral inflammation via vagal nerve innervation (Jin et al., 2024). Additionally, this response can be triggered by toll-like receptor 4 (TLR4) present in the central nervous system. TLR4 can be reached through the bloodstream due to their localization on microglia (which constitute the blood brain barrier (BBB)) suggesting a vital role for TLR4 in also engaging the innate cerebral immune response (Trotta et al., 2014). Depression has been increasingly linked to neuroinflammatory processes. Given that neuroinflammation can be triggered by peripheral immune activation via vagal nerve innervation, it is plausible that sustained peripheral inflammation may contribute to the development or exacerbation of depression symptoms.

There is a strong link between inflammation and depression, so investigating how inflammation affects the brain's chemicals will help provide a deeper understanding of depression's underlying pathology.

Neurotransmission

Signaling within the brain is regulated by chemical messengers termed neurotransmitters. These molecules, secreted by neurons, exert their effects on target cells across synaptic junctions. The role of chemicals as transmitters was first elucidated by Dale and Loewi in the 1920s with the discovery of acetylcholine, marking a seminal discovery in the field and winning the men the Nobel Prize in 1936 (NobelPrize.org, 2025a). From that point on, numerous other neurotransmitters have been identified and extensively studied: dopamine (Carlsson et al., 1957, Carlsson et al., 1958), serotonin (Twarog and Page, 1953), norepinephrine (NobelPrize.org, 2025b), glutamate (Krebs, 1935), and GABA (Roberts and Frankel, 1950) to name a few.

Neurotransmission typically occurs in four steps as shown in **Figure 1.1**. Briefly: 1) an action potential moves down the axon toward the axon terminal, 2) voltage-gated calcium channels open and calcium enters the axon terminals causing the release of the neurotransmitter into the synapse, 3) the neurotransmitter binds to receptors on the post-synaptic neuron and triggers a reaction, and 4) the neurotransmitter is destroyed or taken back into the pre-synaptic cell (Bear et al., 2016).

Neurotransmitters are classified into two major types, excitatory and inhibitory. Excitatory neurotransmitters, such as glutamate, work by allowing an influx of Ca^{2+} or Na^{+} into the post-synaptic cell thereby depolarizing the membrane and bringing the cell closer to an action potential. Conversely, inhibitory neurotransmitters like GABA act by

facilitating an influx of Cl^- or an efflux of K^+ which hyperpolarizes the post-synaptic cell membrane, reducing the likelihood of producing an action potential (Bear et al., 2016).

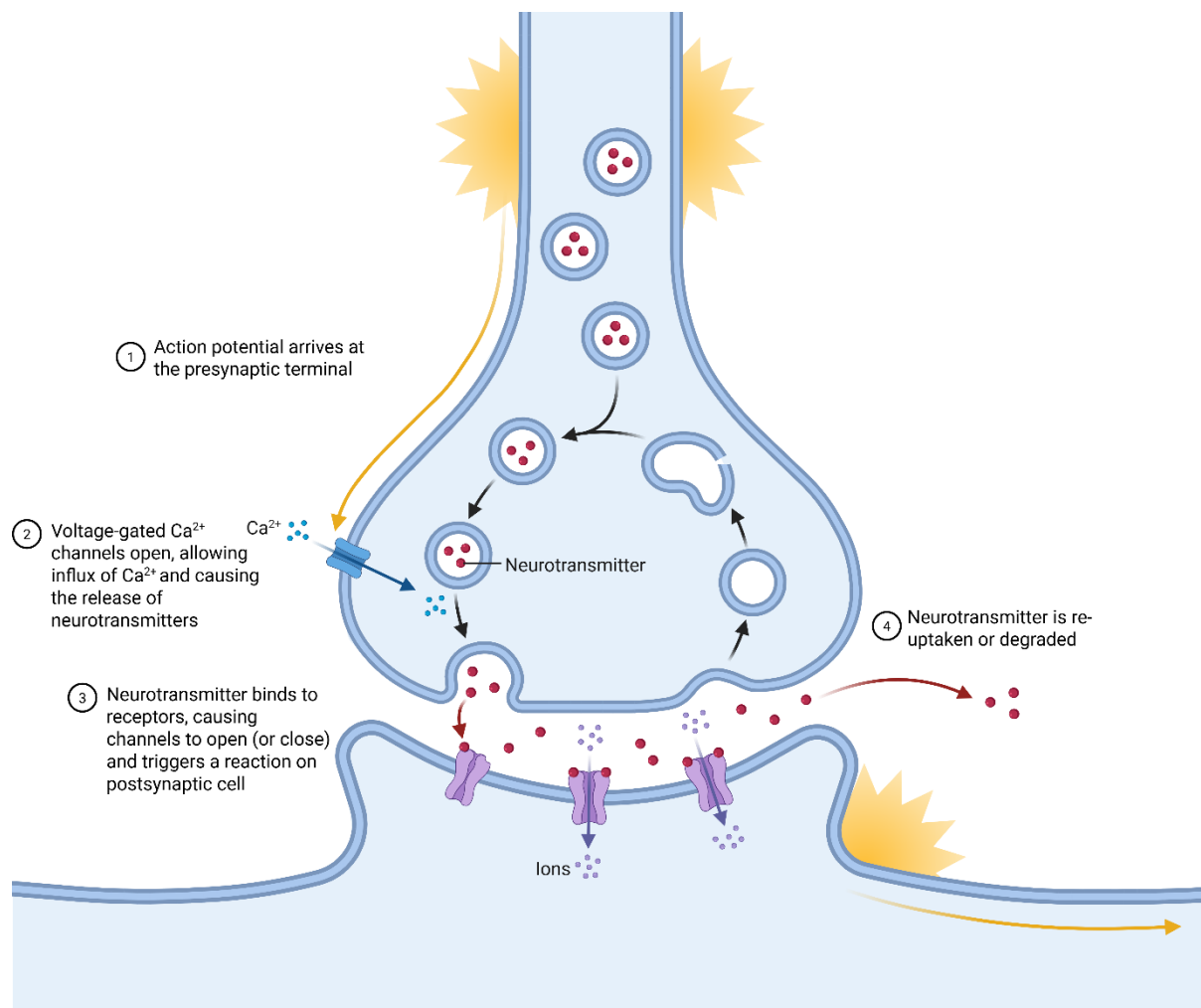


Figure 1.1 Steps of Neurotransmission. 1) Action potential moves down the axon toward the axon terminal, 2) voltage-gated calcium channels open and calcium enters the axon terminals causing the release of the neurotransmitter into the synapse, 3) the neurotransmitter binds to receptors on the postsynaptic neuron and triggers a reaction, 4) the neurotransmitter is destroyed or taken back into the presynaptic cell (Bear et al., 2016). Image created using Biorender.

Neurotransmitter receptors are a class of proteins located on the surface of neurons. These proteins bind to and respond to neurotransmitters, such as serotonin, dopamine, or histamine, when they are released into the synaptic space by presynaptic neurons.

The two main types of receptors are ionotropic and metabotropic. Ionotropic receptors, like NMDA and AMPA receptors, are ligand gated ion channels that allow for the rapid flow of ions like Ca^{2+} , Na^{+} , or K^{+} across the cell membrane leading to changes in the membrane potential (Engelman and MacDermott, 2004). Metabotropic receptors are slower than ionotropic receptors, but their function is more complex and amplified to a greater extent. Activation or inhibition of these receptors ultimately influences neuronal excitability, synaptic plasticity, and signal propagation (Tuteja, 2009).

Neurotransmitter transporters are a type of membrane transport protein that carry neurotransmitters across cell membranes and clear them from the synaptic space. There are two main categories of neurotransmitter transporters: plasma membrane transporters and vesicular transporters. Plasma membrane transporters, such as DATs, NETs, and SERTs, reuptake neurotransmitters from the synaptic space back into the presynaptic neuron. Vesicular transporters, such as VMATs, package neurotransmitters into vesicles for storage and eventually release.

The processes underlying neurotransmission and the roles of various neurotransmitters are of critical importance, as dysfunctions in neurotransmission are central to the pathophysiology of neuropsychiatric disorders, such as depression.

Serotonergic system

The human serotonergic system is a complex and dynamic network which is responsible for many important biological functions.

The serotonergic system originates in the raphe nucleus of the brainstem and projects to many areas of the brain, including the hippocampus, hypothalamus, and cortex (Moore et al., 1978). Serotonin is synthesized in serotonergic neurons from the amino acid L-tryptophan in a two-step process. Tryptophan hydroxylase converts L-tryptophan into 5-hydroxytryptophan and then aromatic L-amino acid decarboxylase converts 5-hydroxytryptophan into serotonin (Tyce, 1990). Serotonin is then packaged and stored in vesicles by VMAT and upon neuronal firing is released by either synaptic or volume transmission acting upon serotonin receptors on the postsynaptic cell (Wang et al., 2024). Serotonin is then reuptaken by a variety of protein transporters via two uptake systems, uptake-1 and uptake-2. Uptake-1 is the uptake of serotonin by 'high-affinity low-efficiency' serotonin transporters (SERTs) while uptake-2 is the uptake of serotonin by 'low-affinity high-efficiency' transporters such as the dopamine transporters (DATs), norepinephrine transporters (NETs), organic cation transporters (OCTs), and plasma membrane monoamine transporters (PMATs) (Shaskan and Snyder, 1970). Once reuptaken, serotonin is either recycled back into vesicles or broken down by monoamine oxidase (MAO) into its metabolite 5-hydroxyindoleacetic acid (5-HIAA) (Bortolato et al., 2010). **Figure 1.2** shows a schematic of this process.

Drugs that target these transporters, such as selective serotonin reuptake inhibitors (SSRIs), are important in the treatment of psychiatric disorders.

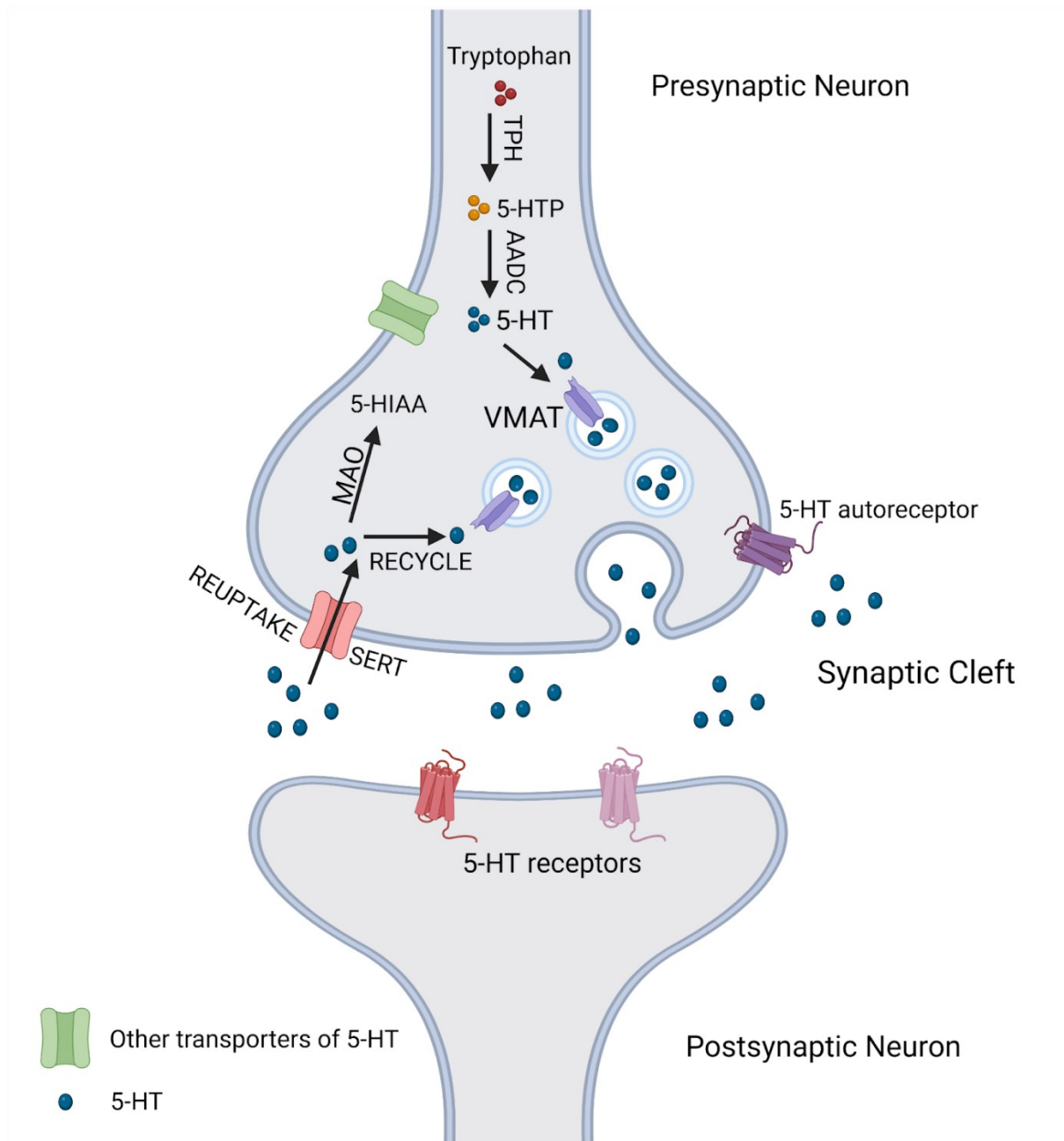


Figure 1.2 Serotonin synthesis and metabolism in the nervous system. TPH - tryptophan hydroxylase; 5-HTP - 5-hydroxytryptophan; AADC - amino acid decarboxylase; 5-HT - serotonin; VMAT - vesicular monoamine transporter; SERT - serotonin transporter; MAO - monoamine oxidase; 5-HIAA - 5-hydroxyindoleacetic acid. Image created using Biorender.

Histaminergic system

The human histaminergic system is an intricate network that plays an important role in regulating various biological functions. This system is responsible for a range of functions including sleep, arousal, appetite, and inflammation (Brown et al., 2001, Tashiro et al., 2002, Passani et al., 2004, Morimoto et al., 2001). Dysregulation of the histaminergic system has been implicated in both neurological and psychiatric disorders, including Alzheimer's and Parkinson's diseases to name a few (Panula et al., 1997, Rinne et al., 2002, Anichtchik et al., 2001).

The histaminergic system originates in the tuberomammillary nucleus of the posterior hypothalamus with an extensive network of projections to almost every brain region, including the cortex, hippocampus, hypothalamus, and brainstem. Histamine is synthesized in histaminergic neurons from the amino acid L-histidine in a process which uses histidine decarboxylase to convert histidine into histamine. Histamine is then packaged and stored in vesicles by VMAT and upon neuronal firing is released acting upon pre- and postsynaptic histamine receptors. It is then broken down by histamine N-methyltransferase (HNMT), converting histamine into tele-methylhistamine (Benarroch, 2010) as shown in **Figure 1.3**. There is no known histamine specific transporter, but it is believed to be partially reuptaken by OCTs and PMATs (Daws, 2021).

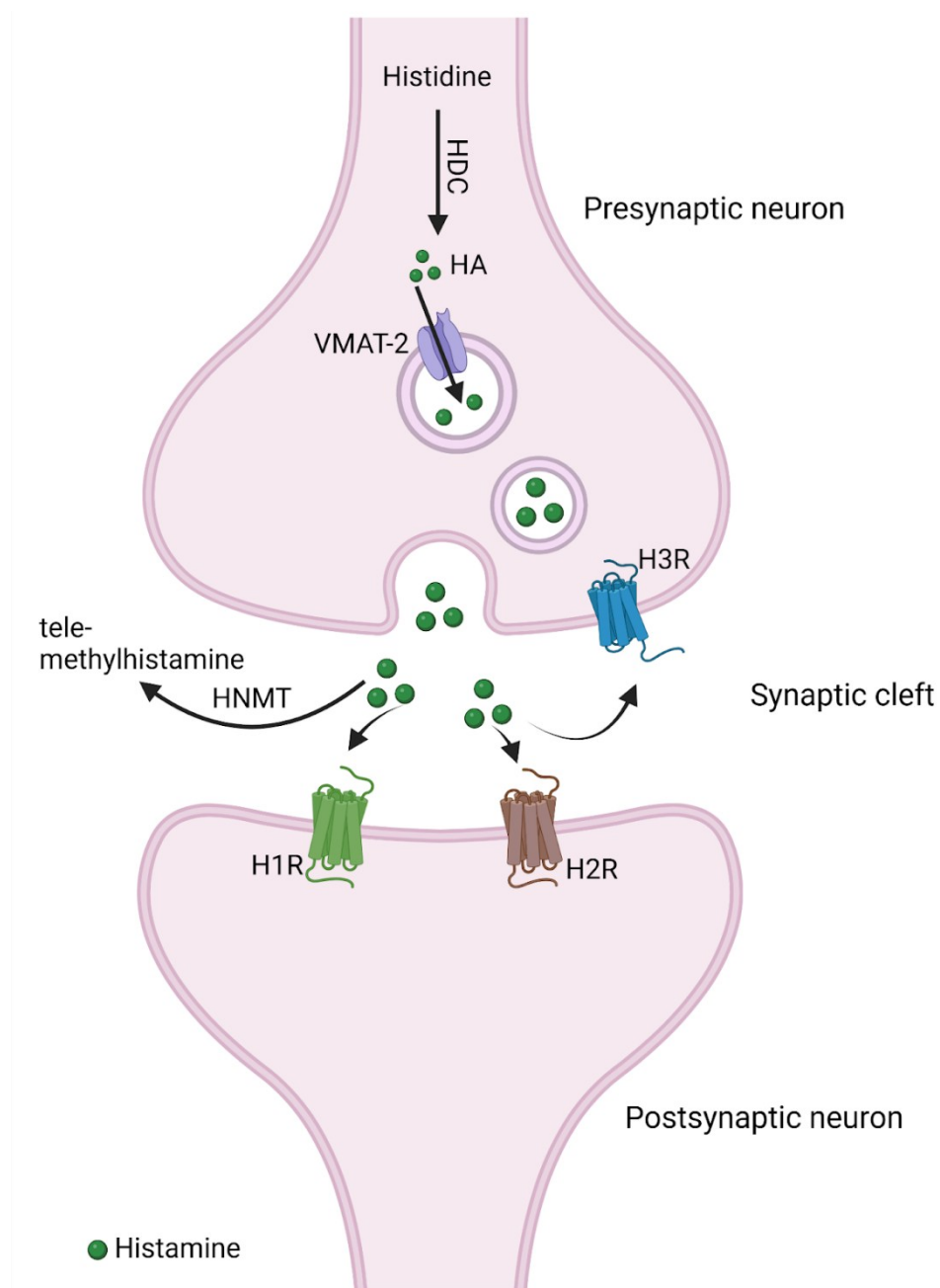


Figure 1.3 Histamine synthesis and metabolism in the nervous system. HDC - histidine decarboxylase; HA - histamine; VMAT-2 - vesicular monoamine transporter 2; HNMT - histamine-N-methyltransferase; H1R - histamine H1 receptor; H2R - histamine H2 receptor; H3R - histamine H3 receptor. Image created using Biorender.

There are four known histamine receptors within the histaminergic system: H1R, H2R, H3R, and H4R. The H1R, H2R, and H3R are expressed in the brain while the H4R is expressed in the periphery (Haas et al., 2008, Jutel et al., 2009, Strakhova et al., 2009, Schneider and Seifert, 2016, Dong et al., 2014). In addition to the brain, The H1R is also expressed in the periphery in cells such as nerves, respiratory epithelium, endothelial cells, hepatic cells, vascular smooth muscle cells, dendritic cells, and lymphocytes. Additionally in the periphery, the H2R is expressed in B cells, T cells, dendritic cells, gastric parietal cells, smooth muscle cells, and cardiac tissues (Thangam et al., 2018a). It is worth noting that the H4R is expressed on mast cells which can cross the BBB (Esposito et al., 2001, Zhuang et al., 1996). The H1R is a postsynaptic excitatory receptor. Common over-the-counter antihistamines target the H1R, such as diphenhydramine, and cause receptor inactivation and block signal propagation (Sadiq et al., 2011, Simon and Simons, 2008). Unlike the H1R, the H2R is an inhibitory postsynaptic receptor and treatments that target the H2R are typically used to reduce gastric acid secretion (Parsons and Ganellin, 2006, Thangam et al., 2018b). The H3R is an inhibitory autoreceptor located on presynaptic histamine neurons and controls the synthesis and release of histamine (Arrang et al., 1987, Torrent et al., 2005, Moreno-Delgado et al., 2006, Gomez-Ramirez et al., 2002). The H3R has also been shown to modulate other neurotransmitters such as dopamine (Varaschin et al., 2018, Vanhanen et al., 2016, Schlicker et al., 1993), serotonin (Samaranayake et al., 2016, Threlfell et al., 2004), acetylcholine (Baux et al., 1992), norepinephrine (Schlicker et al., 1999), glutamate and GABA (Schlicker et al., 1999), making the H3R a viable target for therapeutic treatment (Leurs et al., 2005, Gemkow et al., 2009, Passani and Blandina, 2011).

Serotonin and Histamine Co-Modulation

Histamine has previously been shown to have modulatory effects on serotonin. In 2004, Threlfell and Cragg et al. demonstrated that the H3R mediates serotonin release in the substantia nigra pars reticulata in rat brain slices. The researchers found that the H3R agonists imipramine and (R)-(-)- α -methyl histamine inhibited evoked serotonin release; however, these inhibitory effects were suppressed by the H3R antagonist thioperamide (Threlfell et al., 2004). Additionally, the group revealed that the H2R was implicated in serotonin modulation by revealing how the H2R inverse agonists ranitidine and tiotidine augmented serotonin release whereas release was diminished by the agonist amthamine (Threlfell et al., 2008).

Expanding upon this work, the Hashemi lab revealed that histamine release in the mouse posterior hypothalamus elicited the swift and robust suppression of extracellular serotonin levels that were dependent on concentration and that a large dose of thioperamide (50 mg kg^{-1}) induced a significant and sustained inhibition of serotonin. (Samaranayake et al., 2016). Additionally, the lab demonstrated that a high dose of diphenhydramine, an H1R inverse agonist, rapidly decreased serotonin levels implicating the H1R in serotonin modulation (Berger et al., 2022). Another notable finding was that in mice with higher levels of histamine due to stress induced inflammation, an SSRI alone was not enough to increase extracellular serotonin levels above baseline in the mouse hippocampus. Instead, only by combining the SSRI with a histamine synthesis inhibitor (α -fluoromethylhistidine (FMH)) were extracellular serotonin levels able to

reach above baseline, further implicating histamine in the modulation of serotonin (Hersey et al., 2021a).

These results suggest that histamine functions as a co-modulator of serotonin signaling in the brain and influences serotonin activity through receptor-specific mechanisms.

Neurotransmitter Measurement Techniques

The brain is a dynamic and complex network so monitoring neurotransmission poses unique challenges and requires precise and specialized approaches. The techniques that are used must meet a specific set of requirements as defined by Lama et al. termed the 'Four S's': size, selectivity, sensitivity, and speed (Lama et al., 2012).

The brain is a delicate organ that is highly susceptible to injury. The measurement technique must be minimally invasive in order to cause as little damage to the surrounding tissue as possible making a small probe size is imperative. Additionally, the brain is extremely complex with many different molecules present having similar physical properties, so the method must be able to distinguish the analyte of interest from all other compounds. Next, many neurotransmitters exist in the brain in very low concentrations (picomolar to micromolar range) so the technique must be sensitive enough to detect the analyte over a broad range of concentrations. And finally, neurotransmission occurs on a sub-second timescale so the technique must possess a high temporal resolution in order to accurately analyze the neurotransmitter in real time.

Many techniques have been developed to measure neurotransmission, including microdialysis and fast voltammetry. Both techniques offer unique advantages and limitations in meeting these requirements.

Microdialysis

Microdialysis is a technique that has traditionally been used to measure extracellular neurotransmitter concentrations in the brain. This method works by continuously sampling the interstitial fluid through a semipermeable probe implanted in the brain, as shown in **Figure 1.4**. Small molecules are able to diffuse across the membrane along a concentration gradient. The sample is then analyzed with a separation technique such as high-performance liquid chromatography (HPLC) or mass spectrometry for chemical identification and quantification.

Microdialysis is useful as it allows for the measurement of basal neurotransmitter levels and, when coupled with a separation technique, offers high chemical selectivity. However, this technique has limitations. Because sampling is on the scale of minutes, microdialysis cannot detect rapid changes in neurotransmitter dynamics. Additionally, microdialysis probes are quite large (approximately 200-500 μm in diameter). The large probe size can cause damage to the surrounding tissue and elicit an immune response which has the potential to affect the reliability of neurotransmitter measurements.

While microdialysis remains a valuable tool for measuring basal neurotransmitter levels, its limitations in temporal resolution and tissue damage have led to the development of alternative electrochemical techniques.

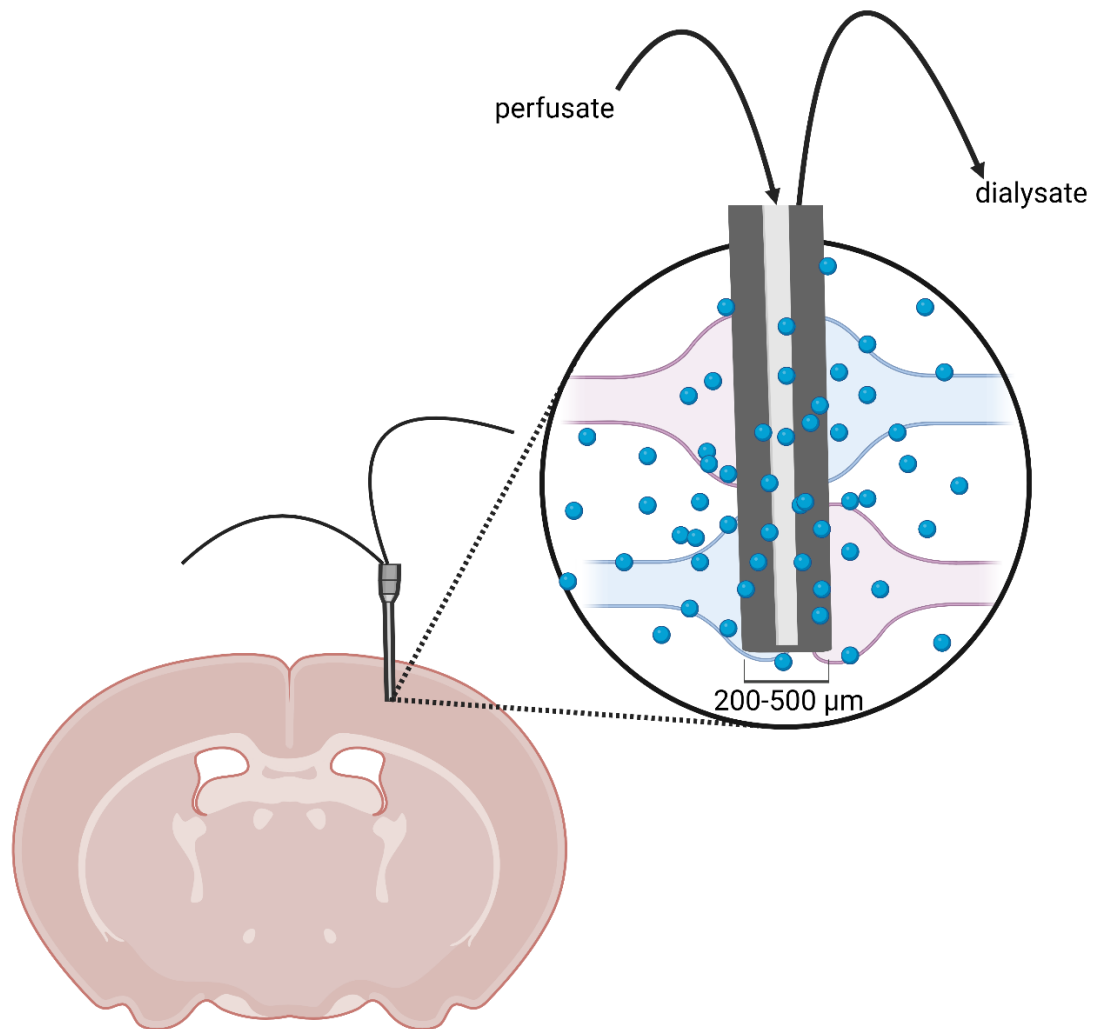


Figure 1.4. Microdialysis schematic. Microdialysis probe (between 200-500 μm in diameter) implanted in the brain continuously perfused with a solution known as the perfusate. Neurochemicals present in the surrounding extracellular space diffuse across the semi-permeable membrane into the probe and are carried out as the dialysate. The dialysate is then collected, and its constituents are separated using high-performance liquid chromatography (HPLC) for subsequent qualitative and quantitative analysis via analytical techniques such as UV/Vis spectroscopy, electrochemistry, or mass spectrometry. Image created using Biorender.

Fast scan cyclic voltammetry

In the 1980s, Millar, Wightman and colleagues developed a technique they coined fast scan cyclic voltammetry (FSCV) to study dopamine neurotransmission in real time (Baur 32

et al., 1988, Millar et al., 1985). Similar to cyclic voltammetry, FSCV is an electrochemical technique that applies a sweeping voltage to an electrode to induce the oxidation and reduction of a molecule at particular potentials depending on the molecule's electrochemistry. This sweeping voltage is known as a waveform and can be adapted and optimized to be selective to different analytes. While cyclic voltammetry typically utilizes scan rates of less than 1 Vs^{-1} and, therefore, does not have a high enough temporal resolution to fully capture sub-second neurotransmission, FSCV uses scan rates of 400 Vs^{-1} or higher enabling accurate real time detection of neurotransmission. However, the disadvantage of these high scan rates is that they generate a large non-faradaic capacitive current that can conceal the faradaic oxidation current generated by the analyte. These capacitive currents (or background currents) must be subtracted, but since they remain relatively stable over time, this background subtraction can be performed without compromising the signal. This background subtraction, however, means that FSCV can only detect changes, or evoked response, to the analyte concentration and not the ambient concentration. In the brain, these changes are typically stimulated by electrical, optical, or pharmacological methods (Venton and Cao, 2020).

FSCV utilizes carbon fiber microelectrodes (CFMs) which possess many unique advantages. CFMs are biocompatible, adsorb cations, have favorable surface kinetics, are minimally invasive ($<7 \text{ }\mu\text{m}$ in diameter), are stable over time, and are low-cost (Wightman, 2006). Additionally, the carbon surface striations allow for enhanced sensitivity (Heien et al., 2003).

Since its advent in the 1980s, FSCV has expanded beyond dopamine and is now used to study serotonin (Hashemi et al., 2009, Jackson et al., 1995), histamine (Samaranayake et al., 2016, Samaranayake, 2015, Berger et al., 2022, Hersey et al., 2022, Hersey et al., 2021b), and norepinephrine (Park et al., 2015, Park et al., 2013, Park et al., 2011), to name a few.

Data collected using FSCV is represented as a 2D color plot where current is represented in false color with green typically being indicative of oxidation and blue of reduction. **Figure 1.5** provides an example of a typical data set obtained for serotonin in the CA2 region of the hippocampus of a mouse. A serotonin specific waveform, known as the ‘Jackson’ waveform (Jackson et al., 1995), is applied to the electrode. Upon electrical stimulation, serotonin is released and undergoes a two-electron transfer generating the color plot. Serotonin oxidation is the green event around 0.7 V and serotonin reduction is the blue event around 0 V. The vertical white line indicated by the green star is used to generate the CV in the bottom right, with oxidation and reduction peak locations allowing for identification of the analyte. The horizontal white line indicated by the purple star generates a current vs. time trace shown in the bottom left, which allows for monitoring the release and reuptake of serotonin.

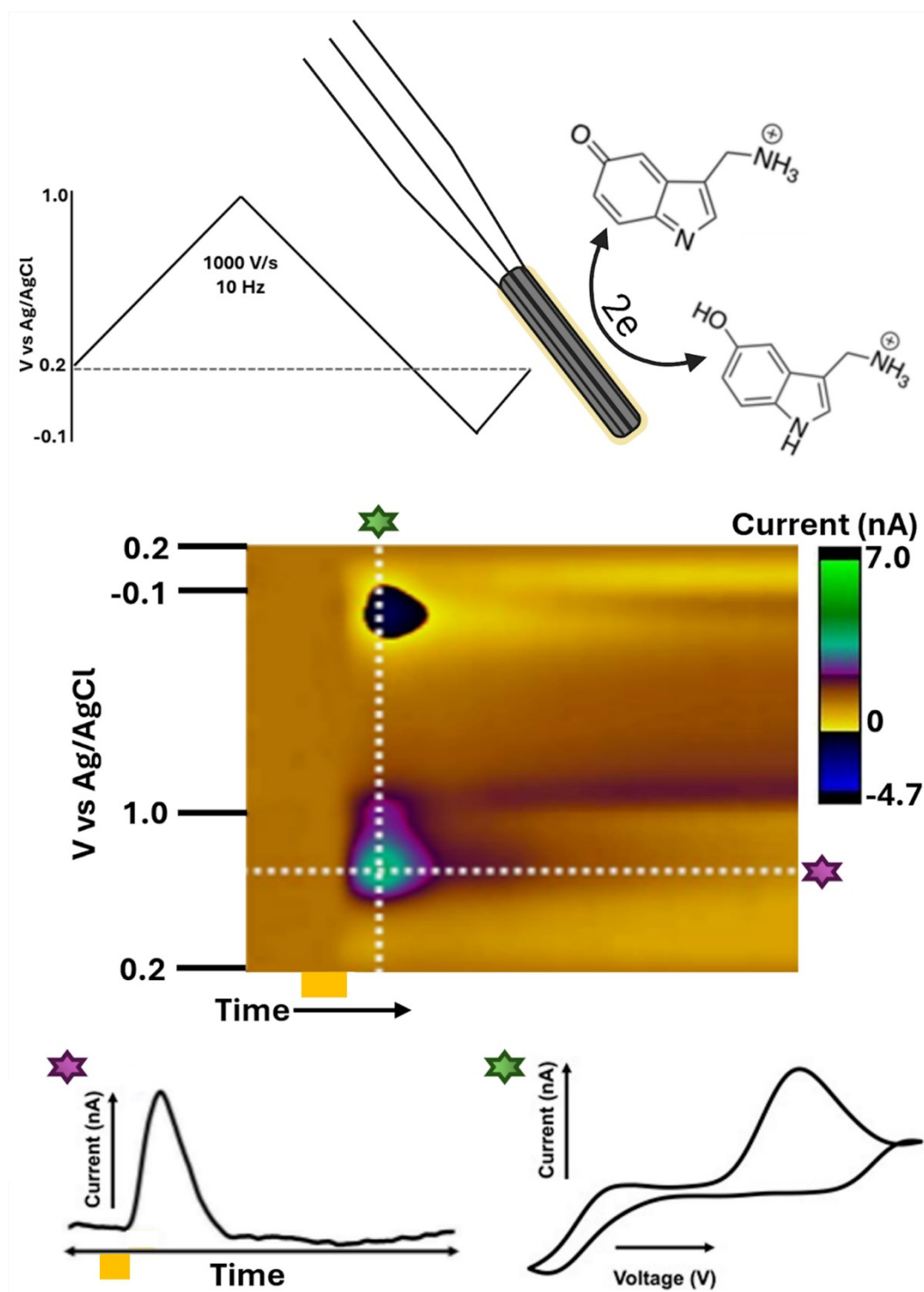


Figure 1.5. FSCV schematic. A CFM applies a serotonin specific waveform and measures the oxidation and reduction of serotonin molecules, depicting information in a color plot. The representative CV and concentration time traces are denoted by the green and purple stars, respectively. Orange boxes represent time of electrical stimulation.

Fast scan controlled adsorption voltammetry

As previously stated, the disadvantage of FSCV is that it is a background subtracted technique and therefore cannot measure ambient levels of neurotransmitters. To address this, in 2013 Heien and colleagues developed fast scan controlled adsorption voltammetry (FSCAV) to measure ambient dopamine concentrations (Atcherley et al., 2013, Atcherley et al., 2015) and was later adapted for serotonin in 2017 (Abdalla et al., 2017). FSCAV is a three step process as shown in **Figure 1.6**. In the first step, the analyte specific waveform is applied at a very high frequency (100 Hz as compared to 10 Hz for FSCV) which forces the analyte to desorb from the electrode producing a 'clean' electrode surface. Second, the electrode is held at a constant potential to allow the analyte to adsorb onto the electrode surface until it reaches equilibrium with the surrounding environment. Finally, the waveform is reapplied, and the charge of the oxidation peak of the first CV is used to determine the analyte concentration. The electrode is then postcalibrated to convert charge into concentration. FSCAV achieves a temporal resolution of 60 s, which is significantly faster than the minutes-long resolution of microdialysis. Moreover, as FSCAV is done on a CFM (diameter = 7 μm), it greatly reduces the damage to the tissue surrounding the probe compared to microdialysis (diameter = 200-500 μm) (Nesbitt et al., 2013). In addition to its improved temporal resolution over other techniques, FSCAV also offers the advantage of being compatible with FSCV on the same electrode, enabling multimodal neurochemical analysis.

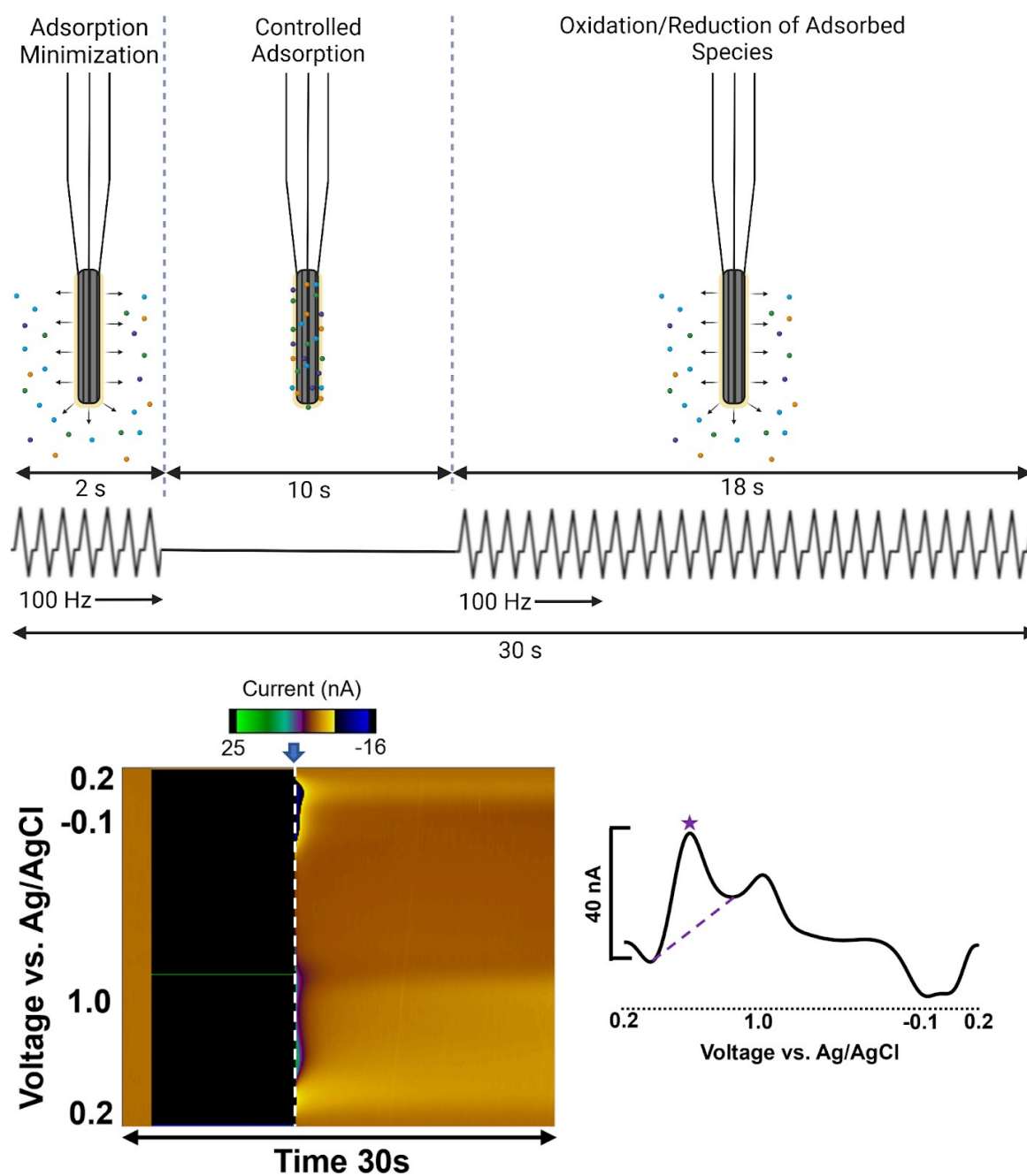


Figure 1.6. FSCAV schematic. The steps of FSCAV are shown from left to right. 1. A 2 s adsorption minimization period where the waveform is applied at a high frequency to repel molecules from the electrode surface; 2. A controlled adsorption period where the electrode is held at a constant potential to allow the analyte to adsorb to the electrode surface; and 3. Oxidation and reduction of the adsorbed species when the waveform is reapplied. An example color plot, CV, and integrated oxidation peak are shown.

Electrode Modifications

Modifications to the carbon surface of CFMs have greatly improved analyte sensitivity and selectivity during FSCV measurements. One commonly used modification involves applying a polymer to the carbon surface. Nafion, a cation exchange polymer containing sulfonated and fluorinated functional groups, is frequently used for biochemical analyses, especially for measurements of serotonin with FSCV. When polymerized onto an electrode surface, Nafion possesses an overall negative charge. This negative charge attracts cations (such as serotonin) and repels anions. Additionally, the structure contains pores or channels which allow smaller molecules, such as neurotransmitters, to pass through while restricting the passage of larger molecules (i.e. proteins) thereby helping to prevent fouling of the electrode surface. This polymer has been shown to repel interferences present in the brain, such as ascorbic acid and the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), while also increasing the sensitivity toward serotonin and limiting the biofouling of the carbon surface (Gerhardt et al., 1984, Singh et al., 2011, Hashemi et al., 2009). Another polymer that has been shown to increase analyte sensitivity with FSCV is poly-(3,4-ethylenedioxythiophene) (PEDOT), which has been used for enhanced dopamine detection (Rafi and Zestos, 2021, Vreeland et al., 2015). Additionally, amino acids provide beneficial advantages as many can be electropolymerized and are already present in the brain matrix. One such amino acid is glutamic acid, which has been shown to polymerize to poly-glutamic acid onto the carbon surface and increase sensitivity to serotonin in vivo (Holmes et al., 2021). In addition to polymers, application of carbon nanotubes (CNTs) have been used to improve the detection of neurotransmitters such as serotonin, dopamine, and

adenosine (Ross and Venton, 2012, Swamy and Venton, 2007) (Schmidt et al., 2013, Hočevár et al., 2005)). CNTs have unique physical properties that facilitate electron transfer reactions, as well as an abundance of reactive functional groups. Their high porosity and large specific surface area, combined with excellent adsorption characteristics make them ideal electrochemical sensors and biosensors. Moreover, CNTs have been demonstrated to effectively reduce electrode surface fouling, further improving sensor performance (Hočevár et al., 2005). Other additional modifications have been used, such as overoxidation of the carbon surface by applying a potential over 1 V, which increases surface oxides and sensitivity toward dopamine (Heien et al., 2003).

Scope of Dissertation

This dissertation aims to use novel approaches to improve FSCV and FSCAV measurements. The overarching research questions that I set out to answer was can we develop a robust research capacity to measure histamine in depressive disorder? There are currently no methods that can measure basal histamine dynamics in real time. This thesis explores the relationship of histamine in depressive disorder and proposes novel means to improve the performance of electrodes. The culmination of the work is a novel method to measure basal histamine in vivo.

Chapter 2: This published article reviews studies that find chemical changes in the brain after administration of the endotoxin LPS, which is an effective method to trigger rapid inflammation. From these studies, we interpret how LPS influences dopamine, serotonin, and norepinephrine levels in various regions in the brain.

Chapter 3: In this chapter, we present the history and development of three theories of depression and discuss the treatments associated with each hypothesis. We conclude

that these theories are interdependent and are each a small part of a bigger picture and suggest that future therapies should take into account all three hypotheses.

Chapter 4: In this chapter, we present critical analytical methods that have been used in depression studies.

Chapter 5: This chapter describes general methods used during this research.

Chapter 6: In this chapter, we describe a novel and robust electrode surface treatment utilizing amino acids to improve ambient serotonin detection with FSCAV.

Chapter 7: This chapter describes the development of FSCAV for the detection of histamine. We apply this method in vivo and observe how anesthetics and an H3 receptor antagonist influence extracellular histamine levels.

Chapter 8: In this chapter, we describe how bespoke electrode coatings and buffers are the best approach to replicate in vivo conditions in vitro.

Chapter 9: This chapter summarizes the work and proposes future directions.

References

- ABDALLA, A., ATCHERLEY, C. W., PATHIRATHNA, P., SAMARANAYAKE, S., QIANG, B., PEÑA, E., MORGAN, S. L., HEIEN, M. L. & HASHEMI, P. 2017. In Vivo Ambient Serotonin Measurements at Carbon-Fiber Microelectrodes. *Analytical Chemistry*, 89, 9703-9711.
- AHMED, A. U. 2011. An overview of inflammation: mechanism and consequences. *Frontiers in Biology*, 6, 274-281.
- AKIRA, S., UEMATSU, S. & TAKEUCHI, O. 2006. Pathogen Recognition and Innate Immunity. *Cell*, 124, 783-801.
- AMBROSINI, E., REMOLI, M. E., GIACOMINI, E., ROSICARELLI, B., SERAFINI, B., LANDE, R., ALOISI, F. & COCCIA, E. M. 2005. Astrocytes produce dendritic cell-attracting chemokines in vitro and in multiple sclerosis lesions. *Journal of Neuropathology & Experimental Neurology*, 64, 706-715.
- ANICHTCHIK, O. V., PEITSARO, N., RINNE, J. O., KALIMO, H. & PANULA, P. 2001. Distribution and modulation of histamine H3 receptors in basal ganglia and frontal cortex of healthy controls and patients with Parkinson's disease. *Neurobiology of disease*, 8, 707-716.
- ARRANG, J. M., GARBARG, M., LANCELO, J. C., LECOMTE, J. M., POLLARD, H., ROBBA, M., SCHUNACK, W. & SCHWARTZ, J. C. 1987. Highly potent and selective ligands for histamine H3-receptors. *Nature*, 327, 117-123.
- 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, 14885-14892.
- 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, 2235-2238.
- BAUR, J. E., KRISTENSEN, E. W., MAY, L. J., WIEDEMANN, D. J. & WIGHTMAN, R. M. 1988. Fast-scan voltammetry of biogenic amines. *Analytical Chemistry*, 60, 1268-1272.
- BAUX, G., FOSSIER, P., TRUDEAU, L. & TAUC, L. 1992. Presynaptic receptors for FMRFamide, histamine and buccalin regulate acetylcholine release at a neuro-neuronal synapse of Aplysia by modulating N-type Ca²⁺ channels. *Journal of Physiology-Paris*, 86, 3-13.
- BEAR, M. F., CONNORS, B. W. & PARADISO, M. A. 2016. *Neuroscience : exploring the brain*, Philadelphia, Wolters Kluwer.
- BENARROCH, E. E. 2010. Histamine in the CNS: multiple functions and potential neurologic implications. *Neurology*, 75, 1472-1479.
- BERGER, S. N., BAUMBERGER, B., SAMARANAYAKE, S., HERSEY, M., MENA, S., BAIN, I., DUNCAN, W., REED, M. C., NIJHOUT, H. F. & BEST, J. 2022. An In Vivo Definition of Brain Histamine Dynamics Reveals Critical Neuromodulatory Roles for This Elusive Messenger. *International journal of molecular sciences*, 23, 14862.
- BORTOLATO, M., CHEN, K. & SHIH, J. C. 2010. CHAPTER 2.4 - The Degradation of Serotonin: Role of MAO. In: MÜLLER, C. P. & JACOBS, B. L. (eds.) *Handbook of Behavioral Neuroscience*. Elsevier.
- BROWN, R. E., STEVENS, D. R. & HAAS, H. L. 2001. The physiology of brain histamine. *Prog Neurobiol*, 63, 637-72.

- BUNNEY, W. E. & DAVIS, J. M. 1965. Norepinephrine in depressive reactions: A review. *Archives of General Psychiatry*, 13, 483-494.
- CARLSSON, A., LINDQVIST, M. & MAGNUSSON, T. 1957. 3, 4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature*, 180, 1200-1200.
- CARLSSON, A., LINDQVIST, M., MAGNUSSON, T. & WALDECK, B. 1958. On the presence of 3-hydroxytyramine in brain. *Science*, 127, 471-471.
- CHOI, D., KOH, J. & PETERS, S. 1988. Pharmacology of glutamate neurotoxicity in cortical cell culture: attenuation by NMDA antagonists. *The Journal of Neuroscience*, 8, 185-196.
- DAWS, L. C. 2021. *Organic cation transporters in the central nervous system*, Springer.
- DONG, H., ZHANG, W., ZENG, X., HU, G., ZHANG, H., HE, S. & ZHANG, S. 2014. Histamine induces upregulated expression of histamine receptors and increases release of inflammatory mediators from microglia. *Molecular neurobiology*, 49, 1487-1500.
- DU, Y., DOU, Y., WANG, M., WANG, Y., YAN, Y., FAN, H., FAN, N., YANG, X. & MA, X. 2024. Efficacy and acceptability of anti-inflammatory agents in major depressive disorder: a systematic review and meta-analysis. *Frontiers in Psychiatry*, 15, 1407529.
- EACK, S. M., GREENO, C. G. & LEE, B.-J. 2006. Limitations of the Patient Health Questionnaire in identifying anxiety and depression in community mental health: many cases are undetected. *Research on social work practice*, 16, 625-631.
- ENGELMAN, H. S. & MACDERMOTT, A. B. 2004. Presynaptic ionotropic receptors and control of transmitter release. *Nature Reviews Neuroscience*, 5, 135-145.
- ESPOSITO, P., GHEORGHE, D., KANDERE, K., PANG, X., CONNOLLY, R., JACOBSON, S. & THEOHARIDES, T. C. 2001. Acute stress increases permeability of the blood-brain-barrier through activation of brain mast cells. *Brain research*, 888, 117-127.
- 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, 1161-1171.
- GEMKOW, M. J., DAVENPORT, A. J., HARICH, S., ELLENBROEK, B. A., CESURA, A. & HALLETT, D. 2009. The histamine H3 receptor as a therapeutic drug target for CNS disorders. *Drug discovery today*, 14, 509-515.
- 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, 390-395.
- GOMEZ-RAMIREZ, J., ORTIZ, J. & BLANCO, I. 2002. Presynaptic H3 autoreceptors modulate histamine synthesis through cAMP pathway. *Molecular pharmacology*, 61, 239-245.
- HAAS, H. L., SERGEEVA, O. A. & SELBACH, O. 2008. Histamine in the nervous system. *Physiological reviews*.
- HANG, X., ZHANG, Y., LI, J., LI, Z., ZHANG, Y., YE, X., TANG, Q. & SUN, W. 2021. Comparative efficacy and acceptability of anti-inflammatory agents on major depressive disorder: a network meta-analysis. *Frontiers in Pharmacology*, 12, 691200.
- HASHEMI, P., DANKOSKI, E. C., PETROVIC, J., KEITHLEY, R. B. & WIGHTMAN, R. 2009. Voltammetric detection of 5-hydroxytryptamine release in the rat brain. *Analytical chemistry*, 81, 9462-9471.

- HÄUSLER, K. G., PRINZ, M., NOLTE, C., WEBER, J. R., SCHUMANN, R. R., KETTENMANN, H. & HANISCH, U. K. 2002. Interferon- γ differentially modulates the release of cytokines and chemokines in lipopolysaccharide-and pneumococcal cell wall-stimulated mouse microglia and macrophages. *European Journal of Neuroscience*, 16, 2113-2122.
- 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 increases sensitivity. *Analyst*, 128, 1413-1419.
- HEITHOFF, B. P., GEORGE, K. K., PHARES, A. N., ZUIDHOEK, I. A., MUNOZ-BALLESTER, C. & ROBEL, S. 2021. Astrocytes are necessary for blood–brain barrier maintenance in the adult mouse brain. *Glia*, 69, 436-472.
- HERSEY, M., RENEAU, M., BERGER, S. N., MENA, S., BUCHANAN, A. M., OU, Y., TAVAKOLI, N., REAGAN, L. P., CLOPATH, C. & HASHEMI, P. 2022. A tale of two transmitters: serotonin and histamine as in vivo biomarkers of chronic stress in mice. *Journal of Neuroinflammation*, 19, 1-20.
- HERSEY, M., SAMARANAYAKE, S., BERGER, S. N., TAVAKOLI, N., MENA, S., NIJHOUT, H. F., REED, M. C., BEST, J., BLAKELY, R. D. & REAGAN, L. P. 2021a. Inflammation-induced histamine impairs the capacity of escitalopram to increase hippocampal extracellular serotonin. *Journal of Neuroscience*, 41, 6564-6577.
- HERSEY, M., WOODRUFF, J. L., MAXWELL, N., SADEK, A. T., BYKALO, M. K., BAIN, I., GRILLO, C. A., PIROLI, G. G., HASHEMI, P. & REAGAN, L. P. 2021b. High-fat diet induces neuroinflammation and reduces the serotonergic response to escitalopram in the hippocampus of obese rats. *Brain, behavior, and immunity*, 96, 63-72.
- HIDE, I., TANAKA, M., INOUE, A., NAKAJIMA, K., KOHSAKA, S., INOUE, K. & NAKATA, Y. 2000. Extracellular ATP triggers tumor necrosis factor- α release from rat microglia. *Journal of neurochemistry*, 75, 965-972.
- HOČEVAR, S. B., WANG, J., DEO, R. P., MUSAMEH, M. & OGOREVC, B. 2005. Carbon Nanotube Modified Microelectrode for Enhanced Voltammetric Detection of Dopamine in the Presence of Ascorbate. *Electroanalysis*, 17, 417-422.
- HOLMES, J., WITT, C. E., KEEN, D., BUCHANAN, A. M., BATEY, L., HERSEY, M. & HASHEMI, P. 2021. Glutamate Electropolymerization on Carbon Increases Analytical Sensitivity to Dopamine and Serotonin: An Auspicious In Vivo Phenomenon in Mice? *Analytical Chemistry*, 93, 10762-10771.
- JACKSON, B. P., DIETZ, S. M. & WIGHTMAN, R. M. 1995. Fast-scan cyclic voltammetry of 5-hydroxytryptamine. *Analytical chemistry*, 67, 1115-1120.
- JACOBS, B. & FORNAL, C. Chronic fluoxetine treatment increases hippocampal neurogenesis in rats: a novel theory of depression. *Soc Neurosci Abstr*, 1999, 714.
- JIN, H., LI, M., JEONG, E., CASTRO-MARTINEZ, F. & ZUKER, C. S. 2024. A body–brain circuit that regulates body inflammatory responses. *Nature*, 1-3.
- JUTEL, M., AKDIS, M. & AKDIS, C. 2009. Histamine, histamine receptors and their role in immune pathology. *Clinical & Experimental Allergy*, 39, 1786-1800.
- KREBS, H. A. 1935. Metabolism of amino-acids: The synthesis of glutamine from glutamic acid and ammonia, and the enzymic hydrolysis of glutamine in animal tissues. *Biochemical Journal*, 29, 1951.
- KUHN, R. 1958. THE TREATMENT OF DEPRESSIVE STATES WITH G 22355 (IMIPRAMINE HYDROCHLORIDE). *American Journal of Psychiatry*, 115, 459-464.

- LAMA, R. D., CHARLSON, K., ANANTHARAM, A. & HASHEMI, P. 2012. Ultrafast Detection and Quantification of Brain Signaling Molecules with Carbon Fiber Microelectrodes. *Analytical Chemistry*, 84, 8096-8101.
- LAU, L. T. & YU, A. C.-H. 2001. Astrocytes produce and release interleukin-1, interleukin-6, tumor necrosis factor alpha and interferon-gamma following traumatic and metabolic injury. *Journal of neurotrauma*, 18, 351-359.
- LEE, Y. B., NAGAI, A. & KIM, S. U. 2002. Cytokines, chemokines, and cytokine receptors in human microglia. *Journal of neuroscience research*, 69, 94-103.
- LEHMANN, H. E., CAHN, C. H. & DE VERTEUIL, R. L. 1958. The Treatment of Depressive Conditions with Imipramine (G 22355). *Canadian Psychiatric Association Journal*, 3, 155-164.
- LEURS, R., BAKKER, R. A., TIMMERMAN, H. & DE ESCH, I. J. 2005. The histamine H3 receptor: from gene cloning to H3 receptor drugs. *Nature reviews Drug discovery*, 4, 107-120.
- LIDDELOW, S. A. & BARRES, B. A. 2017. Reactive Astrocytes: Production, Function, and Therapeutic Potential. *Immunity*, 46, 957-967.
- MAES, M., MELTZER, H. Y., BOSMANS, E., BERGMANS, R., VANDOOOLAEGHE, E., RANJAN, R. & DESNYDER, R. 1995a. Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. *Journal of affective disorders*, 34, 301-309.
- MAES, M., VANDOOOLAEGHE, E., RANJAN, R., BOSMANS, E., BERGMANS, R. & DESNYDER, R. 1995b. Increased serum interleukin-1-receptor-antagonist concentrations in major depression. *Journal of Affective Disorders*, 36, 29-36.
- MARTINEZ-HERNANDEZ, A., BELL, K. P. & NOREMBERG, M. D. 1977. Glutamine synthetase: glial localization in brain. *Science*, 195, 1356-1358.
- MILLAR, J., STAMFORD, J. A., KRUK, Z. L. & WIGHTMAN, R. M. 1985. Electrochemical, pharmacological and electrophysiological evidence of rapid dopamine release and removal in the rat caudate nucleus following electrical stimulation of the median forebrain bundle. *European Journal of Pharmacology*, 109, 341-348.
- MITTAL, M., SIDDIQUI, M. R., TRAN, K., REDDY, S. P. & MALIK, A. B. 2014. Reactive oxygen species in inflammation and tissue injury. *Antioxidants & redox signaling*, 20, 1126-1167.
- MOORE, R. Y., HALARIS, A. E. & JONES, B. E. 1978. Serotonin neurons of the midbrain raphe: ascending projections. *Journal of Comparative Neurology*, 180, 417-438.
- MORENO-DELGADO, D., TORRENT, A., GÓMEZ-RAMÍREZ, J., DE ESCH, I., BLANCO, I. & ORTIZ, J. 2006. Constitutive activity of H3 autoreceptors modulates histamine synthesis in rat brain through the cAMP/PKA pathway. *Neuropharmacology*, 51, 517-523.
- MORIMOTO, T., YAMAMOTO, Y. & YAMATODANI, A. 2001. Brain histamine and feeding behavior. *Behavioural brain research*, 124, 145-150.
- NESBITT, K. M., JAQUINS-GERSTL, A., SKODA, E. M., WIPF, P. & MICHAEL, A. C. 2013. Pharmacological Mitigation of Tissue Damage during Brain Microdialysis. *Analytical Chemistry*, 85, 8173-8179.
- NOBELPRIZE.ORG. 2025a. *The Nobel Prize in Physiology or Medicine 1936* [Online]. Nobel Prize Outreach AB. Available: <https://www.nobelprize.org/prizes/medicine/1936/summary/> [Accessed 21 Jan 2025].

- NOBELPRIZE.ORG. 2025b. *Ulf von Euler* [Online]. Nobel Prize Outreach AB. Available: <https://www.nobelprize.org/prizes/medicine/1970/euler/facts/> [Accessed 19 Feb 2025].
- NUTT, D., SPRIGGS, M. & ERRITZOE, D. 2023. Psychedelics therapeutics: What we know, what we think, and what we need to research. *Neuropharmacology*, 223, 109257.
- O'CONNOR, J. B. 1953. Side effects accompanying use of iproniazid. *American Review of Tuberculosis*, 68, 270-272.
- OLSON, J. K. & MILLER, S. D. 2004. Microglia Initiate Central Nervous System Innate and Adaptive Immune Responses through Multiple TLRs¹. *The Journal of Immunology*, 173, 3916-3924.
- ORGANIZATION, W. H. 2023. *Depressive disorder (Depression)* [Online]. Available: <https://www.who.int/news-room/fact-sheets/detail/depression> [Accessed 12 Jan 2025].
- PANULA, P., RINNE, J., KUOKKANEN, K., ERIKSSON, K., SALLMEN, T., KALIMO, H. & RELJA, M. 1997. Neuronal histamine deficit in Alzheimer's disease. *Neuroscience*, 82, 993-997.
- PARK, J., BUCHER, E. S., BUDYGIN, E. A. & WIGHTMAN, R. M. 2015. Norepinephrine and dopamine transmission in 2 limbic regions differentially respond to acute noxious stimulation. *Pain*, 156, 318-327.
- PARK, J., BUCHER, E. S., FONTILLAS, K., OWESSON-WHITE, C., ARIANSEN, J. L., CARELLI, R. M. & WIGHTMAN, R. M. 2013. Opposing Catecholamine Changes in the Bed Nucleus of the Stria Terminalis During Intracranial Self-Stimulation and Its Extinction. *Biological Psychiatry*, 74, 69-76.
- PARK, J., TAKMAKOV, P. & WIGHTMAN, R. M. 2011. In vivo comparison of norepinephrine and dopamine release in rat brain by simultaneous measurements with fast-scan cyclic voltammetry. *Journal of neurochemistry*, 119, 932-944.
- PARSONS, M. E. & GANELLIN, C. R. 2006. Histamine and its receptors. *British journal of pharmacology*, 147, S127-S135.
- PASSANI, M. B. & BLANDINA, P. 2011. Histamine receptors in the CNS as targets for therapeutic intervention. *Trends in pharmacological sciences*, 32, 242-249.
- PASSANI, M. B., LIN, J.-S., HANCOCK, A., CROCHET, S. & BLANDINA, P. 2004. The histamine H₃ receptor as a novel therapeutic target for cognitive and sleep disorders. *Trends in pharmacological sciences*, 25, 618-625.
- RAFI, H. & ZESTOS, A. G. 2021. Review—Recent Advances in FSCV Detection of Neurochemicals via Waveform and Carbon Microelectrode Modification. *Journal of The Electrochemical Society*, 168, 057520.
- RINNE, J., ANICHTCHIK, O., ERIKSSON, K., KASLIN, J., TUOMISTO, L., KALIMO, H., RÖYTTÄ, M. & PANULA, P. 2002. Increased brain histamine levels in Parkinson's disease but not in multiple system atrophy. *Journal of neurochemistry*, 81, 954-960.
- ROBERTS, E. & FRANKEL, S. 1950. γ -Aminobutyric acid in brain: its formation from glutamic acid.
- ROSS, A. E. & VENTON, B. J. 2012. Nafion–CNT coated carbon-fiber microelectrodes for enhanced detection of adenosine. *Analyst*, 137, 3045-3051.
- SADIQ, M. W., BORGS, A., OKURA, T., SHIMOMURA, K., KATO, S., DEGUCHI, Y., JANSSON, B., BJÖRKMAN, S., TERASAKI, T. & HAMMARLUND-UDENAES, M. 2011. Diphenhydramine active uptake at the blood–brain barrier and its interaction with

- oxycodone in vitro and in vivo. *Journal of pharmaceutical sciences*, 100, 3912-3923.
- SAMARANAYAKE, S., ABDALLA, A., ROBKE, R., NIJHOUT, H. F., REED, M. C., BEST, J. & HASHEMI, P. 2016. A voltammetric and mathematical analysis of histaminergic modulation of serotonin in the mouse hypothalamus. *Journal of neurochemistry*, 138, 374-383.
- SAMARANAYAKE, S. A., A.; ROBKE, R.; WOOD, K. M.; ZEQA, A.; HASHEMI, P. 2015. In Vivo Histamine Voltammetry in the Mouse Premammillary Nucleus. *Analyst*, 140, 3759-3765.
- SAPOLSKY, R. M. 1996. Stress, glucocorticoids, and damage to the nervous system: the current state of confusion. *Stress*, 1, 1-19.
- SCHATZBERG, A. F. 1998. Noradrenergic versus serotonergic antidepressants: predictors of treatment response. *Journal of Clinical Psychiatry*, 59, 15-18.
- SCHILDKRAUT, J. J. 1965. The catecholamine hypothesis of affective disorders: a review of supporting evidence. *American journal of Psychiatry*, 122, 509-522.
- SCHLICKER, E., FINK, K., DETZNER, M. & GÖTHERT, M. 1993. Histamine inhibits dopamine release in the mouse striatum via presynaptic H₃ receptors. *Journal of Neural Transmission/General Section JNT*, 93, 1-10.
- SCHLICKER, E., WERTHWEIN, S. & ZENTNER, J. 1999. Histamine H₃ receptor-mediated inhibition of noradrenaline release in the human brain. *Fundamental & clinical pharmacology*, 13, 120-122.
- SCHMIDT, A. C., WANG, X., ZHU, Y. & SOMBERS, L. A. 2013. Carbon Nanotube Yarn Electrodes for Enhanced Detection of Neurotransmitter Dynamics in Live Brain Tissue. *ACS Nano*, 7, 7864-7873.
- SCHNEIDER, E. H. & SEIFERT, R. 2016. The histamine H₄-receptor and the central and peripheral nervous system: a critical analysis of the literature. *Neuropharmacology*, 106, 116-128.
- 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, 404-418.
- SHAW, D. M., CAMPS, F. E. & ECCLESTON, E. G. 1967. 5-Hydroxytryptamine in the hind-brain of depressive suicides. *The British Journal of Psychiatry*, 113, 1407-1411.
- SHELIN, Y. I., GADO, M. H. & KRAEMER, H. C. 2003. Untreated depression and hippocampal volume loss. *American journal of psychiatry*, 160, 1516-1518.
- SHELIN, Y. I., SANGHAVI, M., MINTUN, M. A. & GADO, M. H. 1999. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *Journal of Neuroscience*, 19, 5034-5043.
- SHELIN, Y. I., WANG, P. W., GADO, M. H., CSERNANSKY, J. G. & VANNIER, M. W. 1996. Hippocampal atrophy in recurrent major depression. *Proceedings of the National Academy of Sciences*, 93, 3908-3913.
- SHENG, W. S., HU, S., FENG, A. & ROCK, R. B. 2013. Reactive oxygen species from human astrocytes induced functional impairment and oxidative damage. *Neurochemical research*, 38, 2148-2159.
- SIMON, F. E. R. & SIMONS, K. J. 2008. H₁ antihistamines: current status and future directions. *World Allergy Organization Journal*, 1, 145-155.

- SIMPSON, D. S. A. & OLIVER, P. L. 2020. ROS Generation in Microglia: Understanding Oxidative Stress and Inflammation in Neurodegenerative Disease. *Antioxidants*, 9, 743.
- SINGH, Y. S., SAWARYNSKI, L. E., DABIRI, P. D., CHOI, W. R. & ANDREWS, A. M. 2011. Head-to-head comparisons of carbon fiber microelectrode coatings for sensitive and selective neurotransmitter detection by voltammetry. *Analytical chemistry*, 83, 6658-6666.
- SMITH-APELDOORN, S. Y., VERAART, J. K., SPIJKER, J., KAMPHUIS, J. & SCHOEVEERS, R. A. 2022. Maintenance ketamine treatment for depression: a systematic review of efficacy, safety, and tolerability. *The Lancet Psychiatry*, 9, 907-921.
- SMITH, R. S. 1991. The macrophage theory of depression. *Medical Hypotheses*, 35, 298-306.
- STRAKHOVA, M. I., NIKKEL, A. L., MANELLI, A. M., HSIEH, G. C., ESBENSHADE, T. A., BRIONI, J. D. & BITNER, R. S. 2009. Localization of histamine H4 receptors in the central nervous system of human and rat. *Brain research*, 1250, 41-48.
- SWAMY, B. K. & VENTON, B. J. 2007. Carbon nanotube-modified microelectrodes for simultaneous detection of dopamine and serotonin in vivo. *Analyst*, 132, 876-884.
- TASHIRO, M., MOCHIZUKI, H., IWABUCHI, K., SAKURADA, Y., ITOH, M., WATANABE, T. & YANAI, K. 2002. Roles of histamine in regulation of arousal and cognition: functional neuroimaging of histamine H1 receptors in human brain. *Life sciences*, 72, 409-414.
- THANGAM, E. B., JEMIMA, E. A., SINGH, H., BAIG, M. S., KHAN, M., MATHIAS, C. B., CHURCH, M. K. & SALUJA, R. 2018a. The Role of Histamine and Histamine Receptors in Mast Cell-Mediated Allergy and Inflammation: The Hunt for New Therapeutic Targets. *Front Immunol*, 9, 1873.
- THANGAM, E. B., JEMIMA, E. A., SINGH, H., BAIG, M. S., KHAN, M., MATHIAS, C. B., CHURCH, M. K. & SALUJA, R. 2018b. The role of histamine and histamine receptors in mast cell-mediated allergy and inflammation: the hunt for new therapeutic targets. *Frontiers in immunology*, 9, 1873.
- THRELFELL, S., CRAGG, S. J., KALLÓ, I., TURI, G. F., COEN, C. W. & GREENFIELD, S. A. 2004. Histamine H3 receptors inhibit serotonin release in substantia nigra pars reticulata. *Journal of Neuroscience*, 24, 8704-8710.
- THRELFELL, S., EXLEY, R., CRAGG, S. J. & GREENFIELD, S. A. 2008. Constitutive histamine H2 receptor activity regulates serotonin release in the substantia nigra. *Journal of Neurochemistry*, 107, 745-755.
- TORRENT, A., MORENO-DELGADO, D., GÓMEZ-RAMÍREZ, J., RODRÍGUEZ-AGUDO, D., RODRÍGUEZ-CASO, C., SÁNCHEZ-JIMÉNEZ, F., BLANCO, I. & ORTIZ, J. 2005. H3 Autoreceptors Modulate Histamine Synthesis through Calcium/Calmodulin- and cAMP-Dependent Protein Kinase Pathways. *Molecular Pharmacology*, 67, 195-203.
- TROTTA, T., PORRO, C., CALVELLO, R. & PANARO, M. A. 2014. Biological role of Toll-like receptor-4 in the brain. *Journal of neuroimmunology*, 268, 1-12.
- TROUBAT, R., BARONE, P., LEMAN, S., DESMIDT, T., CRESSANT, A., ATANASOVA, B., BRIZARD, B., EL HAGE, W., SURGET, A. & BELZUNG, C. 2021. Neuroinflammation and depression: A review. *European journal of neuroscience*, 53, 151-171.

- TURNER, M. D., NEDJAI, B., HURST, T. & PENNINGTON, D. J. 2014. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1843, 2563-2582.
- TUTEJA, N. 2009. Signaling through G protein coupled receptors. *Plant Signal Behav*, 4, 942-7.
- TWAROG, B. M. & PAGE, I. H. 1953. Serotonin content of some mammalian tissues and urine and a method for its determination. *American Journal of Physiology-Legacy Content*, 175, 157-161.
- TYCE, G. M. 1990. Origin and metabolism of serotonin. *Journal of cardiovascular pharmacology*, 16, S1-S7.
- VANHANEN, J. K., NUUTINEN, S., TUOMINEN, M. & PANULA, P. 2016. Histamine H3 receptor regulates sensorimotor gating and dopaminergic signaling in the striatum. *The Journal of pharmacology and experimental therapeutics*, 357, 264-272.
- VARASCHIN, R. K., OSTERSTOCK, G., DUCROT, C., LEINO, S., BOURQUE, M.-J., PRADO, M. A., PRADO, V. F., SALMINEN, O., RANNANPÄÄ, S. & TRUDEAU, L.-E. 2018. Histamine H3 receptors decrease dopamine release in the ventral striatum by reducing the activity of striatal cholinergic interneurons. *Neuroscience*, 376, 188-203.
- VENTON, B. J. & CAO, Q. 2020. Fundamentals of fast-scan cyclic voltammetry for dopamine detection. *Analyst*, 145, 1158-1168.
- VREELAND, R. F., ATCHERLEY, C. W., RUSSELL, W. S., XIE, J. Y., LU, D., LAUDE, N. D., PORRECA, F. & HEIEN, M. L. 2015. Biocompatible PEDOT: Nafion composite electrode coatings for selective detection of neurotransmitters in vivo. *Analytical chemistry*, 87, 2600-2607.
- WANG, Y., ZHANG, P., CHAO, Y., ZHU, Z., YANG, C., ZHOU, Z., LI, Y., LONG, Y., LIU, Y., LI, D., WANG, S. & QU, Q. 2024. Transport and inhibition mechanism for VMAT2-mediated synaptic vesicle loading of monoamines. *Cell Research*, 34, 47-57.
- WIGHTMAN, R. M. 2006. Probing Cellular Chemistry in Biological Systems with Microelectrodes. *Science*, 311, 1570-1574.
- WITTKAMPF, K., VAN RAVESTEIJN, H., BAAS, K., VAN DE HOOGEN, H., SCHENE, A., BINDELS, P., LUCASSEN, P., VAN DE LISDONK, E. & VAN WEERT, H. 2009. The accuracy of Patient Health Questionnaire-9 in detecting depression and measuring depression severity in high-risk groups in primary care. *General hospital psychiatry*, 31, 451-459.
- WONG, D. T., HORNG, J. S., BYMASTER, F. P., HAUSER, K. L. & MOLLOY, B. B. 1974. A selective inhibitor of serotonin uptake: Lilly 110140, 3-(p-Trifluoromethylphenoxy)-n-methyl-3-phenylpropylamine. *Life Sciences*, 15, 471-479.
- WONG, D. T., PERRY, K. W. & BYMASTER, F. P. 2005. The Discovery of Fluoxetine Hydrochloride (Prozac). *Nature Reviews Drug Discovery*, 4, 764-774.
- ZHUANG, X., SILVERMAN, A. J. & SILVER, R. 1996. Brain mast cell degranulation regulates blood-brain barrier. *Journal of neurobiology*, 31, 393-403.

CHAPTER 2 - LIPOPOLYSACCHARIDE EFFECTS ON NEUROTRANSMISSION: IMPLICATIONS FOR DEPRESSION¹

¹ **Batey, L.**, Baumberger, B., Khoshbouei, H., Hashemi, P. Lipopolysaccharide Effects on Neurotransmission: Implications for Depression. *ACS Chemical Neuroscience*. 2024.

Abstract

Immune activation in the body is well studied, however much less is known about how peripheral inflammation changes brain chemistry. Because depression and inflammation are close comorbidities, investigating how inflammation affects the brain's chemicals will help us to better understand depression. The levels of monoamines dopamine, serotonin and norepinephrine are thought to be affected by both inflammation and depression. In this perspective, we review studies that find chemical changes in the brain after administration of the endotoxin LPS, which is a robust method to induce rapid inflammation. From these studies, we interpreted LPS to reduce dopamine and serotonin and increase norepinephrine levels in various regions in the brain. These changes are not a sign of 'dysfunction' but serve an important evolutionary purpose that encourages the body to recover from an immune insult by altering mood.

Introduction

Inflammation is a blanket term used to describe the body's immune response to an irritant. Immune activation in the periphery serves important physiological purposes (*i.e.*, elimination of pathogens/foreign bodies) *via* biochemical cascades, involving several cell types, that are relatively well established (Sompayrac, 2022). In recent times, the literature supports a strong connection between body and brain inflammation (Jin et al., 2024). However, the effect of inflammation on brain chemistry is much less understood. What is clear is that inflammation and mental health disorders, particularly depression, exhibit significant comorbidity.

Researchers have found in animals that 'sickness' and 'depressive' phenotypes bear striking similarities (Dantzer et al., 2008, Maes et al., 2012). Given the comorbidity between depression and inflammation, it is likely that there is a significant or full overlap between sickness and depressive behaviors. Therefore, researchers are studying the underlying pathology of inflammation to better explain depression (Beurel et al., 2020). To this end, observing the detailed effects of inflammation on neurotransmitters, which control mood, would be highly informative.

The neurotransmitters that drive mood likely involve the neuromodulators dopamine (DA), serotonin and norepinephrine (NE) (Bunney and Davis, 1965, Schildkraut, 1965, Woolley and Shaw, 1954). These modulators are thought to be involved because of a long-standing notion arising from the monoamine hypothesis of depression (that the levels of DA, serotonin and NE are lower during depression). While this hypothesis has

cycled in and out of popularity for decades, a rich body of literature supports how monoamines drive affective behaviors (Elhwuegi, 2004, Jiang et al., 2022). Compellingly, there is also extensive literature to support a profound effect of inflammation on these monoamines (reviewed in this chapter).

It is difficult to select an appropriate model to study how inflammation orchestrates monoamine concentrations because immune system activation is complex, heterogeneous, and comorbid with many other brain disorders such as Parkinson's disease and autism spectrum disorder (Liu et al., 2022, Onore et al., 2012). A robust and well-studied way to induce rapid, acute inflammation is *via* the introduction of lipopolysaccharide (LPS) to the peripheral system.

LPS is found in the outer membrane of Gram-negative bacteria and is detected at picomolar levels by toll-like receptor 4 (TLR4). In the periphery, TLR4 is located mostly on immune and nerve cells (Poltorak et al., 1998, Takeuchi et al., 1999) and with respect to the former, TLR4 are densely expressed macrophages, monocytes, dendritic cells (Vaure and Liu, 2014) and interestingly on Schwann cells: "The neuroglia of the peripheral nervous system" (Qin et al., 2012). LPS has been found to activate NF- κ B (an ancient transcription factor that regulates innate immunity) (Albensi, 2019) which induces TNF- α release from Schwann cells (Bunimovich et al., 2017). In macrophages, TLR4 activation triggers biosynthesis of a diverse array of inflammation mediators including TNF- α and IL-1B (Chávez-Sánchez et al., 2010). Although LPS does not go through the blood brain barrier (BBB) (Singh and Jiang, 2004), TLR4 present in the central nervous system can be reached through the bloodstream due to their localization on microglia (which constitute

the BBB) suggesting a vital role for TLR4 in also engaging the innate cerebral immune response (Trotta et al., 2014). As part of this response, LPS rapidly activates the microglia (da Silva et al., 2024) (**Figure 2.1**).

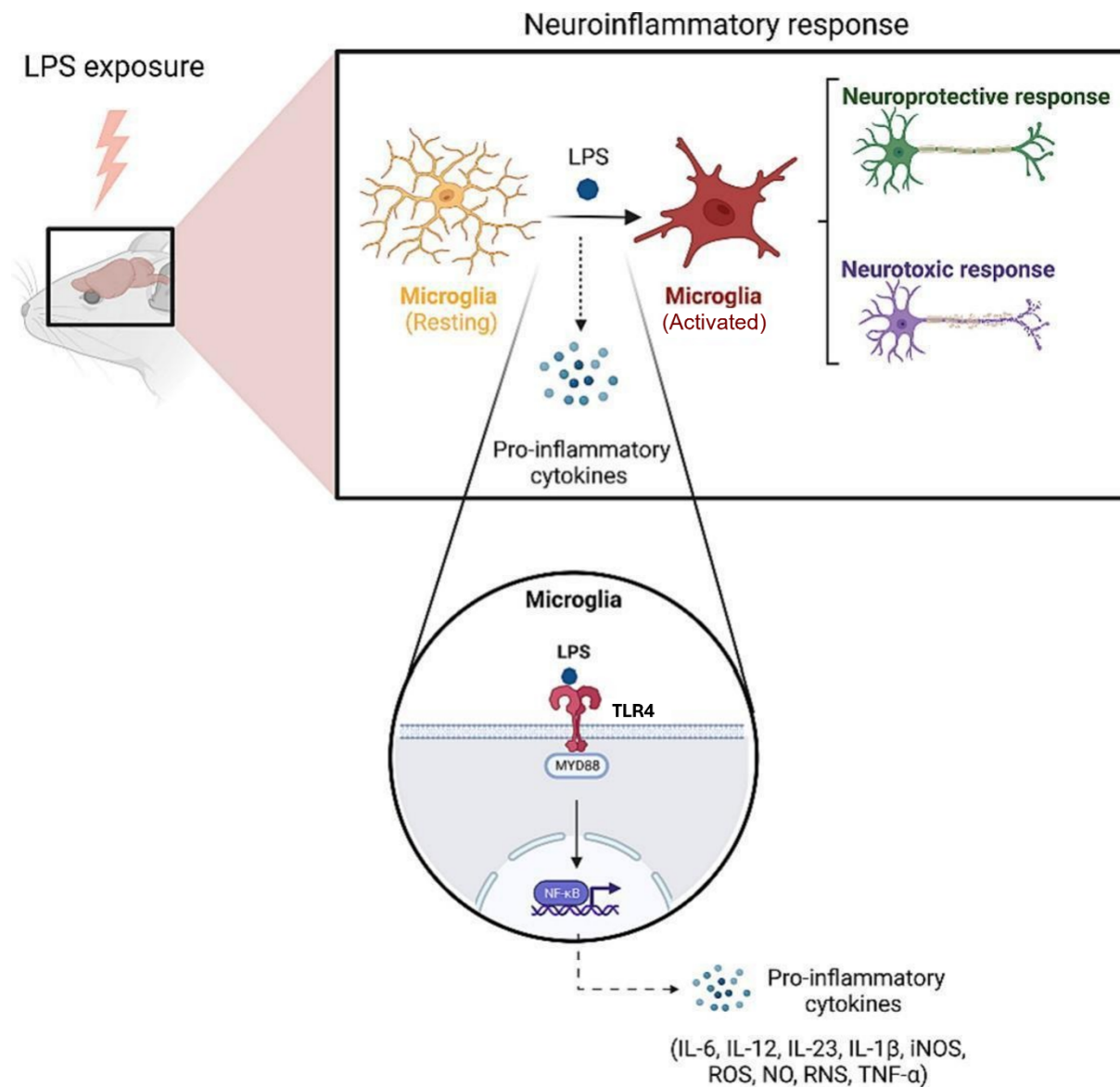


Figure 2.1 - Representation of LPS induced microglial activation (da Silva et al., 2024).

LPS induces rapid sickness behavior in rodents showing that, despite not crossing the BBB, this endotoxin drives behavior (Biesmans et al., 2013, Lacosta et al., 1999). While the mechanisms of LPS induced behaviors are not fully established, more details are emerging such as the importance of the vagus nerve in also transducing the LPS induced inflammation signal from the body to the brain (Jin et al., 2024). The neurotransmitters that are associated with mood are thought to be DA, serotonin and NE. Interestingly, these biogenic amines regulate a range of peripheral immune processes, including cytokine secretion, cell adhesion, cytotoxicity, and chemotaxis (Cosentino et al., 2007, Gaskill et al., 2012, Kipnis et al., 2004, Nolan et al., 2019, Watanabe et al., 2006). Immune system activity can also influence dopaminergic signaling both centrally and peripherally (Kumai et al., 2000, Song et al., 2006). Serotonin concentration decreases in blood and plasma and accumulates in the liver (Endo, 1983, Endo and Nakamura, 1992) during LPS-induced inflammation while NE turnover increases in the rat spleen and lungs in response to LPS (Akiyoshi et al., 1990).

Therefore, it is clear that these monoamines are pivotal in mediating a bidirectional brain/body immune axis. Therefore, in this perspective, we review the changes in the monoamine concentrations in the extracellular space of the brain in response to LPS and correlate these changes to behavior. It is incredibly challenging to measure brain chemistry, and we found studies that included methods such as microdialysis and voltammetry. In the studies, LPS dosage has a big impact on chemical changes. We included only studies that reported measurable impacts on monoamine levels. When accounting for technique artifacts, our interpretation of the literature is that peripheral

LPS:

- a) decreases DA levels due to increased DA turnover, increased dopamine transporter (DAT) activity and the neurodegenerative effects of LPS on DA neurons.
- b) decreases serotonin levels because of increased serotonin turnover, increased serotonin transporter activity and increased inhibition of serotonin *via* H₃ receptors.
- c) increases NE levels because of this messenger's role in modulating microglial activity.

In sum, having reviewed the effects of peripheral LPS on monoamines, it is our opinion that inflammation may serve an important physiological function in altering mood. The sickness/depressed mood may be evolutionarily advantageous to bodily recovery (*i.e.*, lethargy forcing rest).

Dopamine

DA is a versatile modulator with various roles in the brain, including cognition and reward. There is much evidence that DA is an envoy of the inflammation message between the brain and body (Gaskill and Khoshbouei, 2022). For example, hyperdopaminergic systems have been associated with heightened LPS-induced cytokine production in macrophages (Gopinath et al., 2023, Kavelaars et al., 2005, Teunis et al., 2004). In rats, elevating CNS DA levels with L-DOPA affected peripheral T-cells (Ilani et al., 2004). DA was found to suppress pro-inflammatory and promote anti-inflammatory cytokines in mouse splenocytes (Kawano et al., 2018), and direct activation of dopaminergic neurons in the ventral tegmental area (VTA) of mice, using

designer receptors exclusively activated by designer drugs (DREADDs), enhanced the phagocytic activity of splenic dendritic cells and macrophages (Ben-Shaanan et al., 2016). In support of this, Sato *et al.* showed that repeated stimulation of neurons expressing D1 receptors in the mouse brain nucleus accumbens decreased tumor size in the periphery (Sato et al., 2022).

These findings underscore the critical role of dopaminergic neurotransmission in immunoregulation. As a result, evaluating the immunological impact of DA throughout the body is essential when assessing the therapeutic efficacy of dopaminergic drugs.

DA is an important neuromodulator, which has long been implicated in depression. Patients with depression often display motivational and motor impairments and fatigue. These deficits are associated with DA dysfunction in the brain. Several human studies have linked inflammation to reduced motivation, increased fatigue, and motor slowing (Brydon et al., 2008, Capuron et al., 2007, Capuron et al., 2012, Felger and Miller, 2012, Harrison et al., 2016, Majer et al., 2008). One notable study in human volunteers found that after endotoxin injection, there was an increase in self-reported and observer rated depressed mood. These mood states were correlated with altered responsivity in basal ganglia function as measured with fMRI (Eisenberger et al., 2010). Another study showed that treatment with the pro-inflammatory cytokine, IFN-alpha, led to an increase in fatigue and increased glucose metabolism in the basal ganglia (measured with PET), suggesting altered dopaminergic activity (Capuron et al., 2007). A final study of note found that humans treated with the typhoid vaccine had increased rates of fatigue that correlated with the proinflammatory cytokine, IL-6 (measured with ELISA). These studies found that humans with higher levels of IL-6 had slower reaction times (motor deficits)

(Brydon et al., 2008). From these studies, it should follow that DA release would be decreased during inflammation and rapidly after LPS. Few people have measured DA chemically after LPS and predominantly with microdialysis, and there are disparate findings with respect to the effect of LPS on DA levels.

Studies tend to agree that the long term or chronic effect of LPS is a reduction in DA levels, due to LPS's neurodegenerative effects on DA neurons, increased DA turnover and reduction in DAT activity (Bodea et al., 2014, Guo et al., 2016, Hunter et al., 2009, Qin et al., 2007, Van Heesch et al., 2014). However, several studies found that DA levels increased transiently after acute LPS (Guo et al., 2016, Van Heesch et al., 2014), which is at significant odds with the human behavioral and imaging data and the findings of increased DA turnover.

Some notable studies have shed light on this contradiction. For example, LPS has been shown to induce DAT internalization, which acutely increases DA levels but subsequently reduces DA recycling. This reduction in recycling leads to DA depletion, and when combined with increased DA turnover, ultimately results in decreased DA levels over time (Baharnoori et al., 2013, Mackie et al., 2022). Another study showed that LPS-induced increase in DA was mitigated by indomethacin, a non-steroidal anti-inflammatory agent (Lavicky and Dunn, 1995). It is possible that this acute increase in DA arises from local microglia-induced dopaminergic cell death (Brown and Vilalta, 2015, Mastroeni et al., 2009). DA neurons are fragile and do not easily regenerate, in part due to their large energy demand as well as their long, extensive arbors (Pissadaki and Bolam, 2013). It is well documented that microdialysis probe implantation induces immediate

activation of microglia (Kozai et al., 2016, Nesbitt et al., 2013). This effect, combined with the fact that LPS also rapidly activates microglia (Jung et al., 2023), is likely to cause local neuronal death around the microdialysis probe. The acute increase in DA may therefore be a consequence of measuring the contents of these dead neurons. Indeed, direct administration of LPS *in vivo* causes microglia-induced loss of dopaminergic neurons (Arai et al., 2004, De Pablos et al., 2005).

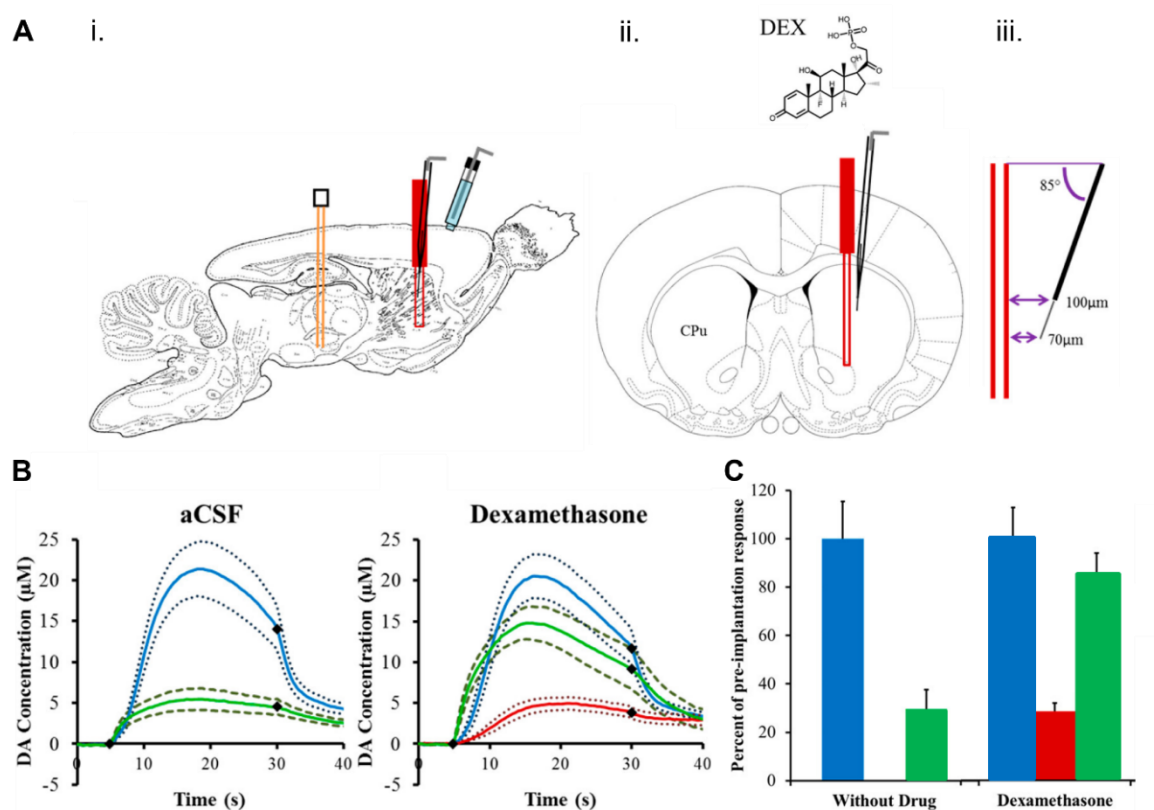


Figure 2.2 Effects of Microdialysis Probe - Figure showing (a) experimental setup to test effect of microdialysis probe on DA neurotransmission (b and c) results showing that the disruption in DA transmission caused by microdialysis probe is rescued with dexamethasone (Nesbitt et al., 2013).

In accord with this notion, Nesbitt *et al.* showed, using voltammetry, that the microdialysis probe significantly disrupts dopaminergic transmission, an effect that could be rescued with dexamethasone (glucocorticoid steroid). This rescue effect was hypothesized by the authors to arise from curtailing glial activation with dexamethasone as seen in **Figure 2.2. (Nesbitt et al., 2013).**

In sum, the literature supports different mechanisms leading to reduced DA transmission in response to LPS, that follows the behavioral roles of dopaminergic dysfunction in depression.

Norepinephrine

NE, also known as noradrenaline, is thought to be involved in the regulation of mood, attention, and the stress response. NE in the body is produced by the adrenal glands, liver and spleen (Benedeczký et al., 1964, Benedeczký et al., 1966, Eisenhofer et al., 1995, Vriend et al., 1993) and plays a strong and well-established role in peripheral inflammation. During inflammation, NE is released from the nerve terminals of lymphoid organs, acting on adrenoceptors on immune cells (Scanzano and Cosentino, 2015). In this respect, NE has been shown to blunt pro-inflammatory and encourage anti-inflammatory cascades. A subset of intestinal macrophages expresses β -adrenergic receptors that promote a tissue-protective phenotype (Gabanyi et al., 2016), while adipose macrophages adjacent to sympathetic terminals express functional norepinephrine transporters (NET), which modulate proinflammatory states and thermogenesis (Pirzgalska et al., 2017). Stolk *et al.* showed that in humans under LPS challenge, NE infusion reduced pro-inflammatory cytokines and promoted a better anti-

inflammatory cytokine balance (Stolk et al., 2020). Furthermore, the group showed that NE inhibited the production of radical oxygen species. This idea was supported in a later review by Thoppil *et al.* who summarized the literature on how NE regulates oxidative metabolism in immune cells (Thoppil et al., 2023).

Specifically in the brain during depression, extracellular NE levels are thought to be reduced. This notion has spurred the development of selective NE reuptake inhibitors (NRIs), which are thought to enhance NE availability in the brain by inhibiting this modulator's reuptake into neurons. However, the premise that depression is associated with decreased NE is not supported by the literature on inflammation and this modulator indicates a more nuanced relationship. Microdialysis studies above showed that DA increased acutely after LPS, and we attributed this to microglial induced dopaminergic cell death. This sort of cell death may not extend to NE neurons. NE is widely accepted to regulate microglia through β_2 -adrenergic receptor activation (O'Donnell et al., 2012). This receptor activation decreases microglia proliferation and cytokine release, while increasing soma migration and phagocytosis of harmful debris (**Figure 2.3**) (Pearson-Leary et al., 2016).

Therefore, we propose that in themselves, NE neurons create a chemically privileged microenvironment that makes them less susceptible to microglial induced cell death. In accord with this, *in vitro* tissue slice preparations produce more NE when exposed to LPS and stay viable throughout the experiment (Francis et al., 2001). Indeed, several *in vivo* studies have found NE to increase in several brain structures after LPS (Linthorst et al., 1996, Linthorst and Reul, 1998).

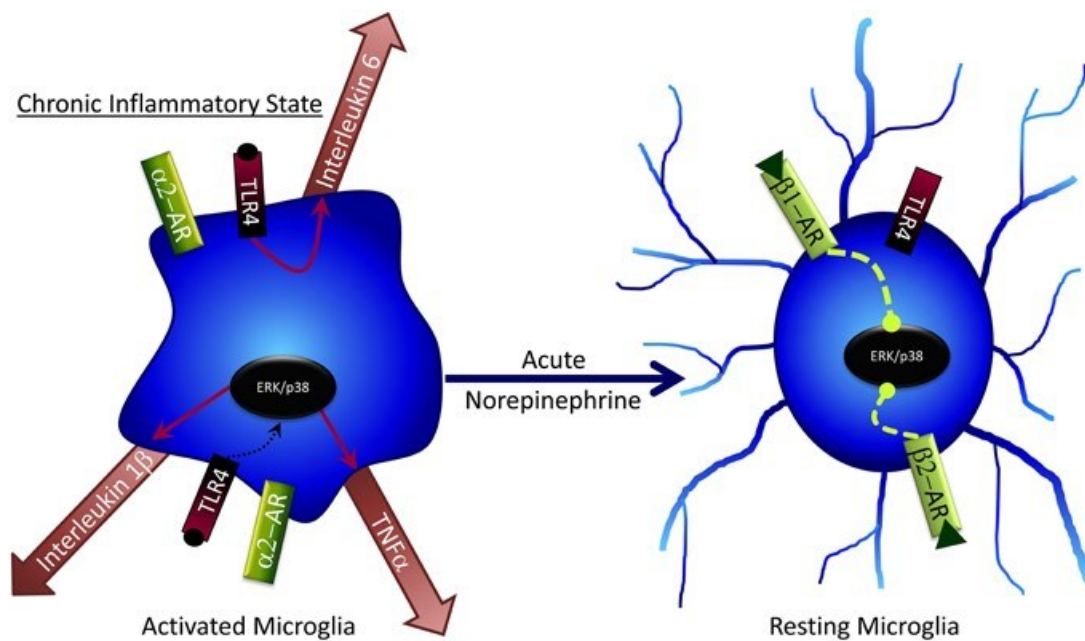


Figure 2.3 - Acute NE exposure can decrease release of cytokines by microglia (Pearson-Leary et al., 2016).

In these studies, the increase in NE is sustained, suggesting a key role for NE during inflammation. Several studies have suggested this role to be neuroprotection of neurons. For example, when rat mesencephalic neuronal cultures were exposed to low doses of NE, the function and survival of the cortical neurons was increased (Madrigal et al., 2005). Other studies supported this finding by showing that depletion of NE accelerates LPS induced cell death *in vivo* (Jiang et al., 2015, Song et al., 2019a, Song et al., 2019b).

In sum, it would appear that inflammation rapidly induces a sustained NE response that serves to modulate microglia and protect DA neurons from cell death. We now refine the original clinical notion that NE is reduced in depression since the literature overwhelmingly supports higher NE levels during inflammation. We hypothesize that NRIs do not compensate for a lack of NE but increase NE above baseline (O'Sullivan et

al., 2009). This increased NE has an anti-inflammatory effect with downstream benefits for depression.

Serotonin

Serotonin is a modulator that is found in high levels in the periphery where it controls digestion, smooth muscle contraction and thermoregulation (Fidalgo et al., 2013). In the body, serotonin is predominantly synthesized by enterochromaffin (EC) cells in the gut. It is well known that serotonin is a potent mediator of peripheral inflammation. With respect to the gut, in particular, there are well-known pathophysiological connections between serotonin and inflammation that cause disorders such as inflammatory bowel disease (IBS) (Bishop et al., 1987, Coates et al., 2004, El-Salhy et al., 1997, Khan and Ghia, 2010). Serotonin receptors and serotonin transporters (SERTs) are expressed on innate immune cells such as monocytes, macrophages, dendritic cells (DCs) and mast cells (Ahern, 2011, Baganz and Blakely, 2013). During acute inflammation, serotonin stimulates recruitment of some of these immune cells (Boehme et al., 2004, Duerschmied et al., 2013, Kushnir-Sukhov et al., 2006, Müller et al., 2009), modifies DC cytokine production, and promotes production of pro-inflammatory cytokines from macrophages (Ahern, 2011, Freire-Garabal et al., 2003, Ghia et al., 2009). Serotonin also plays an important role in circulatory immunity, where platelets have high concentrations of SERTs (Lesch et al., 1993, Qian et al., 1995, Rudnick, 1977). These SERTs take up serotonin into the platelets with high affinity (Jedlitschky et al., 2012) and release their contents upon immune activation (Berger et al., 2009, McNicol and Israels, 1999).

It is well established that peripheral and brain serotonin are connected via the bi-directional vagus immune axis (Jin et al., 2024). Despite only a small fraction of serotonin being present in the brain, this modulator has long been thought to be involved in depression because of the monoamine hypothesis (Bunney and Davis, 1965). This theory states that low extracellular serotonin levels drive the pathology of depression. Selective serotonin reuptake inhibitors (SSRIs) are designed to block the reuptake of serotonin back into cells, thereby increasing extracellular serotonin levels and alleviating depression symptoms (Åberg-Wistedt, 1989). SSRIs have variable efficacy; thus, the serotonergic theory of depression has moved in and out of favor for decades, recently enjoying a resurgence because of clinical trials targeting depression with psychedelics (with high affinity for serotonin receptors) (Duan et al., 2024). An important recent study found, using positron emission tomography, that serotonin release capacity was reduced in depressed human patients compared with healthy controls (Erritzoe et al., 2023).

However, contrary to these notions, microdialysis measurements have found serotonin to transiently increase after LPS (as with DA and NE) (Guo et al., 2016, Linthorst et al., 1996, Linthorst and Reul, 1998, Van Heesch et al., 2014). A theme in these works is increased turnover or metabolism of serotonin (Lavicky and Dunn, 1995) which theoretically should decrease serotonin levels. Indeed, in our own work with voltammetry, we have shown a rapid decrease in basal hippocampal serotonin after LPS (Hersey et al., 2021), which we attributed to activation of inhibitory H₃ receptors by inflammation induced histamine (**Figure 2.4** shows the biochemical model of histaminergic inhibition of serotonin) (Mena et al., 2024). This reduction is supported by

a

Histamine terminal

Leakage

Removal

b

Serotonin terminal

Leakage

Removal

c

SERT processes

64

The increase in serotonin observed by microdialysis can be potentially explained *via* mast cell recruitment around the probe. It is well known that BBB breakage results in rapid mast cell recruitment (Dong et al., 2014, Hendriksen et al., 2017, Silverman et al., 2000) and mast cell degranulation has been reported at the site of implanted devices (Tang et al., 1998). Mast cells contain serotonin and histamine and release both when activated (Pihel et al., 1995). Therefore, we propose that despite LPS induced increased serotonin turnover, increased SERT function and increased inhibition of release *via* H₃ receptors, microdialysis observes an increase in serotonin due to serotonin release from mast cells at the microdialysis site.

Conclusion

Inflammation is synonymous with immune activation and while the effect of inflammation on the body is well researched, there is less known about how peripheral inflammation changes brain chemistry. There is comorbidity between depression and inflammation, thus studying the underlying pathology of inflammation on the brain's chemicals will aid a better understanding of depression. There is compelling evidence to support the monoamines DA, serotonin and NE's roles in both inflammation and depression. In this perspective, we reviewed chemical changes in the brain after LPS, a robust and well-studied way to induce rapid, acute inflammation. From these studies, we interpreted LPS to reduce DA and serotonin and increase NE in the brain. We proposed that these changes serve an important evolutionary purpose (e.g., bodily recovery).

References

- ÅBERG-WISTEDT, A. 1989. The antidepressant effects of 5-HT uptake inhibitors. *The British Journal of Psychiatry*, 155, 32-40.
- AHERN, G. P. 2011. 5-HT and the immune system. *Current opinion in pharmacology*, 11, 29-33.
- AKIYOSHI, M., SHIMIZU, Y. & SAITO, M. 1990. Interleukin-1 increases norepinephrine turnover in the spleen and lung in rats. *Biochemical and biophysical research communications*, 173, 1266-1270.
- ALBENSI, B. C. 2019. What is nuclear factor kappa B (NF-κB) doing in and to the mitochondrion? *Frontiers in cell and developmental biology*, 7, 154.
- ARAI, H., FURUYA, T., YASUDA, T., MIURA, M., MIZUNO, Y. & MOCHIZUKI, H. 2004. Neurotoxic effects of lipopolysaccharide on nigral dopaminergic neurons are mediated by microglial activation, interleukin-1β, and expression of caspase-11 in mice. *Journal of Biological Chemistry*, 279, 51647-51653.
- BAGANZ, N. L. & BLAKELY, R. D. 2013. A dialogue between the immune system and brain, spoken in the language of serotonin. *ACS chemical neuroscience*, 4, 48-63.
- BAHARNOORI, M., BHARDWAJ, S. K. & SRIVASTAVA, L. K. 2013. Effect of maternal lipopolysaccharide administration on the development of dopaminergic receptors and transporter in the rat offspring. *PloS one*, 8, e54439.
- BEN-SHAANAN, T. L., AZULAY-DEBBY, H., DUBOVIK, T., STAROSVETSKY, E., KORIN, B., SCHILLER, M., GREEN, N. L., ADMON, Y., HAKIM, F. & SHEN-ORR, S. S. 2016. Activation of the reward system boosts innate and adaptive immunity. *Nature Medicine*, 22, 940-944.
- BENEDECZKY, I., PUPPI, A., TIGYI, A. & LISSÁK, K. 1964. Various cell types of the adrenal medulla. *Nature*, 204, 591-592.
- BENEDECZKY, I., PUPPI, A., TIGYI, A. & LISSÁK, K. 1966. Various cell types in the adrenal medulla. *Nature*, 209, 592-594.
- BERGER, M., GRAY, J. A. & ROTH, B. L. 2009. The expanded biology of serotonin. *Annu Rev Med*, 60, 355-66.
- BEUREL, E., TOUPS, M. & NEMEROFF, C. B. 2020. The bidirectional relationship of depression and inflammation: double trouble. *Neuron*, 107, 234-256.
- BIESMANS, S., MEERT, T. F., BOUWKNECHT, J. A., ACTON, P. D., DAVOODI, N., DE HAES, P., KUIJLAARS, J., LANGLOIS, X., MATTHEWS, L. J. & VER DONCK, L. 2013. Systemic immune activation leads to neuroinflammation and sickness behavior in mice. *Mediators of inflammation*, 2013, 271359.
- BISHOP, A., PIETROLETTI, R., TAAT, C., BRUMMELKAMP, W. & POLAK, J. 1987. Increased populations of endocrine cells in Crohn's ileitis. *Virchows Archiv A*, 410, 391-396.
- BODEA, L.-G., WANG, Y., LINNARTZ-GERLACH, B., KOPATZ, J., SINKKONEN, L., MUSGROVE, R., KAOMA, T., MULLER, A., VALLAR, L. & DI MONTE, D. A. 2014. Neurodegeneration by activation of the microglial complement-phagosome pathway. *Journal of Neuroscience*, 34, 8546-8556.
- BOEHME, S. A., LIO, F. M., SIKORA, L., PANDIT, T. S., LAVRADOR, K., RAO, S. P. & SRIRAMARAO, P. 2004. Cutting edge: serotonin is a chemotactic factor for eosinophils and functions additively with eotaxin. *J Immunol*, 173, 3599-603.

- BROWN, G. C. & VILALTA, A. 2015. How microglia kill neurons. *Brain Research*, 1628, 288-297.
- BRYDON, L., HARRISON, N. A., WALKER, C., STEPTOE, A. & CRITCHLEY, H. D. 2008. Peripheral inflammation is associated with altered substantia nigra activity and psychomotor slowing in humans. *Biological psychiatry*, 63, 1022-1029.
- BUNIMOVICH, Y. L., KESKINOV, A. A., SHURIN, G. V. & SHURIN, M. R. 2017. Schwann cells: a new player in the tumor microenvironment. *Cancer immunology, immunotherapy*, 66, 959-968.
- BUNNEY, W. E. & DAVIS, J. M. 1965. Norepinephrine in depressive reactions: A review. *Archives of General Psychiatry*, 13, 483-494.
- CAPURON, L., PAGNONI, G., DEMETRASHVILI, M. F., LAWSON, D. H., FORNWALT, F. B., WOOLWINE, B., BERNS, G. S., NEMEROFF, C. B. & MILLER, A. H. 2007. Basal ganglia hypermetabolism and symptoms of fatigue during interferon- α therapy. *Neuropsychopharmacology*, 32, 2384-2392.
- CAPURON, L., PAGNONI, G., DRAKE, D. F., WOOLWINE, B. J., SPIVEY, J. R., CROWE, R. J., VOTAW, J. R., GOODMAN, M. M. & MILLER, A. H. 2012. Dopaminergic mechanisms of reduced basal ganglia responses to hedonic reward during interferon alfa administration. *Archives of general psychiatry*, 69, 1044-1053.
- CHÁVEZ-SÁNCHEZ, L., CHÁVEZ-RUEDA, K., LEGORRETA-HAQUET, M. V., ZENTENO, E., LEDESMA-SOTO, Y., MONTOYA-DÍAZ, E., TESORO-CRUZ, E., MADRID-MILLER, A. & BLANCO-FAVELA, F. 2010. The activation of CD14, TLR4, and TLR2 by mmLDL induces IL-1 β , IL-6, and IL-10 secretion in human monocytes and macrophages. *Lipids in health and disease*, 9, 1-8.
- COATES, M. D., MAHONEY, C. R., LINDEN, D. R., SAMPSON, J. E., CHEN, J., BLASZYK, H., CROWELL, M. D., SHARKEY, K. A., GERSHON, M. D. & MAWE, G. M. 2004. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. *Gastroenterology*, 126, 1657-1664.
- COSENTINO, M., FIETTA, A. M., FERRARI, M., RASINI, E., BOMBELLI, R., CARCANO, E., SAPORITI, F., MELONI, F., MARINO, F. & LECCHINI, S. 2007. Human CD4⁺ CD25⁺ regulatory T cells selectively express tyrosine hydroxylase and contain endogenous catecholamines subserving an autocrine/paracrine inhibitory functional loop. *Blood*, 109, 632-642.
- DA SILVA, A. A. F., FIADEIRO, M. B., BERNARDINO, L. I., FONSECA, C. S. P., BALTAZAR, G. M. F. & CRISTÓVÃO, A. C. B. 2024. "Lipopolysaccharide-induced animal models for neuroinflammation – An overview.". *Journal of Neuroimmunology*, 387, 578273.
- DANTZER, R., O'CONNOR, J. C., FREUND, G. G., JOHNSON, R. W. & KELLEY, K. W. 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature reviews neuroscience*, 9, 46-56.
- DE PABLOS, R. M., HERRERA, A. J., VILLARÁN, R. F., CANO, J. & MACHADO, A. 2005. Dopamine-dependent neurotoxicity of lipopolysaccharide in substantia nigra. *The FASEB journal*, 19, 1-22.
- DONG, H., ZHANG, X. & QIAN, Y. 2014. Mast cells and neuroinflammation. *Medical science monitor basic research*, 20, 200.

- DUAN, W., CAO, D., WANG, S. & CHENG, J. 2024. Serotonin 2A Receptor (5-HT_{2A}R) Agonists: Psychedelics and Non-Hallucinogenic Analogues as Emerging Antidepressants. *Chemical Reviews*, 124, 124-163.
- DUERSCHMIED, D., SUIDAN, G. L., DEMERS, M., HERR, N., CARBO, C., BRILL, A., CIFUNI, S. M., MAULER, M., CICKO, S., BADER, M., IDZKO, M., BODE, C. & WAGNER, D. D. 2013. Platelet serotonin promotes the recruitment of neutrophils to sites of acute inflammation in mice. *Blood*, 121, 1008-15.
- EISENBERGER, N. I., BERKMAN, E. T., INAGAKI, T. K., RAMESON, L. T., MASHAL, N. M. & IRWIN, M. R. 2010. Inflammation-induced anhedonia: endotoxin reduces ventral striatum responses to reward. *Biological psychiatry*, 68, 748-754.
- EISENHOFER, G., ANEMAN, A., HOOPER, D., HOLMES, C., GOLDSTEIN, D. S. & FRIBERG, P. 1995. Production and metabolism of dopamine and norepinephrine in mesenteric organs and liver of swine. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 268, G641-G649.
- EL-SALHY, M., DANIELSSON, Å., STENLING, R. & GRIMELIUS, L. 1997. Colonic endocrine cells in inflammatory bowel disease. *Journal of internal medicine*, 242, 413-419.
- ELHWUEGI, A. S. 2004. Central monoamines and their role in major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 28, 435-451.
- ENDO, Y. 1983. A lipopolysaccharide and concanavalin A induce variations of serotonin levels in mouse tissues. *European journal of pharmacology*, 91, 493-499.
- ENDO, Y. & NAKAMURA, M. 1992. The effect of lipopolysaccharide, interleukin-1 and tumour necrosis factor on the hepatic accumulation of 5-hydroxytryptamine and platelets in the mouse. *British journal of pharmacology*, 105, 613.
- ERRITZOE, D., GODLEWSKA, B. R., RIZZO, G., SEARLE, G. E., AGNORELLI, C., LEWIS, Y., ASHOK, A. H., COLASANTI, A., BOURA, I. & FARRELL, C. 2023. Brain serotonin release is reduced in patients with depression: a [¹¹C] Cimbi-36 positron emission tomography study with a d-amphetamine challenge. *Biological Psychiatry*, 93, 1089-1098.
- FELGER, J. C. & MILLER, A. H. 2012. Cytokine effects on the basal ganglia and dopamine function: the subcortical source of inflammatory malaise. *Frontiers in neuroendocrinology*, 33, 315-327.
- FIDALGO, S., IVANOV, D. K. & WOOD, S. H. 2013. Serotonin: from top to bottom. *Biogerontology*, 14, 21-45.
- FRANCIS, J., MOHANKUMAR, P. S. & MOHANKUMAR, S. M. J. 2001. Lipopolysaccharide stimulates norepinephrine efflux from the rat hypothalamus in vitro: blockade by soluble IL-1 receptor. *Neuroscience Letters*, 308, 71-74.
- FREIRE-GARABAL, M., NÚÑEZ, M. J., BALBOA, J., LÓPEZ-DELGADO, P., GALLEGO, R., GARCÍA-CABALLERO, T., FERNÁNDEZ-ROEL, M. D., BRENLLA, J. & REY-MÉNDEZ, M. 2003. Serotonin upregulates the activity of phagocytosis through 5-HT_{1A} receptors. *Br J Pharmacol*, 139, 457-63.
- GABANYI, I., MULLER, P. A., FEIGHERY, L., OLIVEIRA, T. Y., COSTA-PINTO, F. A. & MUCIDA, D. 2016. Neuro-immune interactions drive tissue programming in intestinal macrophages. *Cell*, 164, 378-391.
- GASKILL, P. J., CARVALLO, L., EUGENIN, E. A. & BERMAN, J. W. 2012. Characterization and function of the human macrophage dopaminergic system: implications for CNS disease and drug abuse. *Journal of neuroinflammation*, 9, 1-14.

- GASKILL, P. J. & KHOSHBOUEI, H. 2022. Dopamine and norepinephrine are embracing their immune side and so should we. *Current opinion in neurobiology*, 77, 102626.
- GHIA, J. E., LI, N., WANG, H., COLLINS, M., DENG, Y., EL-SHARKAWY, R. T., CÔTÉ, F., MALLET, J. & KHAN, W. I. 2009. Serotonin has a key role in pathogenesis of experimental colitis. *Gastroenterology*, 137, 1649-60.
- GOPINATH, A., MACKIE, P. M., PHAN, L. T., MIRABEL, R., SMITH, A. R., MILLER, E., FRANKS, S., SYED, O., RIAZ, T. & LAW, B. K. 2023. Who knew? Dopamine transporter activity is critical in innate and adaptive immune responses. *Cells*, 12, 269.
- GUO, Y., CAI, H., CHEN, L., LIANG, D., YANG, R., DANG, R. & JIANG, P. 2016. Quantitative profiling of neurotransmitter abnormalities in the hippocampus of rats treated with lipopolysaccharide: focusing on kynurenine pathway and implications for depression. *Journal of neuroimmunology*, 295, 41-46.
- HARRISON, N. A., VOON, V., CERCIGNANI, M., COOPER, E. A., PESSIGLIONE, M. & CRITCHLEY, H. D. 2016. A neurocomputational account of how inflammation enhances sensitivity to punishments versus rewards. *Biological psychiatry*, 80, 73-81.
- HENDRIKSEN, E., VAN BERGEIJK, D., OOSTING, R. S. & REDEGELD, F. A. 2017. Mast cells in neuroinflammation and brain disorders. *Neuroscience & Biobehavioral Reviews*, 79, 119-133.
- HERSEY, M., SAMARANAYAKE, S., BERGER, S. N., TAVAKOLI, N., MENA, S., NIJHOUT, H. F., REED, M. C., BEST, J., BLAKELY, R. D. & REAGAN, L. P. 2021. Inflammation-induced histamine impairs the capacity of escitalopram to increase hippocampal extracellular serotonin. *Journal of Neuroscience*, 41, 6564-6577.
- HUNTER, R. L., CHENG, B., CHOI, D. Y., LIU, M., LIU, S., CASS, W. A. & BING, G. 2009. Intrastriatal lipopolysaccharide injection induces parkinsonism in C57/B6 mice. *Journal of Neuroscience Research*, 87, 1913-1921.
- ILANI, T., STROUS, R. D. & FUCHS, S. 2004. Dopaminergic regulation of immune cells via D3 dopamine receptor: a pathway mediated by activated T cells. *The FASEB journal*, 18, 1600-1602.
- JEDLITSCHKY, G., GREINACHER, A. & KROEMER, H. K. 2012. Transporters in human platelets: physiologic function and impact for pharmacotherapy. *Blood*, 119, 3394-402.
- JIANG, L., CHEN, S. H., CHU, C. H., WANG, S. J., OYARZABAL, E., WILSON, B., SANDERS, V., XIE, K., WANG, Q. & HONG, J. S. 2015. A novel role of microglial NADPH oxidase in mediating extra-synaptic function of norepinephrine in regulating brain immune homeostasis. *Glia*, 63, 1057-1072.
- JIANG, Y., ZOU, D., LI, Y., GU, S., DONG, J., MA, X., XU, S., WANG, F. & HUANG, J. H. 2022. Monoamine neurotransmitters control basic emotions and affect major depressive disorders. *Pharmaceuticals*, 15, 1203.
- JIN, H., LI, M., JEONG, E., CASTRO-MARTINEZ, F. & ZUKER, C. S. 2024. A body-brain circuit that regulates body inflammatory responses. *Nature*, 1-3.
- JUNG, H., LEE, D., YOU, H., LEE, M., KIM, H., CHEONG, E. & UM, J. W. 2023. LPS induces microglial activation and GABAergic synaptic deficits in the hippocampus accompanied by prolonged cognitive impairment. *Scientific Reports*, 13, 6547.

- KAVELAARS, A., COBELENS, P. M., TEUNIS, M. A. & HEIJNEN, C. J. 2005. Changes in innate and acquired immune responses in mice with targeted deletion of the dopamine transporter gene. *Journal of neuroimmunology*, 161, 162-168.
- KAWANO, M., TAKAGI, R., SAIKA, K., MATSUI, M. & MATSUSHITA, S. 2018. Dopamine regulates cytokine secretion during innate and adaptive immune responses. *International immunology*, 30, 591-606.
- KHAN, W. & GHIA, J. 2010. Gut hormones: emerging role in immune activation and inflammation. *Clinical & Experimental Immunology*, 161, 19-27.
- KIPNIS, J., CARDON, M., AVIDAN, H., LEWITUS, G. M., MORDECHAY, S., ROLLS, A., SHANI, Y. & SCHWARTZ, M. 2004. Dopamine, through the extracellular signal-regulated kinase pathway, downregulates CD4⁺ CD25⁺ regulatory T-cell activity: implications for neurodegeneration. *Journal of Neuroscience*, 24, 6133-6143.
- KOZAI, T. D. Y., JAQUINS-GERSTL, A. S., VAZQUEZ, A. L., MICHAEL, A. C. & CUI, X. T. 2016. Dexamethasone retrodialysis attenuates microglial response to implanted probes in vivo. *Biomaterials*, 87, 157-169.
- KUMAI, T., TATEISHI, T., TANAKA, M., WATANABE, M., SHIMIZU, H. & KOBAYASHI, S. 2000. Effect of interferon- α on tyrosine hydroxylase and catecholamine levels in the brain of rats. *Life sciences*, 67, 663-669.
- KUSHNIR-SUKHOV, N. M., GILFILLAN, A. M., COLEMAN, J. W., BROWN, J. M., BRUENING, S., TOTH, M. & METCALFE, D. D. 2006. 5-hydroxytryptamine induces mast cell adhesion and migration. *J Immunol*, 177, 6422-32.
- LACOSTA, S., MERALI, Z. & ANISMAN, H. 1999. Behavioral and neurochemical consequences of lipopolysaccharide in mice: anxiogenic-like effects. *Brain research*, 818, 291-303.
- LAVICKY, J. & DUNN, A. J. 1995. Endotoxin administration stimulates cerebral catecholamine release in freely moving rats as assessed by microdialysis. *Journal of neuroscience research*, 40, 407-413.
- LESCH, K.-P., WOLOZIN, B. L., MURPHY, D. L. & RIEDERER, P. 1993. Primary Structure of the Human Platelet Serotonin Uptake Site: Identity with the Brain Serotonin Transporter. *Journal of Neurochemistry*, 60, 2319-2322.
- LINTHORST, A. C. E., FLACHSKAMM, C., HOLSBÖER, F. & REUL, J. M. H. M. 1996. Activation of serotonergic and noradrenergic neurotransmission in the rat hippocampus after peripheral administration of bacterial endotoxin: Involvement of the cyclo-oxygenase pathway. *Neuroscience*, 72, 989-997.
- LINTHORST, A. C. E. & REUL, J. M. H. M. 1998. Brain Neurotransmission during Peripheral Inflammation. *Annals of the New York Academy of Sciences*, 840, 139-152.
- LIU, T.-W., CHEN, C.-M. & CHANG, K.-H. 2022. Biomarker of neuroinflammation in Parkinson's disease. *International journal of molecular sciences*, 23, 4148.
- MACKIE, P. M., GOPINATH, A., MONTAS, D. M., NIELSEN, A., SMITH, A., NOLAN, R. A., RUNNER, K., MATT, S. M., MCNAMEE, J. & RIKLAN, J. E. 2022. Functional characterization of the biogenic amine transporters on human macrophages. *JCI insight*, 7.
- MADRIGAL, J. L. M., FEINSTEIN, D. L. & RUSSO, C. D. 2005. Norepinephrine protects cortical neurons against microglial-induced cell death. *Journal of Neuroscience Research*, 81, 390-396.

- MAES, M., BERK, M., GOEHLER, L., SONG, C., ANDERSON, G., GAŁECKI, P. & LEONARD, B. 2012. Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways. *BMC medicine*, 10, 1-19.
- MAJER, M., WELBERG, L. A., CAPURON, L., PAGNONI, G., RAISON, C. L. & MILLER, A. H. 2008. IFN- α -induced motor slowing is associated with increased depression and fatigue in patients with chronic hepatitis C. *Brain, behavior, and immunity*, 22, 870-880.
- MASTROENI, D., GROVER, A., LEONARD, B., JOYCE, J. N., COLEMAN, P. D., KOZIK, B., BELLINGER, D. L. & ROGERS, J. 2009. Microglial responses to dopamine in a cell culture model of Parkinson's disease. *Neurobiology of aging*, 30, 1805-1817.
- MCNICOL, A. & ISRAELS, S. J. 1999. Platelet dense granules: structure, function and implications for haemostasis. *Thromb Res*, 95, 1-18.
- MENA, S., CRUIKSHANK, A., BEST, J., NIJHOUT, H., REED, M. C. & HASHEMI, P. 2024. Modulation of serotonin transporter expression by escitalopram under inflammation. *Communications Biology*, 7, 710.
- MÜLLER, T., DÜRK, T., BLUMENTHAL, B., GRIMM, M., CICKO, S., PANTHER, E., SORICHTER, S., HEROUY, Y., DI VIRGILIO, F., FERRARI, D., NORGAUER, J. & IDZKO, M. 2009. 5-hydroxytryptamine modulates migration, cytokine and chemokine release and T-cell priming capacity of dendritic cells in vitro and in vivo. *PLoS One*, 4, e6453.
- NESBITT, K. M., JAQUINS-GERSTL, A., SKODA, E. M., WIPF, P. & MICHAEL, A. C. 2013. Pharmacological Mitigation of Tissue Damage during Brain Microdialysis. *Analytical Chemistry*, 85, 8173-8179.
- NOLAN, R., MUIR, R., RUNNER, K., HADDAD, E. & GASKILL, P. 2019. Role of macrophage dopamine receptors in mediating cytokine production: implications for neuroinflammation in the context of HIV-associated neurocognitive disorders. *Journal of Neuroimmune Pharmacology*, 14, 134-156.
- O'SULLIVAN, J. B., RYAN, K. M., CURTIN, N. M., HARKIN, A. & CONNOR, T. J. 2009. Noradrenaline reuptake inhibitors limit neuroinflammation in rat cortex following a systemic inflammatory challenge: implications for depression and neurodegeneration. *International Journal of Neuropsychopharmacology*, 12, 687-699.
- O'DONNELL, J., ZEPPENFELD, D., MCCONNELL, E., PENA, S. & NEDERGAARD, M. 2012. Norepinephrine: a neuromodulator that boosts the function of multiple cell types to optimize CNS performance. *Neurochemical research*, 37, 2496-2512.
- ONORE, C., CAREAGA, M. & ASHWOOD, P. 2012. The role of immune dysfunction in the pathophysiology of autism. *Brain, behavior, and immunity*, 26, 383-392.
- PEARSON-LEARY, J., OSBORNE, D. M. & MCNAY, E. C. 2016. Role of glia in stress-induced enhancement and impairment of memory. *Frontiers in integrative neuroscience*, 9, 63.
- PIHEL, K., HSIEH, S., JORGENSON, J. W. & WIGHTMAN, R. M. 1995. Electrochemical detection of histamine and 5-hydroxytryptamine at isolated mast cells. *Analytical chemistry*, 67, 4514-4521.
- PIRZGALSKA, R. M., SEIXAS, E., SEIDMAN, J. S., LINK, V. M., SÁNCHEZ, N. M., MAHÚ, I., MENDES, R., GRES, V., KUBASOVA, N. & MORRIS, I. 2017. Sympathetic neuron-associated macrophages contribute to obesity by importing and metabolizing norepinephrine. *Nature medicine*, 23, 1309-1318.

- PISSADAKI, E. K. & BOLAM, J. P. 2013. The energy cost of action potential propagation in dopamine neurons: clues to susceptibility in Parkinson's disease. *Frontiers in computational neuroscience*, 7, 13.
- POLTORAK, A., HE, X., SMIRNOVA, I., LIU, M.-Y., HUFFEL, C. V., DU, X., BIRDWELL, D., ALEJOS, E., SILVA, M. & GALANOS, C. 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science*, 282, 2085-2088.
- QIAN, Y., MELIKIAN, H. E., RYE, D. B., LEVEY, A. I. & BLAKELY, R. D. 1995. Identification and characterization of antidepressant-sensitive serotonin transporter proteins using site-specific antibodies. *J Neurosci*, 15, 1261-74.
- QIN, L., WU, X., BLOCK, M. L., LIU, Y., BREESE, G. R., HONG, J. S., KNAPP, D. J. & CREWS, F. T. 2007. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia*, 55, 453-462.
- QIN, Y., HUA, M., DUAN, Y., GAO, Y., SHAO, X., WANG, H., TAO, T., SHEN, A. & CHENG, C. 2012. TNF- α expression in Schwann cells is induced by LPS and NF- κ B-dependent pathways. *Neurochemical research*, 37, 722-731.
- RUDNICK, G. 1977. Active transport of 5-hydroxytryptamine by plasma membrane vesicles isolated from human blood platelets. *J Biol Chem*, 252, 2170-4.
- SATO, D., HAMADA, Y., NARITA, M., MORI, T., TEZUKA, H., SUDA, Y., TANAKA, K., YOSHIDA, S., TAMURA, H. & YAMANAKA, A. 2022. Tumor suppression and improvement in immune systems by specific activation of dopamine D1-receptor-expressing neurons in the nucleus accumbens. *Molecular brain*, 15, 17.
- SCANZANO, A. & COSENTINO, M. 2015. Adrenergic regulation of innate immunity: a review. *Frontiers in pharmacology*, 6, 171.
- SCHILDKRAUT, J. J. 1965. The catecholamine hypothesis of affective disorders: a review of supporting evidence. *American journal of Psychiatry*, 122, 509-522.
- SILVERMAN, A.-J., SUTHERLAND, A. K., WILHELM, M. & SILVER, R. 2000. Mast Cells Migrate from Blood to Brain. *The Journal of Neuroscience*, 20, 401-408.
- SINGH, A. K. & JIANG, Y. 2004. How does peripheral lipopolysaccharide induce gene expression in the brain of rats? *Toxicology*, 201, 197-207.
- SOMPAYRAC, L. M. 2022. *How the immune system works*, John Wiley & Sons.
- SONG, C., HORROBIN, D. & LEONARD, B. 2006. The comparison of changes in behavior, neurochemistry, endocrine, and immune functions after different routes, doses and durations of administrations of IL-1 β in rats. *Pharmacopsychiatry*, 39, 88-99.
- SONG, S., JIANG, L., OYARZABAL, E. A., WILSON, B., LI, Z., SHIH, Y.-Y. I., WANG, Q. & HONG, J.-S. 2019a. Loss of Brain Norepinephrine Elicits Neuroinflammation-Mediated Oxidative Injury and Selective Caudo-Rostral Neurodegeneration. *Molecular Neurobiology*, 56, 2653-2669.
- SONG, S., WANG, Q., JIANG, L., OYARZABAL, E., RIDDICK, N. V., WILSON, B., MOY, S. S., SHIH, Y.-Y. I. & HONG, J.-S. 2019b. Noradrenergic dysfunction accelerates LPS-elicited inflammation-related ascending sequential neurodegeneration and deficits in non-motor/motor functions. *Brain, Behavior, and Immunity*, 81, 374-387.
- STOLK, R. F., VAN DER PASCH, E., NAUMANN, F., SCHOUWSTRA, J., BRESSERS, S., VAN HERWAARDEN, A. E., GERRETSEN, J., SCHAMBERGEN, R., RUTH, M. M. & VAN DER HOEVEN, J. G. 2020. Norepinephrine dysregulates the immune response and compromises host defense during sepsis. *American journal of respiratory and critical care medicine*, 202, 830-842.

- TAKEUCHI, O., HOSHINO, K., KAWAI, T., SANJO, H., TAKADA, H., OGAWA, T., TAKEDA, K. & AKIRA, S. 1999. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity*, 11, 443-451.
- TANG, L., JENNINGS, T. A. & EATON, J. W. 1998. Mast cells mediate acute inflammatory responses to implanted biomaterials. *Proceedings of the National Academy of Sciences*, 95, 8841-8846.
- TEUNIS, M. A., HEIJNEN, C. J., COOLS, A. R. & KAVELAARS, A. 2004. Reduced splenic natural killer cell activity in rats with a hyperreactive dopaminergic system. *Psychoneuroendocrinology*, 29, 1058-1064.
- THOPPIL, J., MEHTA, P., BARTELS, B., SHARMA, D. & FARRAR, J. D. 2023. Impact of norepinephrine on immunity and oxidative metabolism in sepsis. *Frontiers in immunology*, 14, 1271098.
- TROTTA, T., PORRO, C., CALVELLO, R. & PANARO, M. A. 2014. Biological role of Toll-like receptor-4 in the brain. *Journal of neuroimmunology*, 268, 1-12.
- VAN HEESCH, F., PRINS, J., KONSMAN, J. P., KORTE-BOUWS, G. A., WESTPHAL, K. G., RYBKA, J., OLIVIER, B., KRANEVELD, A. D. & KORTE, S. M. 2014. Lipopolysaccharide increases degradation of central monoamines: an in vivo microdialysis study in the nucleus accumbens and medial prefrontal cortex of mice. *European Journal of Pharmacology*, 725, 55-63.
- VAURE, C. & LIU, Y. 2014. A comparative review of toll-like receptor 4 expression and functionality in different animal species. *Frontiers in immunology*, 5, 316.
- VRIEND, C. Y., ZUO, L., DYCK, D. G., NANCE, D. M. & GREENBERG, A. H. 1993. Central administration of interleukin-1 β increases norepinephrine turnover in the spleen. *Brain research bulletin*, 31, 39-42.
- WATANABE, Y., NAKAYAMA, T., NAGAKUBO, D., HIESHIMA, K., JIN, Z., KATOU, F., HASHIMOTO, K. & YOSHIE, O. 2006. Dopamine selectively induces migration and homing of naive CD8⁺ T cells via dopamine receptor D3. *The journal of immunology*, 176, 848-856.
- WOOLLEY, D. W. & SHAW, E. 1954. A biochemical and pharmacological suggestion about certain mental disorders. *Proceedings of the National Academy of Sciences*, 40, 228-231.
- ZHU, C.-B., LINDLER, 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, 2510-2520.

CHAPTER 3 – THREE PIECES OF THE SAME PUZZLE: HOW DEPRESSION THEORIES CONVERGE AT INFLAMMATION²

² Baumberger, B., **Batey, L.**, Hashemi, P. Three Pieces of the Same Puzzle: How Depression Theories Converge at Inflammation. *Discover Mental Health*. 2025. *Under Review*

Abstract

Three main theories of depression have been developed: the monoamine, plasticity and inflammation theories. While each theory has been bolstered by decades of excellent scientific evidence, individually each idea falls short for developing universally effective treatments for depression. In this perspective, we present the history and development of each theory and discuss the therapies that follow each hypothesis. We conclude that these theories complement each other and are smaller parts of a larger puzzle. We suggest that future therapies should involve all three: monoamines, plasticity and inflammation.

Introduction

Human health has improved in many ways over the last century with one exception; mental health. Incidences of mental health disorders are continually rising, with an estimated 300 million people globally diagnosed with depression alone. This number (which is likely an underestimate due to stigma and lack of access to healthcare) renders depression one of the globe's most prevalent disorders.

Diagnosis and treatment of depression are inconsistent and controversial because the underlying pathology of this illness has yet to be fully elucidated. There have been three major theories of depression: the monoamine, plasticity and inflammation theories. There is compelling evidence for each theory, but individually they fail to explain the phenotype and treat the symptoms of depression. In this perspective, we will cover the development of these major theories and discuss their associated therapies. We speculate that these theories are not discrete but rather form small and synergistic pieces of the larger puzzle of depression pathology, around the central theme of inflammation. Finally, we suggest that future therapies should be designed around all three aspects: monoamines, plasticity and inflammation.

Theories of Depression

Until the 1950s, depression was so ill-understood that treatments were arbitrary and, at times, cruel. Thousands of years ago, depression was a spiritual issue, and treatments involved trephinations (Robison et al., 2012). In the 1600s, the ancient Greeks employed bloodletting and purging to restore balance to the humors of the body (Giraldi, 2017).

Post 1600s, depressed individuals were institutionalized and isolated from other humans, treated with ice baths and restraint techniques (Braslow, 2023). Little progress was made until the early 1900s, when the world entered a modern age of medicine that included important drug discoveries, including insulin (Rosenfeld, 2002). Insulin coma therapy was the first time drugs were used for depression. Here, patients were forced into a hypoglycemic coma under the rationale that this state would “reset” the brain (Sakel, 1938). Around the same time, Metrazol, a nervous system stimulant, was used to induce seizures to the same effect (Cummins, 1940). This precipitated the highly controversial electroconvulsive therapy (ECT) in the 1940s as an alternative to Metrazol because it enabled more precise control of the duration and intensity of the seizures (Shorter and Healy, 2007). Perhaps the most infamous depression treatment was the lobotomy, which was first introduced around the same time. A celebrated therapy at this time (Egas Moniz won the Nobel Prize in Medicine for the lobotomy in 1949) (AB, 1949), this treatment was later recognized as unethical and harmful. These early 20th century treatments became obsolete in the 1950s, when chemical theories of depression began to be developed.

Monoamine Theory of Depression

“Depression is caused by a deficiency of serotonin, dopamine and norepinephrine” Mid 1960s.

The 1950s were a time of rapid growth for biochemistry and drug development and transformed how the scientific and medical communities viewed depression. Scientists at Hoffmann La Roche discovered interesting ‘side effects’ of a new compound they were

trialing for tuberculosis (O'Connor, 1953). These side effects included 'euphoria', improvements in sleep and better appetite. This drug, iproniazid, kills bacteria by inhibiting the synthesis of mycolic acid, a key component of the bacterial cell wall, but is also a monoamine oxidase inhibitor (MAOI). Monoamine oxidase is found at high concentrations in the brain and is the key enzyme in the brain that is tasked with catabolizing monoamine transmitters (serotonin, dopamine and norepinephrine). Iproniazid was the first antidepressant and was used off-label to treat depression in 1958. At the same time, scientists started to investigate the roles of neurotransmitters in depression and to develop more selective drugs.

Imipramine, first synthesized by Hafliger and Schindler (Schindler and Häfliger, 1954), was originally investigated as an antipsychotic treatment. This agent did not have antipsychotic effects in patients but was found to improve depression symptoms (Lehmann et al., 1958, KUHN, 1958). Imipramine was approved by the FDA to treat depression in 1959. It is a tricyclic antidepressant (TCA) that inhibits the reuptake of norepinephrine and serotonin back into neurons (Schatzberg, 1998).

Seminal studies in the mid-1960s found that serotonin levels were lower in the brain of depressed suicide patients (Shaw et al., 1967), and norepinephrine levels were reduced in the cerebrospinal fluid and urine of depression patients (Bunney and Davis, 1965). These findings, taken together with the pharmacological success of MAOIs and TCAs, propelled the development of a new theory of depression. This new theory, the monoamine hypothesis of depression (Schildkraut, 1965), postulates that depression is caused by a deficiency of serotonin, dopamine and norepinephrine. As such, Eli Lilly

started developing agents to selectively inhibit serotonin reuptake *via* the serotonin transporters, under the rationale that this effect would correct a serotonin deficiency. In 1974, the first paper describing a selective serotonin reuptake inhibitor (SSRI) was published (Wong et al., 1974, Fuller et al., 1974), the agent was named LY110140 or fluoxetine and approved by the FDA and marketed as Prozac ^(™) in 1987 (Wong et al., 2005).

A rich and varied body of experimental literature has studied monoamine roles in depressive disorder (Jauhar et al., 2023, Fakhoury, 2016, Belujon and Grace, 2017, Felger, 2017, Seki et al., 2018, Maletic et al., 2017). While it is not yet possible to measure human brain monoamines as a biomarker for depression, exciting recent work in humans using positron emission tomography showed that the release of serotonin was reduced in depressed vs. control patients (Erritzoe et al., 2023). Thus, this might represent an exciting new direction for biomarker research. Since Prozac ^(™), an array of serotonin, norepinephrine and dopamine targeting agents have been approved (and some taken off the market) for depression (Cipriani et al., 2018, Choi, 2003). Of these, a small subset is routinely prescribed to vast numbers of people. These agents have helped millions of people and represent huge advances in depression treatment over the last century. However, in recent years, the low success rate, bad side effects and delayed onset (patients typically must take SSRIs for weeks for clinical effectiveness) of therapeutic actions of SSRIs have spurred the medical community to seek alternative theories of depression.

“Depression is caused by impairments in the brain’s ability to adapt and generate new neurons and neuronal connections” Mid 1990s

A healthy brain can reorganize itself by forming new neural connections. This process, called neuroplasticity, allows the brain to learn new information, recover from damage and adapt in response to new experiences and changes in the environment. In two specific brain regions, the hippocampus and the subventricular zone, the brain can also actually regenerate itself, in a process called neurogenesis (Alvarez-Buylla and García-Verdugo, 2002, Eriksson et al., 1998).

The hippocampus became a critical area of interest in depression studies in the mid-1990s because scientists found, utilizing fMRI, loss of hippocampal volume in a group of older women with recurrent major depression (Sheline et al., 1996). The same group confirmed their original report in a later study and showed a correlation between hippocampal volume and the duration of depression (Sheline et al., 1999). Other work during this period found a similar loss of hippocampal volume in post-traumatic stress disorder patients (Bremner et al., 1995). These clinical findings were supported by a series of animal studies earlier that decade, which found that acute stress suppressed the rate of dentate gyrus (part of the hippocampal formation) neurogenesis in adults of several species (Sapolsky, 1996a).

The plasticity hypothesis of depression was solidified in a seminal Society for Neuroscience Meeting in 1999 where Barry Jacobs and his colleagues presented a paper entitled “Chronic Fluoxetine Treatment Increases Hippocampal Neurogenesis in Rats: A

80

Novel Theory of Depression”. Here, they showed that chronic fluoxetine increased hippocampal neurogenesis in rats (70% increase in the number of cells) (Jacobs and Fornal, 1999). Other work supported this finding into the 2000s (Encinas et al., 2006, Malberg et al., 2000), and eventually the same was found clinically in humans (Frodl et al., 2008, Sheline et al., 2003). However, hippocampal volume was not successful as a biomarker for depression because of individual variability in hippocampal size and overlap with other illnesses (Amico et al., 2011, Ruchinskias et al., 2022, Gosnell et al., 2020).

The proposed mechanism for the neuroplasticity theory is that stress and depression induce glucocorticoids (stress hormones) which decrease glutamate (Di et al., 2009) and serotonin levels (Haleem, 2022, Lanfumey et al., 2008, Sapolsky, 1996b). Decreased glutamate can cause widespread morphological changes and even cell death in the hippocampus (Zhang and Bhavnani, 2006). Serotonin is a well-known neuroplastic factor (Kraus et al., 2017), however the inherent question here is can glutamate itself be pharmacologically targeted for depression?

Ketamine

Ketamine is an NMDA (glutamate) receptor antagonist (Moghaddam et al., 1997) which caused much excitement as a potential antidepressant in 2000 with the publication of its first double-blind placebo-controlled trial for major depression (Berman et al., 2000). A contributing factor to the buzz around ketamine was the rapidity of onset as an antidepressant. Whereas SSRIs typically need to be administered for several weeks for clinical effects, several studies have shown that antidepressant effects could be

achieved within hours or days of a single subanesthetic dose of ketamine given intravenously (Berman et al., 2000, Zarate et al., 2006, Aan Het Rot et al., 2010, Murrough et al., 2013a, Murrough et al., 2013b).

The mechanisms for this rapid onset of action are still being studied (Zanos and Gould, 2018, Krystal et al., 2024), but it has been found that a single ketamine dose rapidly increases the number and function of synapses. This plasticity is thought to arise from a surge of glutamate that causes the release of brain-derived neurotrophic factor (BDNF), activation of mechanistic target of rapamycin complex 1 (mTORC1) (Li et al., 2010) and tropomyosin-related kinase receptor B - protein kinase B (TrkB-Akt) signaling, and the rapid synthesis of synaptic proteins that contribute to the formation of new synapses (see **Figure 3.1**) (Duman, 2018, Li et al., 2010, Li et al., 2011).

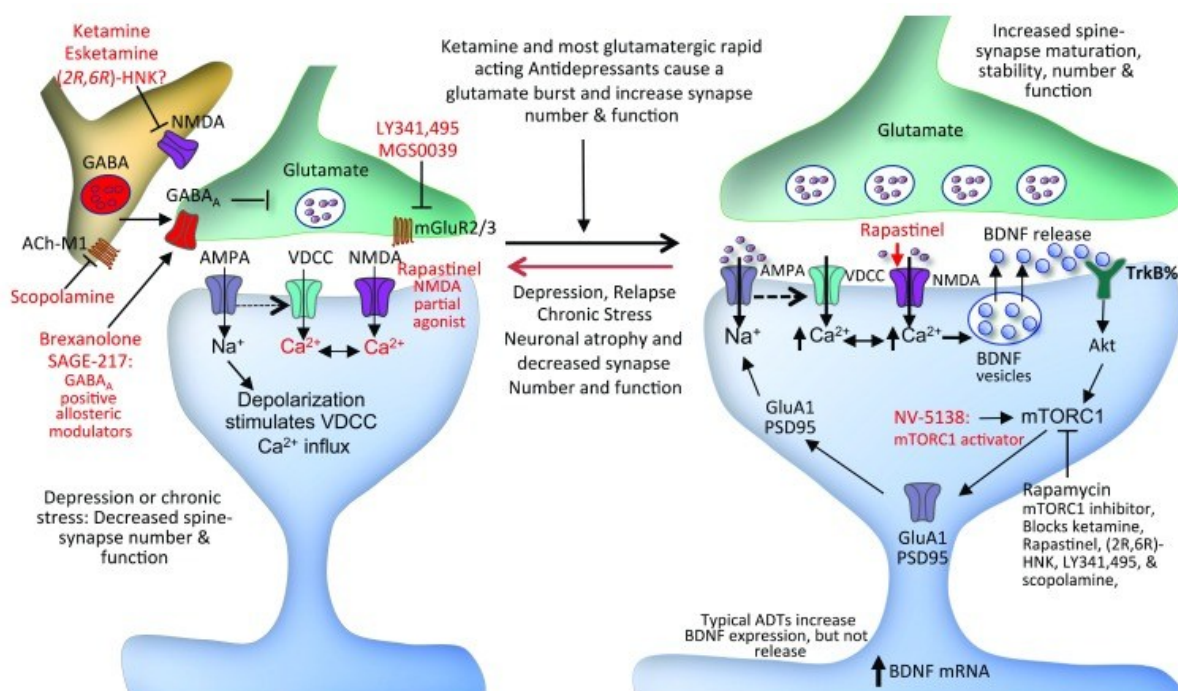


Figure 3.1 - Mechanism of ketamine action on plasticity, taken from Duman (Duman, 2018).

While the effects of ketamine on plasticity are inconsistent, one study found that the antidepressant effects of ketamine are independent of the effects on neuroplasticity, implying other nuances in the mechanisms of action (Soumier et al., 2016). In addition, ketamine's effectiveness as an antidepressant is variable and very difficult to compare to SSRIs. This is because typically, only patients who do not respond to SSRIs undergo ketamine therapy. Moreover, there are ethical and safety concerns around ketamine because of its potential for abuse and its toxicity (Zhang et al., 2016, Singh et al., 2017, Zhang and Ho, 2017).

As ketamine was being explored for its antidepressant effects, a parallel new idea was brewing. It is well known that psychedelics are neuroplastic agents (Vollenweider and Kometer, 2010), and after some clinical success using psychedelics to treat obsessive compulsive disorder (Moreno et al., 2006) and tobacco (Johnson et al., 2014) and alcohol addiction (Bogenschutz et al., 2015), a natural new direction was to explore psychedelics in depression.

Psychedelics

Psychedelics, such as psilocybin, are powerful serotonergic hallucinogens that alter perception and affect cognitive processes (Nichols, 2016). Importantly, these agents are known to affect mood (Nichols, 2016). There were clinical studies in the mid-2010s, using small patient cohorts (10-20 patients per study), that found promise in psychedelic therapy for recurrent or treatment resistant depression (Gasser et al., 2014, Griffiths et al., 2016, Grob et al., 2011, Ross et al., 2016).

A key feature of these studies is that the psychedelic was administered in conjunction with psychotherapy. Psychotherapy is known to change brain function (Straub et al., 2015) in a way that suggests plasticity is involved (Goldapple et al., 2004, Kunas et al., 2019). One notion is that the connections associated with painful memories/stress/trauma are gradually replaced and/or overwritten with a larger number of connections that cue more positive emotions.

Therefore, one way to integrate psychotherapy with psychedelic therapy is to suppose that serotonergic psychedelics render the effect of psychotherapy more effective and efficient. Indeed, it was hypothesized by Carhart Harris *et al.* that the therapeutic effects of psychedelics are “*fundamentally reliant on context*” (Carhart-Harris et al., 2018). Hence, providing psychological support and a positive environment (good atmosphere) is a key for clinical success. Not providing the correct environment or support means that the treatment may not only be clinically ineffective but also potentially harmful (Erritzoe and Richards, 2017). As such, the state of the art psychedelic treatment involves the patients being treated with psilocybin in a custom-designed treatment room with low lighting, music and full support from two psychiatrists at all times (Carhart-Harris et al., 2016). The neuroplastic effects of the psychedelics, therefore, are very much guided by a positive environment and psychological guidance.

In sum, ketamine and psychedelics are exciting avenues of research for depression that are being actively trialed and explored. There are, however, concerns that both are controlled substances with abuse potential and limited effectiveness. Therefore, the

community is eagerly anticipating the results of large-scale trials for drug efficacy and safety.

Cytokine/Inflammation Theory of Depression

“Depression is caused by immune activation” Early 1990s

In parallel to the plasticity theory of depression, another hypothesis was being developed, based on inflammation. In 1991, Ronald Smith, professor of Chemistry and Nutrition at Gavilan College, published a new hypothesis of depression in a paper entitled “The Macrophage Theory of Depression” (Smith, 1991). In the work, he argued that cytokine secretion was the cause of depression under several points of rationale, but most importantly, human volunteers that were treated with cytokines developed depressive-like behaviors (Niiranen et al., 1988). This notion was quickly backed up in 2 papers from Maes *et al.* who suggested that sickness behavior and depression share common pathways in the form of immune activation (Maes et al., 1995a, Maes et al., 1995b). Since these early papers, a superfluity of studies have linked inflammation to depression, with a comprehensive summary of the literature provided in a review by Nemeroff and colleagues (Beurel et al., 2020).

It is essentially undisputed that chronic illnesses (including chronic stress) and depression are comorbid and the precise mechanisms of how inflammation drives behavior are still being elucidated. This mechanism is clearly considerably complicated involving blood brain barrier function (Turkheimer et al., 2023) and overproduction of proinflammatory cytokines, e.g. tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-85

6), and interleukin-1 beta (IL-1 β) (Raison et al., 2010). Cytokines have been shown to directly affect brain function by altering neurotransmitters (Dunn, 2006, Miller et al., 2013), in particular the mood driving serotonin, dopamine, and norepinephrine. The effects of inflammation on these monoamine levels in the brain has recently been reviewed by Batey *et al* (Batey et al., 2024). Lipopolysaccharide (LPS) induced inflammation, through varied and nuanced mechanisms involving the blood brain barrier (Medina-Rodriguez and Beurel, 2022), reduces the brain's serotonin and dopamine signaling and increases norepinephrine signaling (Batey et al., 2024). This effect on mood-altering neurotransmitters begs the question, why does inflammation induce depressive behavior? Early evolutionary immune pressures were primarily pathogenic in nature and the mood response was directed towards energy conservation for healing and recovery. In modern society, where pathogens are less of a challenge, social issues (e.g. stress) have become the predominant immune activator, as seen in **Figure 3.2**. The latter is less tangibly resolved and can often be chronic (Miller and Raison, 2016).

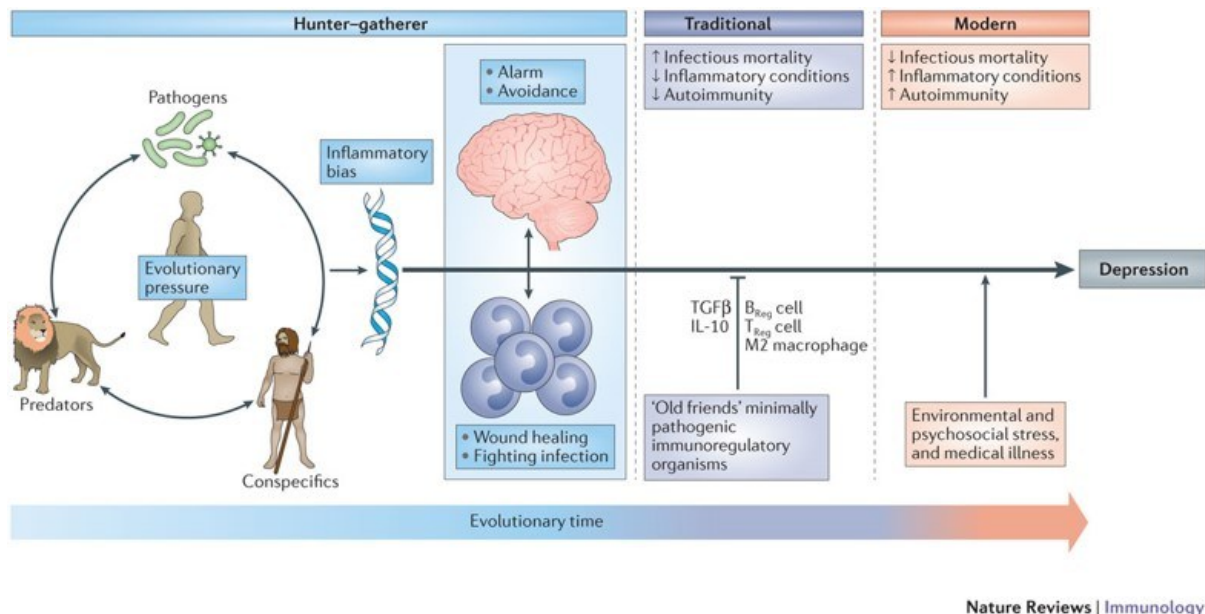


Figure 3.2 - Evolutionary hypothesis of inflammation and depression. The human immune system has inherited an evolutionary propensity toward inflammatory responses, which were historically shaped by pressures from pathogens, predators, and social threats. This ‘inflammatory bias’ involved immune and behavioral adjustments designed to conserve energy for wound healing and infection control, while increasing alertness to potential dangers. Throughout most of human evolution, this inflammatory bias was likely regulated by frequent exposure to microorganisms in traditional, rural environments. Such exposure promoted immunoregulatory mechanisms, including the induction of TReg and BReg cells, M2 macrophages, and the secretion of anti-inflammatory cytokines like IL-10 and TGFβ. However, in modern urban societies where exposure to infectious agents is minimal and psychological stressors are abundant, this immunoregulatory balance is disrupted. Therefore, evolutionary immune and behavioral patterns may be inappropriately activated, causing higher rates of inflammation-associated conditions, such as depression. Taken from Miller et al (Miller and Raison, 2016).

There is compelling evidence to show that patients with high levels of inflammation are more likely to be resistant to conventional SSRI treatment (Haroon et al., 2018). In animal work, SSRIs were shown to be less capable of restoring serotonin levels under inflammation. This was due to inflammation-induced brain histamine which inhibits

serotonin *via* H₃ receptors (Hersey et al., 2021). **Figure 3.3** shows basal serotonin measurements in the mouse hippocampus. Two cohorts (red and purple) received peripheral LPS and very rapidly the serotonin levels declined. Serotonin levels could not be recovered to control values with escitalopram alone (red). The co-application of an SSRI with a pharmacological agent that inhibited histamine synthesis (purple) was necessary to resolve the serotonin levels to control values. Therefore, utilizing anti-inflammatory therapies may represent an exciting new avenue for depression.

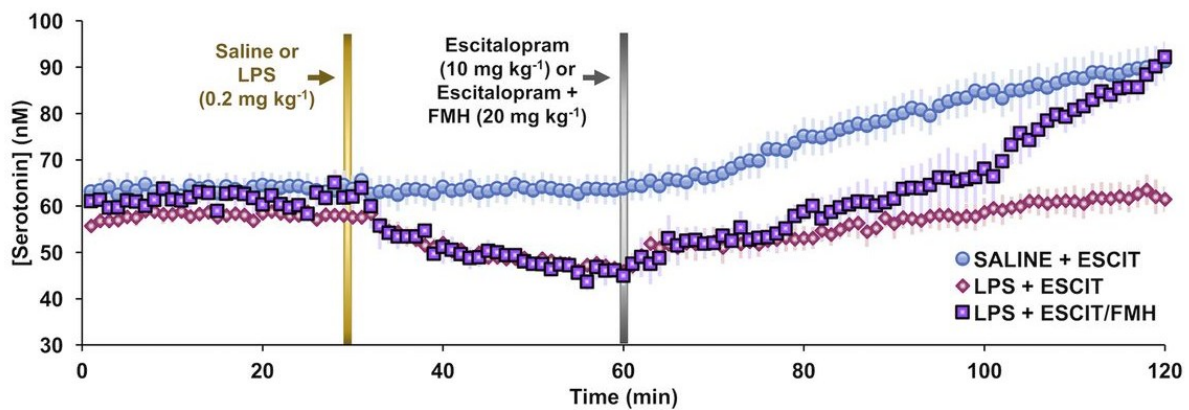


Figure 3.3 - Peripheral LPS effects on brain serotonin. LPS – lipopolysaccharide; FMH – α -fluoromethylhistidine; ESCIT – escitalopram. Taken and modified from Hersey et al (Hersey et al., 2021).

The literature on using anti-inflammatories as a depression treatment is mixed but shows some promise (Köhler et al., 2014, Köhler-Forsberg et al., 2019, Bai et al., 2020b, Husain et al., 2017). Non-steroidal anti-inflammatory drugs (NSAIDs) and cytokine inhibitors are the main therapies used (Du et al., 2024, Hang et al., 2021), but there are also many adjuncts such as statins (Köhler-Forsberg et al., 2019). Interestingly, ketamine and psychedelics are also considered to have anti-inflammatory properties (Nichols, 2022).

A challenge with this anti-inflammatory treatment is dosage. Recent reviews of the clinical literature that correlate markers of inflammation to depression concluded that inflammatory responses in depressed patients were different from control patients in approximately one-third of subjects (similar to SSRIs). The studies reviewed disagreed on the direction and the magnitude of the differences (Strawbridge et al., 2017, Bai et al., 2020a, Köhler-Forsberg et al., 2019). This is an extremely important aspect to decode when trying to treat depression with anti-inflammatories since there is evidence showing that using anti-inflammatory agents in patients without inflammation can lead to adverse outcomes (Arteaga-Henríquez et al., 2019).

In sum, the inflammation theory of depression describes a potential behavioral outcome of the evolutionary response to acute and chronic illnesses and to modern stressors. Anti-inflammatories serve as an exciting new therapy for the treatment of depression with the remaining challenges for type, dose and side effects.

Three Theories: One Story

It is clear to see, now, that the three main theories of depression are small parts of the same jigsaw puzzle. During inflammation, the body has evolved to conserve energy to recover, thus, mood-driving monoamine levels change. While the sickness behavior serves its purpose evolutionarily, in modern sanitized societies, the response is synonymous with stress. Low serotonin is not a cause but a consequence of depression and can serve as an indicator to diagnose and monitor depression progression. Targeting serotonin does not necessarily help patients recover from depression, since the cause

of depression lies in inflammation rather than a singular marker in serotonin. This issue leaves the majority of depressed patients (with differing levels of inflammation) partially or totally unresponsive to SSRIs.

This lower serotonin, along with lower glutamate and other facets of inflammation, decreases hippocampal plasticity which can be recovered to differing extents with SSRIs, ketamine and psychedelics, though none are universally efficacious. Again, this is because the loss of neuronal connections is a result (marker) and not the cause of depression.

The issue of targeting inflammation is complicated. A big challenge is creating a robust system to measure neuroinflammation as a biomarker of depression. Without this, we don't know how and when to intervene in the inflammation chain to stop the detrimental effects on mood.

To conclude, we propose that the monoamine, plasticity and inflammation theories of depression are not discrete or in competition with each other. They are, at their core, foundational parts of a larger story. Therefore, improving future treatments is critically dependent on accurately and chemically defining 'neuroinflammation' status and considering all three theories for drug discovery.

References

- AAN HET ROT, M., COLLINS, K. A., MURROUGH, J. W., PEREZ, A. M., REICH, D. L., CHARNEY, D. S. & MATHEW, S. J. 2010. Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. *Biological psychiatry*, 67, 139-145.
- AB, N. P. O. 1949. "The Nobel Prize in Physiology or Medicine 1949 - António Egas Moniz".
- ALVAREZ-BUYLLA, A. & GARCÍA-VERDUGO, J. M. 2002. Neurogenesis in Adult Subventricular Zone. *The Journal of Neuroscience*, 22, 629-634.
- AMICO, F., MEISENZAHN, E., KOUTSOULERIS, N., REISER, M., MÖLLER, H.-J. & FRODL, T. 2011. Structural MRI correlates for vulnerability and resilience to major depressive disorder. *Journal of Psychiatry and Neuroscience*, 36, 15-22.
- ARTEAGA-HENRÍQUEZ, G., SIMON, M. S., BURGER, B., WEIDINGER, E., WIJKHUIJS, A., AROLT, V., BIRKENHAGER, T. K., MUSIL, R., MÜLLER, N. & DREXHAGE, H. A. 2019. Low-grade inflammation as a predictor of antidepressant and anti-inflammatory therapy response in MDD patients: a systematic review of the literature in combination with an analysis of experimental data collected in the EU-MOODINFLAME consortium. *Frontiers in psychiatry*, 10, 458.
- BAI, S., GUO, W., FENG, Y., DENG, H., LI, G., NIE, H., GUO, G., YU, H., MA, Y., WANG, J., CHEN, S., JING, J., YANG, J., TANG, Y. & TANG, Z. 2020a. Efficacy and safety of anti-inflammatory agents for the treatment of major depressive disorder: a systematic review and meta-analysis of randomised controlled trials. *Journal of Neurology, Neurosurgery & Psychiatry*, 91, 21-32.
- BAI, S., GUO, W., FENG, Y., DENG, H., LI, G., NIE, H., GUO, G., YU, H., MA, Y., WANG, J., CHEN, S., JING, J., YANG, J., TANG, Y. & TANG, Z. 2020b. Efficacy and safety of anti-inflammatory agents for the treatment of major depressive disorder: a systematic review and meta-analysis of randomised controlled trials. *Journal of Neurology, Neurosurgery & Psychiatry*, 91, 21-32.
- BATEY, L., BAUMBERGER, B., KHOSHBOUEI, H. & HASHEMI, P. 2024. Lipopolysaccharide Effects on Neurotransmission: Understanding Implications for Depression. *ACS Chemical Neuroscience*.
- BELUJON, P. & GRACE, A. A. 2017. Dopamine system dysregulation in major depressive disorders. *International Journal of Neuropsychopharmacology*, 20, 1036-1046.
- BERMAN, R. M., CAPPIELLO, A., ANAND, A., OREN, D. A., HENINGER, G. R., CHARNEY, D. S. & KRYSTAL, J. H. 2000. Antidepressant effects of ketamine in depressed patients. *Biological Psychiatry*, 47, 351-354.
- BEUREL, E., TOUPS, M. & NEMEROFF, C. B. 2020. The Bidirectional Relationship of Depression and Inflammation: Double Trouble. *Neuron*, 107, 234-256.
- BOGENSCHUTZ, M. P., FORCEHIMES, A. A., POMMY, J. A., WILCOX, C. E., BARBOSA, P. & STRASSMAN, R. J. 2015. Psilocybin-assisted treatment for alcohol dependence: A proof-of-concept study. *Journal of Psychopharmacology*, 29, 289-299.
- BRASLOW, J. 2023. *Mental ills and bodily cures: Psychiatric treatment in the first half of the twentieth century*, Univ of California Press.
- BREMNER, J. D., RANDALL, P., SCOTT, T. M., BRONEN, R. A., SEIBYL, J. P., SOUTHWICK, S. M., DELANEY, R. C., MCCARTHY, G., CHARNEY, D. S. & INNIS, R. B. 1995. MRI-

- based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. *Am J Psychiatry*, 152, 973-81.
- BUNNEY, W. E. & DAVIS, J. M. 1965. Norepinephrine in depressive reactions: A review. *Archives of General Psychiatry*, 13, 483-494.
- CARHART-HARRIS, R. L., BOLSTRIDGE, M., RUCKER, J., DAY, C. M. J., ERRITZOE, D., KAELEN, M., BLOOMFIELD, M., RICKARD, J. A., FORBES, B., FEILDING, A., TAYLOR, D., PILLING, S., CURRAN, V. H. & NUTT, D. J. 2016. Psilocybin with psychological support for treatment-resistant depression: an open-label feasibility study. *The Lancet Psychiatry*, 3, 619-627.
- CARHART-HARRIS, R. L., ROSEMAN, L., HAIJEN, E., ERRITZOE, D., WATTS, R., BRANCHI, I. & KAELEN, M. 2018. Psychedelics and the essential importance of context. *Journal of Psychopharmacology*, 32, 725-731.
- CHOI, S. 2003. Nefazodone (Serzone) withdrawn because of hepatotoxicity. *Cmaj*, 169, 1187.
- CIPRIANI, A., FURUKAWA, T. A., SALANTI, G., CHAIMANI, A., ATKINSON, L. Z., OGAWA, Y., LEUCHT, S., RUHE, H. G., TURNER, E. H., HIGGINS, J. P. T., EGGER, M., TAKESHIMA, N., HAYASAKA, Y., IMAI, H., SHINOHARA, K., TAJIKA, A., IOANNIDIS, J. P. A. & GEDDES, J. R. 2018. Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. *The Lancet*, 391, 1357-1366.
- CUMMINS, J. A. 1940. THE TREATMENT OF MENTAL ILLNESSES WITH METRAZOL : (Preliminary Report on Seventy-six Patients). *Can Med Assoc J*, 42, 39-45.
- DI, S., MAXSON, M. M., FRANCO, A. & TASKER, J. G. 2009. Glucocorticoids regulate glutamate and GABA synapse-specific retrograde transmission via divergent nongenomic signaling pathways. *J Neurosci*, 29, 393-401.
- DU, Y., DOU, Y., WANG, M., WANG, Y., YAN, Y., FAN, H., FAN, N., YANG, X. & MA, X. 2024. Efficacy and acceptability of anti-inflammatory agents in major depressive disorder: a systematic review and meta-analysis. *Frontiers in Psychiatry*, 15, 1407529.
- DUMAN, R. S. 2018. Ketamine and rapid-acting antidepressants: a new era in the battle against depression and suicide. *F1000Res*, 7.
- DUNN, A. J. 2006. Effects of cytokines and infections on brain neurochemistry. *Clinical Neuroscience Research*, 6, 52-68.
- ENCINAS, J. M., VAAHTOKARI, A. & ENIKOLOPOV, G. 2006. Fluoxetine targets early progenitor cells in the adult brain. *Proceedings of the National Academy of Sciences*, 103, 8233-8238.
- ERIKSSON, P. S., PERFILIEVA, E., BJÖRK-ERIKSSON, T., ALBORN, A.-M., NORDBORG, C., PETERSON, D. A. & GAGE, F. H. 1998. Neurogenesis in the adult human hippocampus. *Nature medicine*, 4, 1313-1317.
- ERRITZOE, D., GODLEWSKA, B. R., RIZZO, G., SEARLE, G. E., AGNORELLI, C., LEWIS, Y., ASHOK, A. H., COLASANTI, A., BOURA, I. & FARRELL, C. 2023. Brain serotonin release is reduced in patients with depression: a [11C] Cimbi-36 positron emission tomography study with a d-amphetamine challenge. *Biological Psychiatry*, 93, 1089-1098.
- ERRITZOE, D. & RICHARDS, W. A. 2017. Lessons to be learned from early psychedelic therapy in Denmark. Taylor & Francis.

- FAKHOURY, M. 2016. Revisiting the serotonin hypothesis: implications for major depressive disorders. *Molecular neurobiology*, 53, 2778-2786.
- FELGER, J. C. 2017. The role of dopamine in inflammation-associated depression: mechanisms and therapeutic implications. *Inflammation-Associated Depression: Evidence, Mechanisms and Implications*, 199-219.
- FRODL, T., JÄGER, M., SMAJSTRLOVA, I., BORN, C., BOTTLENDER, R., PALLADINO, T., REISER, M., MÖLLER, H.-J. & MEISENZAHN, E. M. 2008. Effect of hippocampal and amygdala volumes on clinical outcomes in major depression: a 3-year prospective magnetic resonance imaging study. *Journal of Psychiatry and Neuroscience*, 33, 423-430.
- 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, 1161-1171.
- GASSER, P., HOLSTEIN, D., MICHEL, Y., DOBLIN, R., YAZAR-KLOSINSKI, B., PASSIE, T. & BRENNEISEN, R. 2014. Safety and Efficacy of Lysergic Acid Diethylamide-Assisted Psychotherapy for Anxiety Associated With Life-threatening Diseases. *The Journal of Nervous and Mental Disease*, 202, 513-520.
- GIRALDI, T. 2017. Melancholy and Depression. In: GIRALDI, T. (ed.) *Unhappiness, Sadness and 'Depression': Antidepressants and the Mental Disorder Epidemic*. Cham: Springer International Publishing.
- GOLDAPPLE, K., SEGAL, Z., GARSON, C., LAU, M., BIELING, P., KENNEDY, S. & MAYBERG, H. 2004. Modulation of Cortical-Limbic Pathways in Major Depression: Treatment-Specific Effects of Cognitive Behavior Therapy. *Archives of General Psychiatry*, 61, 34-41.
- GOSNELL, S. N., MEYER, M. J., JENNINGS, C., RAMIREZ, D., SCHMIDT, J., OLDHAM, J. & SALAS, R. 2020. Hippocampal volume in psychiatric diagnoses: should psychiatry biomarker research account for comorbidities? *Chronic Stress*, 4, 2470547020906799.
- GRIFFITHS, R. R., JOHNSON, M. W., CARDUCCI, M. A., UMBRIGHT, A., RICHARDS, W. A., RICHARDS, B. D., COSIMANO, M. P. & KLINEDINST, M. A. 2016. Psilocybin produces substantial and sustained decreases in depression and anxiety in patients with life-threatening cancer: A randomized double-blind trial. *Journal of Psychopharmacology*, 30, 1181-1197.
- GROB, C. S., DANFORTH, A. L., CHOPRA, G. S., HAGERTY, M., MCKAY, C. R., HALBERSTADT, A. L. & GREER, G. R. 2011. Pilot Study of Psilocybin Treatment for Anxiety in Patients With Advanced-Stage Cancer. *Archives of General Psychiatry*, 68, 71-78.
- HALEEM, D. J. 2022. Glucocorticoids in the physiological and transcriptional regulation of 5-HT_{1A} receptor and the pathogenesis of depression. *The Neuroscientist*, 28, 59-68.
- HANG, X., ZHANG, Y., LI, J., LI, Z., ZHANG, Y., YE, X., TANG, Q. & SUN, W. 2021. Comparative efficacy and acceptability of anti-inflammatory agents on major depressive disorder: a network meta-analysis. *Frontiers in Pharmacology*, 12, 691200.
- HAROON, E., DAGUANNO, A. W., WOOLWINE, B. J., GOLDSMITH, D. R., BAER, W. M., WOMMACK, E. C., FELGER, J. C. & MILLER, A. H. 2018. Antidepressant treatment

- resistance is associated with increased inflammatory markers in patients with major depressive disorder. *Psychoneuroendocrinology*, 95, 43-49.
- HERSEY, M., SAMARANAYAKE, S., BERGER, S. N., TAVAKOLI, N., MENA, S., NIJHOUT, H. F., REED, M. C., BEST, J., BLAKELY, R. D. & REAGAN, L. P. 2021. Inflammation-induced histamine impairs the capacity of escitalopram to increase hippocampal extracellular serotonin. *Journal of Neuroscience*, 41, 6564-6577.
- HUSAIN, M. I., STRAWBRIDGE, R., STOKES, P. R. & YOUNG, A. H. 2017. Anti-inflammatory treatments for mood disorders: Systematic review and meta-analysis. *Journal of Psychopharmacology*, 31, 1137-1148.
- JACOBS, B. & FORNAL, C. Chronic fluoxetine treatment increases hippocampal neurogenesis in rats: a novel theory of depression. *Soc Neurosci Abstr*, 1999, 714.
- JAUHAR, S., COWEN, P. J. & BROWNING, M. 2023. Fifty years on: Serotonin and depression. *Journal of psychopharmacology*, 37, 237-241.
- JOHNSON, M. W., GARCIA-ROMEY, A., COSIMANO, M. P. & GRIFFITHS, R. R. 2014. Pilot study of the 5-HT_{2A}R agonist psilocybin in the treatment of tobacco addiction. *Journal of Psychopharmacology*, 28, 983-992.
- KÖHLER-FORSBERG, O., N. LYDHOLM, C., HJORTHØJ, C., NORDENTOFT, M., MORS, O. & BENROS, M. E. 2019. Efficacy of anti-inflammatory treatment on major depressive disorder or depressive symptoms: meta-analysis of clinical trials. *Acta Psychiatrica Scandinavica*, 139, 404-419.
- KÖHLER, O., BENROS, M. E., NORDENTOFT, M., FARKOUH, M. E., IYENGAR, R. L., MORS, O. & KROGH, J. 2014. Effect of Anti-inflammatory Treatment on Depression, Depressive Symptoms, and Adverse Effects: A Systematic Review and Meta-analysis of Randomized Clinical Trials. *JAMA Psychiatry*, 71, 1381-1391.
- KRAUS, C., CASTRÉN, E., KASPER, S. & LANZENBERGER, R. 2017. Serotonin and neuroplasticity - Links between molecular, functional and structural pathophysiology in depression. *Neurosci Biobehav Rev*, 77, 317-326.
- KRYSTAL, J. H., KAVALLALI, E. T. & MONTEGGIA, L. M. 2024. Ketamine and rapid antidepressant action: new treatments and novel synaptic signaling mechanisms. *Neuropsychopharmacology*, 49, 41-50.
- KUHN, R. 1958. THE TREATMENT OF DEPRESSIVE STATES WITH G 22355 (IMIPRAMINE HYDROCHLORIDE). *American Journal of Psychiatry*, 115, 459-464.
- KUNAS, S. L., YANG, Y., STRAUBE, B., KIRCHER, T., GERLACH, A. L., PFLEIDERER, B., AROLT, V., WITTMANN, A., STROEHLE, A., WITTCHEN, H.-U. & LUEKEN, U. 2019. The impact of depressive comorbidity on neural plasticity following cognitive-behavioral therapy in panic disorder with agoraphobia. *Journal of Affective Disorders*, 245, 451-460.
- LANFUMEY, L., MONGEAU, R., COHEN-SALMON, C. & HAMON, M. 2008. Corticosteroid-serotonin interactions in the neurobiological mechanisms of stress-related disorders. *Neuroscience & Biobehavioral Reviews*, 32, 1174-1184.
- LEHMANN, H. E., CAHN, C. H. & DE VERTEUIL, R. L. 1958. The Treatment of Depressive Conditions with Imipramine (G 22355). *Canadian Psychiatric Association Journal*, 3, 155-164.
- LI, N., LEE, B., LIU, R.-J., BANASR, M., DWYER, J. M., IWATA, M., LI, X.-Y., AGHAJANIAN, G. & DUMAN, R. S. 2010. mTOR-Dependent Synapse Formation Underlies the Rapid Antidepressant Effects of NMDA Antagonists. *Science*, 329, 959-964.

- LI, N., LIU, R.-J., DWYER, J. M., BANASR, M., LEE, B., SON, H., LI, X.-Y., AGHAJANIAN, G. & DUMAN, R. S. 2011. Glutamate N-methyl-D-aspartate Receptor Antagonists Rapidly Reverse Behavioral and Synaptic Deficits Caused by Chronic Stress Exposure. *Biological Psychiatry*, 69, 754-761.
- MAES, M., MELTZER, H. Y., BOSMANS, E., BERGMANS, R., VANDOOOLAEGHE, E., RANJAN, R. & DESNYDER, R. 1995a. Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. *Journal of affective disorders*, 34, 301-309.
- MAES, M., VANDOOOLAEGHE, E., RANJAN, R., BOSMANS, E., BERGMANS, R. & DESNYDER, R. 1995b. Increased serum interleukin-1-receptor-antagonist concentrations in major depression. *Journal of Affective Disorders*, 36, 29-36.
- MALBERG, J. E., EISCH, A. J., NESTLER, E. J. & DUMAN, R. S. 2000. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *Journal of Neuroscience*, 20, 9104-9110.
- MALETIC, V., ERAMO, A., GWIN, K., OFFORD, S. J. & DUFFY, R. A. 2017. The role of norepinephrine and its α -adrenergic receptors in the pathophysiology and treatment of major depressive disorder and schizophrenia: a systematic review. *Frontiers in psychiatry*, 8, 42.
- MEDINA-RODRIGUEZ, E. M. & BEUREL, E. 2022. Blood brain barrier and inflammation in depression. *Neurobiology of Disease*, 175, 105926.
- MILLER, A. H., HAROON, E., RAISON, C. L. & FELGER, J. C. 2013. Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. *Depression and anxiety*, 30, 297-306.
- MILLER, A. H. & RAISON, C. L. 2016. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nature Reviews Immunology*, 16, 22-34.
- MOGHADDAM, B., ADAMS, B., VERMA, A. & DALY, D. 1997. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci*, 17, 2921-7.
- MORENO, F. A., WIEGAND, C. B., TAITANO, E. K. & DELGADO, P. L. 2006. Safety, tolerability, and efficacy of psilocybin in 9 patients with obsessive-compulsive disorder. *Journal of clinical Psychiatry*, 67, 1735-1740.
- MURROUGH, J. W., IOSIFESCU, D. V., CHANG, L. C., AL JURDI, R. K., GREEN, C. E., PEREZ, A. M., IQBAL, S., PILLEMER, S., FOULKES, A. & SHAH, A. 2013a. Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. *American Journal of Psychiatry*, 170, 1134-1142.
- MURROUGH, J. W., PEREZ, A. M., PILLEMER, S., STERN, J., PARIDES, M. K., AAN HET ROT, M., COLLINS, K. A., MATHEW, S. J., CHARNEY, D. S. & IOSIFESCU, D. V. 2013b. Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment-resistant major depression. *Biological psychiatry*, 74, 250-256.
- NICHOLS, C. D. 2022. Psychedelics as potent anti-inflammatory therapeutics. *Neuropharmacology*, 219, 109232.
- NICHOLS, D. E. 2016. Psychedelics. *Pharmacol Rev*, 68, 264-355.

- NIIRANEN, A., LAAKSONEN, R., LIVANAINEN, M., MATTSON, K., FÄRKILÄ, M. & CANTELL, K. 1988. Behavioral assessment of patients treated with alpha-interferon. *Acta Psychiatrica Scandinavica*, 78, 622-626.
- O'CONNOR, J. B. 1953. Side effects accompanying use of iproniazid. *American Review of Tuberculosis*, 68, 270-272.
- RAISON, C. L., DANTZER, R., KELLEY, K. W., LAWSON, M. A., WOOLWINE, B. J., VOGT, G., SPIVEY, J. R., SAITO, K. & MILLER, A. H. 2010. CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN- α : relationship to CNS immune responses and depression. *Molecular psychiatry*, 15, 393-403.
- ROBISON, R. A., TAGHVA, A., LIU, C. Y. & APUZZO, M. L. J. 2012. Surgery of the Mind, Mood, and Conscious State: An Idea in Evolution. *World Neurosurgery*, 77, 662-686.
- ROSENFELD, L. 2002. Insulin: Discovery and Controversy. *Clinical Chemistry*, 48, 2270-2288.
- ROSS, S., BOSSIS, A., GUSS, J., AGIN-LIEBES, G., MALONE, T., COHEN, B., MENNENGA, S. E., BELSER, A., KALLIONTZI, K., BABB, J., SU, Z., CORBY, P. & SCHMIDT, B. L. 2016. Rapid and sustained symptom reduction following psilocybin treatment for anxiety and depression in patients with life-threatening cancer: a randomized controlled trial. *Journal of Psychopharmacology*, 30, 1165-1180.
- RUCHINSKAS, R., NGUYEN, T., WOMACK, K., KHERA, A., FANG, F. Y. & KELLEY, B. J. 2022. Diagnostic utility of hippocampal volumetric data in a memory disorder clinic setting. *Cognitive and Behavioral Neurology*, 35, 66-75.
- SAKEL, M. 1938. 4. THE NATURE AND ORIGIN OF THE HYPOGLYCEMIC TREATMENT OF PSYCHOSES. *American Journal of Psychiatry*, 94, 24-40.
- SAPOLSKY, R. M. 1996a. Stress, glucocorticoids, and damage to the nervous system: the current state of confusion. *Stress*, 1, 1-19.
- SAPOLSKY, R. M. 1996b. Why Stress Is Bad for Your Brain. *Science*, 273, 749-750.
- SCHATZBERG, A. F. 1998. Noradrenergic versus serotonergic antidepressants: predictors of treatment response. *Journal of Clinical Psychiatry*, 59, 15-18.
- SCHILDKRAUT, J. J. 1965. The catecholamine hypothesis of affective disorders: a review of supporting evidence. *American journal of Psychiatry*, 122, 509-522.
- SCHINDLER, W. & HÄFLIGER, F. 1954. Über derivate des iminodibenzyls. *Helvetica Chimica Acta*, 37, 472-483.
- SEKI, K., YOSHIDA, S. & JAISWAL, M. K. 2018. Molecular mechanism of noradrenaline during the stress-induced major depressive disorder. *Neural regeneration research*, 13, 1159-1169.
- SHAW, D. M., CAMPS, F. E. & ECCLESTON, E. G. 1967. 5-Hydroxytryptamine in the hind-brain of depressive suicides. *The British Journal of Psychiatry*, 113, 1407-1411.
- SHELINE, Y. I., GADO, M. H. & KRAEMER, H. C. 2003. Untreated depression and hippocampal volume loss. *American journal of psychiatry*, 160, 1516-1518.
- SHELINE, Y. I., SANGHAVI, M., MINTUN, M. A. & GADO, M. H. 1999. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *Journal of Neuroscience*, 19, 5034-5043.
- SHELINE, Y. I., WANG, P. W., GADO, M. H., CSERNANSKY, J. G. & VANNIER, M. W. 1996. Hippocampal atrophy in recurrent major depression. *Proceedings of the National Academy of Sciences*, 93, 3908-3913.

- SHORTER, E. & HEALY, D. 2007. *Shock therapy: a history of electroconvulsive treatment in mental illness*, Rutgers University Press.
- SINGH, I., MORGAN, C., CURRAN, V., NUTT, D., SCHLAG, A. & MCSHANE, R. 2017. Ketamine treatment for depression: opportunities for clinical innovation and ethical foresight. *The Lancet Psychiatry*, 4, 419-426.
- SMITH, R. S. 1991. The macrophage theory of depression. *Medical Hypotheses*, 35, 298-306.
- SOUMIER, A., CARTER, R. M., SCHOENFELD, T. J. & CAMERON, H. A. 2016. New hippocampal neurons mature rapidly in response to ketamine but are not required for its acute antidepressant effects on neophagia in rats. *eneuro*, 3.
- STRAUB, J., PLENER, P. L., SPROEBER, N., SPRENGER, L., KOELCH, M. G., GROEN, G. & ABLER, B. 2015. Neural correlates of successful psychotherapy of depression in adolescents. *Journal of Affective Disorders*, 183, 239-246.
- STRAWBRIDGE, R., YOUNG, A. H. & CLEARE, A. J. 2017. Biomarkers for depression: recent insights, current challenges and future prospects. *Neuropsychiatric disease and treatment*, 1245-1262.
- TURKHEIMER, F. E., VERONESE, M., MONDELLI, V., CASH, D. & PARIANTE, C. M. 2023. Sickness behaviour and depression: An updated model of peripheral-central immunity interactions. *Brain, Behavior, and Immunity*, 111, 202-210.
- VOLLENWEIDER, F. X. & KOMETER, M. 2010. The neurobiology of psychedelic drugs: implications for the treatment of mood disorders. *Nature Reviews Neuroscience*, 11, 642-651.
- WONG, D. T., HORNG, J. S., BYMASTER, F. P., HAUSER, K. L. & MOLLOY, B. B. 1974. A selective inhibitor of serotonin uptake: Lilly 110140, 3-(p-Trifluoromethylphenoxy)-n-methyl-3-phenylpropylamine. *Life Sciences*, 15, 471-479.
- WONG, D. T., PERRY, K. W. & BYMASTER, F. P. 2005. The Discovery of Fluoxetine Hydrochloride (Prozac). *Nature Reviews Drug Discovery*, 4, 764-774.
- ZANOS, P. & GOULD, T. D. 2018. Mechanisms of ketamine action as an antidepressant. *Molecular Psychiatry*, 23, 801-811.
- ZARATE, C. A., JR., SINGH, J. B., CARLSON, P. J., BRUTSCHE, N. E., AMELI, R., LUCKENBAUGH, D. A., CHARNEY, D. S. & MANJI, H. K. 2006. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry*, 63, 856-64.
- ZHANG, M. W., HARRIS, K. M. & HO, R. C. 2016. Is Off-label repeat prescription of ketamine as a rapid antidepressant safe? Controversies, ethical concerns, and legal implications. *BMC Medical Ethics*, 17, 4.
- ZHANG, M. W. & HO, R. C. 2017. Ethical Considerations for Clinical Research and Off-label Use of Ketamine to Treat Mood Disorders: The Balance Between Risks and Benefits. *Ethics & Behavior*, 27, 681-699.
- ZHANG, Y. & BHAVNANI, B. R. 2006. Glutamate-induced apoptosis in neuronal cells is mediated via caspase-dependent and independent mechanisms involving calpain and caspase-3 proteases as well as apoptosis inducing factor (AIF) and this process is inhibited by equine estrogens. *BMC Neurosci*, 7, 49.

CHAPTER 4 – HOW ELECTROCHEMICAL MEASUREMENTS IN THE BRAIN HAVE SHAPED OUR UNDERSTANDING OF DEPRESSION³

³ **Batey, L.**, Baumberger, B., Prieto, P., Hashemi, P. How Electrochemical Measurements in the Brain Have Shaped Our Understanding of Depression. *Annual Reviews of Analytical Chemistry*. 2025. *Under Review*

Abstract

Mental health disorders, such as depression, represent a growing global challenge. Depression is difficult to diagnose and treat. These difficulties stem from an insufficient understanding of the underlying mechanisms of the disorder. It is incredibly challenging to improve diagnostic and therapeutic approaches without a clear understanding of depression pathology. Neurotransmitters are low in concentration and fluctuate rapidly, making them difficult to investigate. Progress in methods to study the brain has uncovered clues to the pathology of depression and antidepressant mechanisms. In this review, we first describe the three medical hypotheses of depression: the monoamine, plasticity and inflammation theories. We highlight key analytical methods that have been employed in depression studies. Lastly, we show how these investigations have advanced our knowledge of depression mechanisms and treatment strategies. Thus, via this review, we present the status quo of how chemical measurements are guiding our understanding of the chemical underpinnings of depression and pointing the community towards new antidepressant treatment targets.

Introduction

Depression presents one of the globe's most pertinent challenges, affecting an estimated 300 million individuals worldwide. It is very difficult to diagnose depression and there remains much controversy around depression treatments. Furthermore, large scale drug discovery efforts for antidepressants have been dramatically toned down over the last two decades. These issues are grounded in our incomplete understanding of the underlying pathology of this disease. Without a clear grasp of the brain's intricate chemical landscape, identifying and addressing the root causes of depression remains challenging. Brain chemistry is notoriously difficult to study due to the rapid, low-concentration, and nanoscale dynamics of neurotransmitter signaling.

However, recent advancements in neurochemical analysis have begun to shed light on the biochemical underpinnings of depression and the mechanisms of antidepressant therapies. We begin this review with a description of major depressive disorder. We then critically outline the three salient theories of depression: the monoamine, plasticity and inflammation hypotheses. We then describe key analytical chemistry methods that researchers have used to probe depression. Finally, we critically explore how these chemical studies have informed our understanding of underlying depression pathology and therapeutic approaches for this disorder.

We therefore present a state-of-the-art picture of how chemical measurements are shaping our understanding of the chemical underpinnings and uncovering potential new treatment targets for depression.

Diagnosis and Treatment of Depression

Depression is one of the globe's leading health problems. As we enter into a post-COVID era, we are braced for an unprecedented increase in psychiatric disease. Diagnosis and treatment of depression are imprecise and inconsistent. Many do not seek help because of lack of access and stigma. For those that do, current diagnoses are based on questionnaires which often fail (Eack et al., 2006, Wittkamp et al., 2009). There are many reasons for the difficulty of a diagnosis, including the different ways patients seek help, how they describe their symptoms, and how their concerns are processed by different healthcare practitioners.

Depression treatments are also variable across the board. Selective serotonin reuptake inhibitors (SSRIs) are some of the world's most prescribed medicines and carry with them side effects and a clinical regime whereby type and dosage are altered on a trial-and-error basis. Importantly, there is speculation about whether clinical responses to antidepressants are significantly meaningful with respect to placebo, exercise or psychotherapy (Recchia et al., 2022). A further, and alarming, problem is that because there are no reliable pre-clinical screening tools for antidepressants; pharmaceutical companies have dramatically toned-down antidepressant drug discovery and there are few, if any, new antidepressants on the horizon. The new potential antidepressant strategies have been discovered in smaller academic labs, and are based on existing, controlled substances (such as ketamine and psychedelics) (Nutt et al., 2023, Smith-Apeldoorn et al., 2022).

Despite decades of research by some of the world's best scientists, a biological or chemical pathophysiological basis of depression has not been universally agreed upon. Such an understanding would naturally 'de-mystify' depression (that is for the most part invisible) but has been hampered by lack of technology.

Studying the Brain Requires Exquisite Technology

Tools that can capture fast biochemical processes from functioning brain cells have steadily been developed and applied over the past few decades. The brain is chemically complex, physically delicate and the chemical processes (neurotransmission) that change during pathology occur on the subsecond time scale. Measuring brain chemistry is particularly important for translating mental health research to humans because clinical diagnoses/therapeutics benefit greatly from a chemical output. Micro- and nano-electrode technology have changed this game and analytical techniques for neurotransmitters using such micro and nano devices include:

- Direct electrochemical methods such as chronoamperometry and voltammetry
- Biosensors based on enzymes or other biological molecules.

In the next sections we will briefly discuss the three pathological theories of depression. We will then briefly describe the methods that exist to measure brain chemistry and review how chemical measurements with these analytical methods are shaping our understanding of these theories and precipitating new treatments.

The Three Theories of Depression

Monoamine Hypothesis of Depression

In the 1960s several studies found that serotonin levels were lower in the brain of depressed suicide patients (Shaw et al., 1967), and norepinephrine levels were lower in the cerebral spinal fluid and urine of patients with depression (Bunney and Davis, 1965, Schildkraut, 1965). It was also discovered during that time that monoamine targeting drugs (such as monoamine oxidase inhibitors) could alleviate depression (West and Dally, 1959). Thus was born the Monoamine Hypothesis of Depression. This theory (that extracellular serotonin, norepinephrine and dopamine levels are lower during depression) has moved in and out of favor for decades, recently enjoying a resurgence because of clinical trials targeting depression with psychedelics (high affinity for serotonin receptors). A recent review article posited that serotonin does not underlie depression in humans (Moncrieff et al., 2022), reviving intense debate in this topic. In recent years a growing body of evidence suggests that plasticity and inflammation have central roles in the pathogenesis of depression.

Neuroplasticity Theory of Depression

Using fMRI in the 1990s, researchers found loss of hippocampal volume in patients with recurrent depression (Sheline et al., 1996). Animal studies found that acute stress suppressed the rate of hippocampal neurogenesis (Sapolsky, 1996a). Taken together these findings precipitated the Neuroplasticity Theory of Depression. The notion here is that stress decreases glutamate levels (Sapolsky, 1996b, Di et al., 2009). Because glutamate is a key factor for neuroplasticity, low levels of this transmitter can cause

dramatic and widespread morphological changes and, at worst, cell death in the hippocampus (Zhang and Bhavnani, 2006). Ketamine and psychedelics are known to improve plasticity and are currently being trialed as antidepressants (Nutt et al., 2023, Smith-Apeldoorn et al., 2022).

Inflammation Theory of Depression

The inflammation or cytokine theory of depression was developed in parallel to the plasticity theory. This theory was based on studies that found that human volunteers developed depression-like behaviors after being treated with cytokines (inflammation signaling molecules) (Niiranen et al., 1988). Cytokines themselves directly affect serotonin, dopamine, and norepinephrine, but the story here is likely much bigger because inflammation is complex and involves many cascades.

There is compelling evidence to show that patients with high levels of inflammation are more likely to be resistant to conventional SSRI treatment (Haroon et al., 2018). Targeting inflammation with anti-inflammatory medication is challenging because it is difficult to establish the level of inflammation, and thus to titrate the anti-inflammatory therapies accordingly. Nonetheless, these strategies show promise (Strawbridge et al., 2017, Bai et al., 2020, Köhler-Forsberg et al., 2019, Arteaga-Henríquez et al., 2019).

These are the main theories of the pathology of depression. In the next section, we will discuss the analytical methods that have the capacity to measure the neurotransmitters that are implicated in these theories.

Analytical Methods for Brain Chemistry

Chronoamperometry is an electrochemical technique that applies a square wave potential to a carbon fiber microelectrode (CFM), measuring redox-driven changes in current to analyze neurotransmitter dynamics. It has been widely used to study monoamine systems, particularly serotonin reuptake, by calculating clearance kinetics across different brain regions. This method offers real-time measurements of neurotransmitter transporter activity in vivo, providing insights into the effects of drugs, environmental factors, and physiological conditions on neurotransmitter dynamics.

The technique has limited chemical selectivity and necessitates careful experimental design to accurately interpret the data. Such careful designs have been achieved by Lynn Daws using high-speed chronoamperometry. This version of the method involves coupling the technique with multi-barreled glass micropipettes, allowing for precise microinjection of neurotransmitters and pharmacological agents into specific brain regions. This approach enhances the spatial resolution and versatility of chronoamperometric recordings, enabling detailed investigation of serotonin reuptake (Zhu et al., 2010, Annamalai et al., 2020, Daws et al., 2016).

Voltammetry is another direct electrochemical method that applies a waveform ramp to an electrode. Current is measured at increments across this waveform and analytes are identified based on unique redox potential peaks and quantified via the amplitudes of these same peaks. Fast scan cyclic voltammetry (FSCV) involves applying rapid voltage sweeps ($400\text{--}1000\text{ Vs}^{-1}$) across microelectrodes and ultramicroelectrodes allowing for sub second timescale redox reactions of analytes. FSCV has seen a rise in popularity due

to its capability for investigating neurotransmitter dynamics in real-time (Rodeberg et al., 2017).

Biosensors are typically electrodes that employ a biological recognition element interacting with a transducer to selectively detect analytes and other biological characteristics (e.g. cell morphology) (Keese and Giaever, 1994). Biological molecules such as enzymes and antibodies provide unparalleled selectivity for analytes of interest. Transducers are designed to engage electron transfer processes from the analyte-biological molecule interaction to provide a measurable signal (Mohanty and Kougiannos, 2006).

Biosensors have dramatically evolved beyond glucose detection with glucose oxidase (Clark and Lyons, 1962, Turner, 2013). Currently, there is a vast collection of methods and analytes that can be measured with biosensors. Among these sensors, relevant to this review, enzyme-based and DNA biosensors.

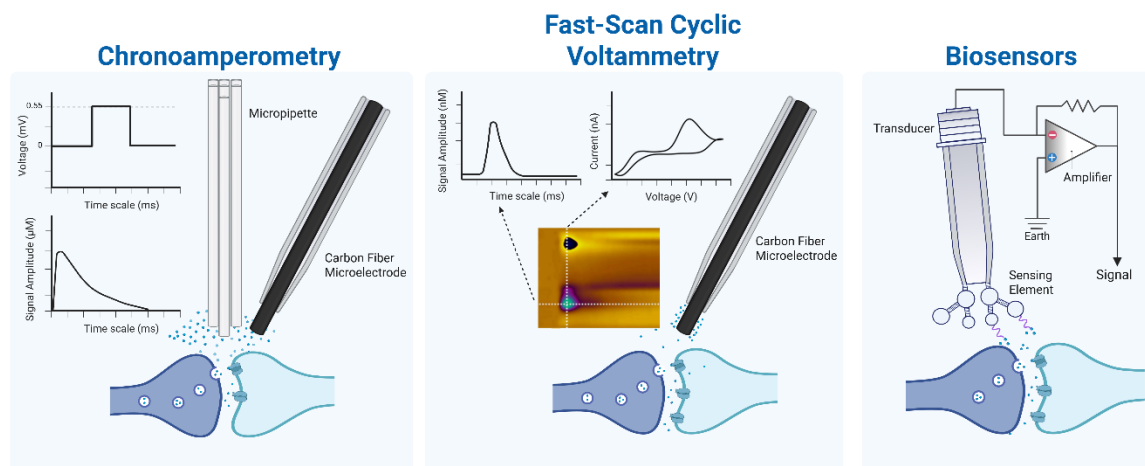


Figure 4.1 - Overview of three analytic techniques used in neurotransmitter research: Chronoamperometry, showing square-wave potential and resulting current response; FSCV, illustrating a color plot, as well as resulting current-voltage and current-time responses; Biosensor-based detection using a biological recognition element, transducer and amplifier to generate a signal. Image created using Biorender.

Figure 4.1 outlines the experimental and data aspects of these methods. We next review the use of these sensors to measure neurotransmitters in depression research and discuss the relevance of the work to furthering our understanding of the chemical underpinnings of depression.

Chemical Underpinnings of Depression

Chronoamperometry

In 1997, chronoamperometry described the effects of lesions and SSRIs on serotonin reuptake (Daws et al., 1997), firmly establishing the method for further studies of serotonin in depression. A seminal study using this method came in 1998 where the group showed that a norepinephrine transporter (NET) inhibitor, desipramine, contributed to the active clearance of serotonin (Daws et al., 1998). This finding complemented a much earlier discovery by Shaskan and Snyder who found two distinct mechanisms of serotonin reuptake; a high affinity, low efficiency mechanism and a low affinity, high efficiency mechanism (Shaskan and Snyder, 1970). These mechanisms were coined Uptake 1 (serotonin transporters (SERTs)) and Uptake 2 (NETs, dopamine transporters (DATs) and organic cation transporters (OCTs)), respectively. Evidence for serotonin transport through OCTs was provided through a series of elegant experiments

in the early 2000s (Engel et al., 2004, Engel and Wang, 2005, Gasser et al., 2006, Schömig et al., 2006), some of which are highlighted below.

In 2004, The Daws group used an engineered mouse with a targeted disruption of the serotonin transporter gene, meaning that knock out mice had little or no SERTs and heterozygotes had approximately 50% less SERTs than wildtypes. The group found no differences in serotonin clearance under control conditions between the heterozygotes and the wild type mice, whereas serotonin reuptake was severely disrupted in the SERT knockout (KO) mice. After injection with an SSRI however, differences were apparent between the wildtype and heterozygous mice (heterozygotes were more sensitive to SSRI). It was speculated that the lack of effect on serotonin clearance in control and heterozygotes was due to a compensatory mechanism that was overwhelmed after SSRI (Montañez et al., 2003).

Some clues about these compensatory mechanisms came in 2008 where it was shown that OCT expression was increased in heterozygotes and in the SERT KO mice (Baganz et al., 2008). This phenomenon was previously independently shown via semi-quantitative competitive polymerase chain reaction (PCR) and in situ hybridization (Schmitt et al., 2003). Administration of an OCT3 inhibitor, decynium-22 (D22) had no effect on control and wildtype mice but was associated with slowed serotonin reuptake in heterozygotes and in KOs. This experiment showed that OCTs may be the compensatory mechanism and can reuptake serotonin when SERTs are less able to (either because there are fewer of them or that they are blocked). Accordingly, this drug potentiated the effects of SSRIs

and showed stronger antidepressant-like behavior in wildtype mice. These effects were reduced in OCT3 KO mice (Horton et al., 2013).

The group next showed that acute stress (as modeled with repeated swim) impaired serotonin clearance; a phenomenon that may serve as a compensatory mechanism to temporarily elevate extracellular serotonin levels (Baganz et al., 2010). However, with prolonged or chronic stress, additional neuromodulators such as histamine appear to become involved, as a result of inflammation, that inhibit serotonergic signaling (Hersey et al., 2022). The group have also studied how alcohol and brain-derived neurotrophic factor (BDNF) modulate serotonin clearance (Daws et al., 2007, Daws et al., 2006).

Most recently, researchers are concentrating on the atypical antidepressant, ketamine. First, they found that another class of uptake 2 transporters, the plasma membrane monoamine transporters (PMATs) are critical for ketamine's actions. In wild-type mice, ketamine inhibited serotonin clearance in the CA3 region of the hippocampus (a finding recently complemented with FSCV in drosophila) (Dunham et al., 2024), but this effect was completely lost in mice lacking either SERTs or PMATs, see **Figure 4.2** below (Bowman et al., 2020). Similar results were found for OCT deficient mice (Bowman et al., 2019). It was concluded that both PMATs and OCTs are involved in the antidepressant effects of ketamine in mice.

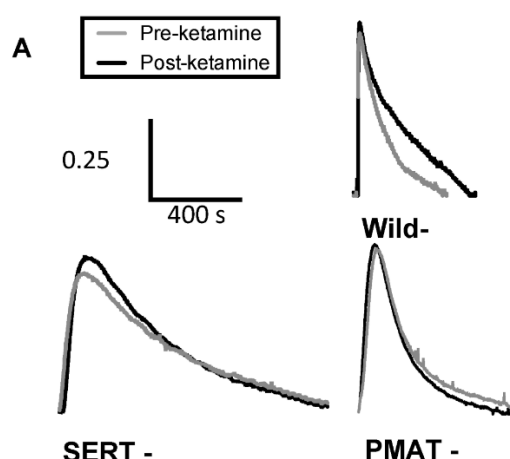


Figure 4.2 - Chronoamperometry measurements of serotonin clearance in the CA3 region of a mouse hippocampus. Ketamine inhibits serotonin clearance in wild type but not SERT or PMAT deficient mice. Adapted from Bowman et al. (Bowman et al., 2020).

This body of work using chronoamperometry, very much led by Lynn Daw's group, is highly relevant to depression because it has shed light on a previously ignored transport mechanism for serotonin (Uptake 2 via OCTs and PMATs). Modern antidepressants primarily target serotonin transporters; thus, this finding is paving the way towards fundamentally new drug targets as antidepressants (Bowman et al., 2017).

Voltammetry

Voltammetry has been used for decades to study dopamine (Venton and Cao, 2020); however, little work has investigated dopamine transmission with respect to depression. Since serotonin is a strong molecule of interest in depression, in the late 90s researchers started to look at using voltammetry to study the dynamics of this messenger. In 1990, Jon Stamford, Ziggy Kruk and Julian Millar performed an elegant experiment whereby they administered high doses of the serotonin precursor, 5-hydroxytryptophan, to a mouse, and pharmacologically abolished striatal dopamine release. Under these conditions,

they were able to measure in vivo serotonin release at carbon fiber microelectrodes, which was dependent on the 5-HTP dose (Stamford et al., 1990). Serotonin electrooxidation is complex, with many unstable electrooxidation products that can polymerize onto the electrode and deteriorate the response (Hashemi et al., 2009). In 1995 Brad Jackson and Mark Wightman developed a new electrochemical waveform, the 'Jackson' waveform, that mitigates serotonin's electrooxidation side products and maintains the integrity of the electrode for longer (Jackson et al., 1995). Jackson and Wightman repeated the Stamford experiment using this waveform (Jackson and Mark Wightman, 1995).

In the following decade, FSCV was used to study serotonin dynamics in mast cells (Travis et al., 2000) and tissue slice preparations (Bunin and Wightman, 1998). With respect to the latter, some basic aspects of serotonin transmission were ascertained. Serotonin was shown to engage in paracrine or volume transmission (Bunin and Wightman, 1998). Additionally, serotonin reuptake rates were reduced to differing degrees after SSRIs depending on the brain region (Bunin et al., 1998).

Serotonin remained very difficult to measure in vivo, this is because of a combination of very low releasable levels of serotonin in vivo, and the fact that it's not just serotonin, but also serotonin metabolites (that are highly concentrated in vivo) that can foul electrodes. In 2009, Hashemi and Wightman made the first measurements of serotonin in vivo with FSCV, by coating the carbon fiber with Nafion, and identifying a brain region where serotonin was abundant (Hashemi et al., 2009). In parallel work, Venton employed channel rhodopsin optogenetics in the single larval *Drosophila* ventral nerve cord to

induce serotonin optically and measure release and reuptake with FSCV. They found that serotonin synthesis plays a significant role in shaping the serotonergic response to reuptake inhibition (Xiao et al., 2014).

In 2012, Hashemi and Wightman simultaneously measured serotonin and dopamine in one rat in different brain regions and showed that very different mechanisms control release and reuptake of the two modulators. Notably serotonin release was not as sensitive to inhibition of synthesis and vesicular packaging as dopamine but was much more sensitive to inhibition of reuptake and metabolism (Hashemi et al., 2012). Related to this, Kile et. al. showed that synapsins (that control the vesicle availability for release) reduced dopamine release but did not affect serotonin release (Kile et al., 2010). Hashemi went on over the next decade to show that, in mice, responses to the SSRI escitalopram were dynamic (Wood and Hashemi, 2013), that Uptake 1 and 2 can be mathematically modeled (Wood et al., 2014), to predict the local architecture of the tissue around the probe (Hashemi et al., 2015), that male and female mice have different serotonin responses to SSRIs (Saylor et al., 2019), and release and reuptake sites in the brain are connected (West et al., 2019). In 2015, Hashemi and Heien developed an FSCV offshoot method they coined fast scan controlled adsorption voltammetry (FSCAV) to measure basal or ambient levels of serotonin in vivo (Abdalla et al., 2017). They found that different brain regions had slightly different basal levels of serotonin and that this was related to serotonin transporter (SERT) density (Abdalla et al., 2020). The group then developed a method to co-detect serotonin and histamine and showed that histamine release inhibits serotonin via H3 receptors (Samaranayake et al., 2016), an idea that came from earlier work using slice voltammetry from Stephanie Cragg (Threlfell et al., 2012)

2004). Interestingly, histamine also mediates serotonin reuptake, potentially showing interconnectivity between release and reuptake dynamics (Annamalai et al., 2020). Adenosine has also been shown to inhibit serotonin release via activation of 5HT_{1A} autoreceptors (Shrestha and Venton, 2024).

Using the new tools for ambient serotonin and histamine, the Hashemi group went on to show that chronic stress induced depression in mice reduces basal, but not stimulated, serotonin in the hippocampus while increasing histamine release in the hypothalamus (Hersey et al., 2022). This result was independently recapitulated recently in mice with chronic restraint stress using field effect transistors (Li et al., 2025). The exact chemical signature seen in the chronically stressed mice (low basal serotonin and increased histamine release) was repeated with acute inflammation via lipopolysaccharide (LPS) (Hersey et al., 2021). Given the almost exclusive comorbidity of depression with inflammation, this work shows that the chemical signatures of the two are interchangeable. The group also showed that serotonin is a biomarker of Parkinsonism (Parkinson's disease is also highly comorbid with depression (Buchanan et al., 2024). In all 3 scenarios (stress, LPS and Parkinsonism), SSRIs were less able to increase serotonin levels than in control conditions. The group showed that this is because histamine reuptake is also blocked by SSRIs (Hersey et al., 2021).

The group next used experimental and modeling approaches to show that antidepressants of differing modes of action all increase basal serotonin levels via different mechanisms that fall in with the different hypotheses of depression. For example, SSRI and NRIs increase serotonin via inhibition of Uptake 1 and 2 transporters

and ketamine increases serotonin due to its anti-inflammatory effects (Witt et al., 2023). The Venton group performed a parallel study in *Drosophila*, with similar findings (Dunham and Venton, 2022).

Most recently, researchers have developed a mathematical framework to model phenomena that are experimentally difficult to perform (such as chronic SSRI administration). They found that the serotonin system needs significant time to reach steady state during clinical type regimes (explaining why patients need to take SSRIs for some weeks to have therapeutic effect). **Figure 4i** shows how the model predicts escitalopram to fluctuate between doses during a chronic regime in mice, reaching steady state after a couple of days. **Figure 4ii blue trace** shows that it takes extracellular serotonin (e5HT) significantly longer (8 days) to stabilize. Also, the model showed that under inflammation (**Figure 4ii red trace**) serotonin levels are lower due to inflammation induced histamine acting to inhibit serotonin. Under this condition, SSRIs at therapeutic doses could increase serotonin levels to control levels, which may explain the clinical variability of SSRIs (Mena et al., 2024).

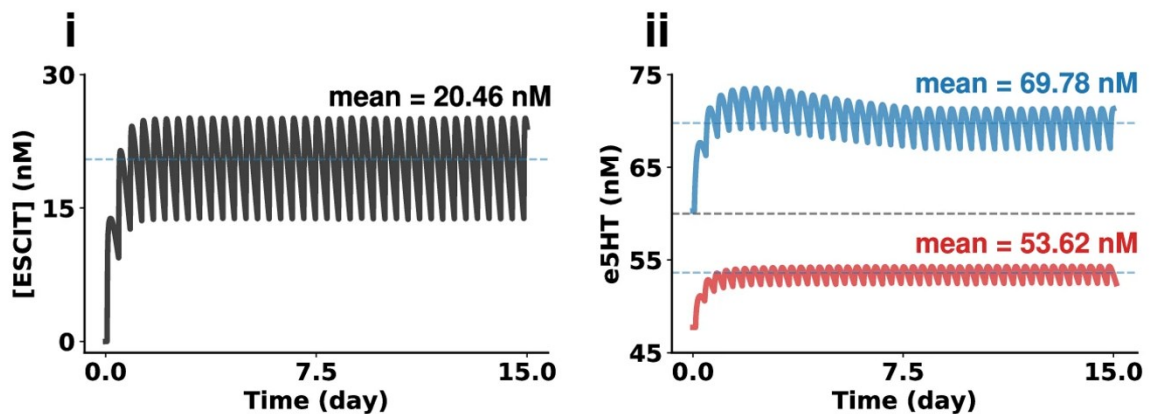


Figure 4.3 - Modeling of oral chronic dosing escitalopram (5 mg pill, $\sim 1.02 \text{ mg kg}^{-1}$) effects on brain (i) escitalopram and (ii) serotonin during control state (blue) and inflammation (red). For the inflammation simulation, mast cell and glia production and release of histamine is triggered 35 days before the first dose. Administration is then repeated every 8 h. Adapted from Mena et al. (Mena et al., 2024).

Thus, voltammetry has been used to explain basic aspects of serotonin transmission, shown that serotonin is a biomarker of stress, depression, inflammation and Parkinsonism and that most antidepressants affect the serotonin system (i.e. a biomarker of antidepressant efficacy). This method has also shown why people might need to take antidepressants for weeks for clinical effect and why these agents may not work for all people.

Biosensors

There are relatively fewer reports of using in vivo biosensors in depression research. There are some reports of enzyme-based biosensors in depression research. For example, Greg Gerhardt developed Fast Analytic Sensing Technology (FAST) sensors. Here, ceramic sensors are modified with glutamate oxidase and when coupled to a custom software enable rapid glutamate measurements (Burmeister et al., 2002, Hascup et al., 2008). These FAST sensors were used by Vesna Lazarevic et al, in mice and found that ketamine elicited significant reduction of KCl-evoked glutamate release (Lazarevic et al., 2021). Another group showed, using enzyme-based microelectrode arrays selective for glutamate, that resting glutamate levels were higher and there were more glutamate transients in genetically depressed mice (Hascup et al., 2011).

Aptamer modified field effect transistor (FET) biosensors are an emerging method for neurotransmitter sensing (Zhao et al., 2021, Nakatsuka, 2022, Nakatsuka et al., 2021,

Nakatsuka et al., 2018). In a recent study, an acupuncture needle-type extended-gated FET was engineered with gold membranes and the high-performance nanomaterial MXene and functionalized with 5-HT-specific aptamers. The sensors were applied to gut/brain axis studies in mouse models of chronic restraint stress and high fat diet. The authors found that in animals that were exposed to stress and high fat diet, the serotonin levels were significantly lower (Li et al., 2025).

Conclusion and Future Perspectives

Depression is very difficult to accurately diagnose. The lack of brain measurement tools means that depression pathology remains elusive. However, rapid progress has been made in the last decade towards precision analytical tools for brain chemistry. In this review, we described the three most popular hypotheses of depression. We outlined the electroanalytical methods employed to study depression and we highlighted how these efforts have furthered our understanding of depression and antidepressants. As new tools, such as biosensors, are being developed and refined, we will uncover ever more nuanced aspects of depression pathology and acquire different clues that will help us better diagnose and treat this disorder.

References

- ABDALLA, A., ATCHERLEY, C. W., PATHIRATHNA, P., SAMARANAYAKE, S., QIANG, B., PEÑA, E., MORGAN, S. L., HEIEN, M. L. & HASHEMI, P. 2017. In Vivo Ambient Serotonin Measurements at Carbon-Fiber Microelectrodes. *Analytical Chemistry*, 89, 9703-9711.
- ABDALLA, A., WEST, A., JIN, Y., SAYLOR, R. A., QIANG, B., PEÑA, E., LINDEN, D. J., NIJHOUT, H. F., REED, M. C. & BEST, J. 2020. Fast serotonin voltammetry as a versatile tool for mapping dynamic tissue architecture: I. Responses at carbon fibers describe local tissue physiology. *Journal of neurochemistry*, 153, 33-50.
- ANNAMALAI, B., RAGU VARMAN, D., HORTON, R. E., DAWS, L. C., JAYANTHI, L. D. & RAMAMOORTHY, S. 2020. Histamine Receptors Regulate the Activity, Surface Expression, and Phosphorylation of Serotonin Transporters. *ACS Chem Neurosci*, 11, 466-476.
- ARTEAGA-HENRÍQUEZ, G., SIMON, M. S., BURGER, B., WEIDINGER, E., WIJKHUIJS, A., AROLT, V., BIRKENHAGER, T. K., MUSIL, R., MÜLLER, N. & DREXHAGE, H. A. 2019. Low-grade inflammation as a predictor of antidepressant and anti-inflammatory therapy response in MDD patients: a systematic review of the literature in combination with an analysis of experimental data collected in the EU-MOODINFLAME consortium. *Frontiers in psychiatry*, 10, 458.
- BAGANZ, N., HORTON, R., MARTIN, K., HOLMES, A. & DAWS, L. C. 2010. Repeated swim impairs serotonin clearance via a corticosterone-sensitive mechanism: organic cation transporter 3, the smoking gun. *J Neurosci*, 30, 15185-95.
- BAGANZ, N. L., HORTON, R. E., CALDERON, A. S., OWENS, W. A., MUNN, J. L., WATTS, L. T., KOLDZIC-ZIVANOVIC, N., JESKE, N. A., KOEK, W. & TONEY, G. M. 2008. Organic cation transporter 3: Keeping the brake on extracellular serotonin in serotonin-transporter-deficient mice. *Proceedings of the National Academy of Sciences*, 105, 18976-18981.
- BAI, S., GUO, W., FENG, Y., DENG, H., LI, G., NIE, H., GUO, G., YU, H., MA, Y., WANG, J., CHEN, S., JING, J., YANG, J., TANG, Y. & TANG, Z. 2020. Efficacy and safety of anti-inflammatory agents for the treatment of major depressive disorder: a systematic review and meta-analysis of randomised controlled trials. *Journal of Neurology, Neurosurgery & Psychiatry*, 91, 21-32.
- BOWMAN, M. A., VITELA, M., CLARKE, K. M., KOEK, W. & DAWS, L. C. 2020. Serotonin transporter and plasma membrane monoamine transporter are necessary for the antidepressant-like effects of ketamine in mice. *International Journal of Molecular Sciences*, 21, 7581.
- BOWMAN, M. A., VITELA, M., OWENS, W. A., KOEK, W. & DAWS, L. 2017. Antidepressant-like Effects of Ketamine in Serotonin Transporter Knockout Mice and Organic Cation Transporter 3 Knockout Mice. *The FASEB Journal*, 31, 813.9-813.9.
- BOWMAN, M. A., VITELA, M., OWENS, W. A., KOEK, W. & DAWS, L. C. 2019. Investigating the Role of Serotonin Transporter, Plasma Membrane Monoamine Transporter, and Organic Cation Transporter 3 in the Antidepressant-like Effects of Ketamine. *The FASEB Journal*, 33, 807.6-807.6.

- BUCHANAN, A. M., MENA, S., CHOUKARI, I., VASA, A., CRAWFORD, J. N., FADEL, J., MAXWELL, N., REAGAN, L., CRUIKSHANK, A. & BEST, J. 2024. Serotonin as a biomarker of toxin-induced Parkinsonism. *Molecular Medicine*, 30, 33.
- 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 Reticulata of the Rat Brain. *Journal of Neurochemistry*, 70, 1077-1087.
- BUNIN, M. A. & WIGHTMAN, R. M. 1998. Quantitative Evaluation of 5-Hydroxytryptamine (Serotonin) Neuronal Release and Uptake: An Investigation of Extrasynaptic Transmission. *The Journal of Neuroscience*, 18, 4854-4860.
- BUNNEY, W. E. & DAVIS, J. M. 1965. Norepinephrine in depressive reactions: A review. *Archives of General Psychiatry*, 13, 483-494.
- BURMEISTER, J. J., POMERLEAU, F., PALMER, M., DAY, B. K., HUETTL, P. & GERHARDT, G. A. 2002. Improved ceramic-based multisite microelectrode for rapid measurements of l-glutamate in the CNS. *Journal of Neuroscience Methods*, 119, 163-171.
- CLARK, L. C., JR. & LYONS, C. 1962. Electrode systems for continuous monitoring in cardiovascular surgery. *Ann N Y Acad Sci*, 102, 29-45.
- DAWS, L., MUNN, J., VALDEZ, M., FROSTO-BURKE, T. & HENSLER, J. 2007. Serotonin transporter function, but not expression, is dependent on brain-derived neurotrophic factor (BDNF): in vivo studies in BDNF-deficient mice. *Journal of Neurochemistry*, 101, 641-651.
- DAWS, L. C., MONTANEZ, S., MUNN, J. L., OWENS, W. A., BAGANZ, N. L., BOYCE-RUSTAY, J. M., MILLSTEIN, R. A., WIEDHOLZ, L. M., MURPHY, D. L. & HOLMES, A. 2006. Ethanol inhibits clearance of brain serotonin by a serotonin transporter-independent mechanism. *Journal of Neuroscience*, 26, 6431-6438.
- DAWS, L. C., OWENS, W. A. & TONEY, G. M. 2016. Using high-speed chronoamperometry to measure biogenic amine release and uptake in vivo. *Neurotransmitter Transporters: Investigative Methods*, 53-81.
- 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, 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, 967-976.
- DI, S., MAXSON, M. M., FRANCO, A. & TASKER, J. G. 2009. Glucocorticoids regulate glutamate and GABA synapse-specific retrograde transmission via divergent nongenomic signaling pathways. *J Neurosci*, 29, 393-401.
- DUNHAM, K. E., KHALED, K. H., WEIZMAN, L. & VENTON, B. J. 2024. Microdosing ketamine in Drosophila does not block serotonin reuptake, but causes complex behavioral changes mediated by glutamate and serotonin receptors. *Journal of Neurochemistry*, 168, 1097-1112.
- DUNHAM, K. E. & VENTON, B. J. 2022. SSRI antidepressants differentially modulate serotonin reuptake and release in Drosophila. *Journal of neurochemistry*, 162, 404-416.

- EACK, S. M., GREENO, C. G. & LEE, B.-J. 2006. Limitations of the Patient Health Questionnaire in identifying anxiety and depression in community mental health: many cases are undetected. *Research on social work practice*, 16, 625-631.
- ENGEL, K. & WANG, J. 2005. Interaction of organic cations with a newly identified plasma membrane monoamine transporter. *Molecular pharmacology*, 68, 1397-1407.
- ENGEL, K., ZHOU, M. & WANG, J. 2004. Identification and characterization of a novel monoamine transporter in the human brain. *Journal of Biological Chemistry*, 279, 50042-50049.
- GASSER, P. J., LOWRY, C. A. & ORCHINIK, M. 2006. Corticosterone-sensitive monoamine transport in the rat dorsomedial hypothalamus: potential role for organic cation transporter 3 in stress-induced modulation of monoaminergic neurotransmission. *Journal of Neuroscience*, 26, 8758-8766.
- HAROON, E., DAGUANNO, A. W., WOOLWINE, B. J., GOLDSMITH, D. R., BAER, W. M., WOMMACK, E. C., FELGER, J. C. & MILLER, A. H. 2018. Antidepressant treatment resistance is associated with increased inflammatory markers in patients with major depressive disorder. *Psychoneuroendocrinology*, 95, 43-49.
- HASCUP, K. N., HASCUP, E. R., POMERLEAU, F., HUETTL, P. & GERHARDT, G. A. 2008. Second-by-Second Measures of l-Glutamate in the Prefrontal Cortex and Striatum of Freely Moving Mice. *The Journal of Pharmacology and Experimental Therapeutics*, 324, 725-731.
- HASCUP, K. N., HASCUP, E. R., STEPHENS, M. L., GLASER, P. E., YOSHITAKE, T., MATHÉ, A. A., GERHARDT, G. A. & KEHR, J. 2011. Resting glutamate levels and rapid glutamate transients in the prefrontal cortex of the Flinders Sensitive Line rat: a genetic rodent model of depression. *Neuropsychopharmacology*, 36, 1769-77.
- HASHEMI, P., ABDALLA, A., JIN, Y., BEST, J., LINDEN, D. & REED, M. In Vivo Serotonin Chemistry and Local Cytoarchitecture: A Combined Voltammetric, Mathematical and Microscopy Study. *NEUROPSYCHOPHARMACOLOGY*, 2015. NATURE PUBLISHING GROUP MACMILLAN BUILDING, 4 CRINAN ST, LONDON N1 9XW, ENGLAND, S522-S523.
- HASHEMI, P., DANKOSKI, E. C., LAMA, R., WOOD, K. M., TAKMAKOV, P. & WIGHTMAN, R. M. 2012. Brain dopamine and serotonin differ in regulation and its consequences. *Proceedings of the National Academy of Sciences*, 109, 11510-11515.
- HASHEMI, P., DANKOSKI, E. C., PETROVIC, J., KEITHLEY, R. B. & WIGHTMAN, R. 2009. Voltammetric detection of 5-hydroxytryptamine release in the rat brain. *Analytical chemistry*, 81, 9462-9471.
- HERSEY, M., RENEAU, M., BERGER, S. N., MENA, S., BUCHANAN, A. M., OU, Y., TAVAKOLI, N., REAGAN, L. P., CLOPATH, C. & HASHEMI, P. 2022. A tale of two transmitters: serotonin and histamine as in vivo biomarkers of chronic stress in mice. *Journal of Neuroinflammation*, 19, 1-20.
- HERSEY, M., SAMARANAYAKE, S., BERGER, S. N., TAVAKOLI, N., MENA, S., NIJHOUT, H. F., REED, M. C., BEST, J., BLAKELY, R. D. & REAGAN, L. P. 2021. Inflammation-induced histamine impairs the capacity of escitalopram to increase hippocampal extracellular serotonin. *Journal of Neuroscience*, 41, 6564-6577.
- HORTON, R. E., APPLE, D. M., OWENS, W. A., BAGANZ, N. L., CANO, S., MITCHELL, N. C., VITELA, M., GOULD, G. G., KOEK, W. & DAWS, L. C. 2013. Decynium-22

- enhances SSRI-induced antidepressant-like effects in mice: uncovering novel targets to treat depression. *Journal of Neuroscience*, 33, 10534-10543.
- JACKSON, B. P., DIETZ, S. M. & WIGHTMAN, R. M. 1995. Fast-scan cyclic voltammetry of 5-hydroxytryptamine. *Analytical chemistry*, 67, 1115-1120.
- JACKSON, B. P. & MARK WIGHTMAN, R. 1995. Dynamics of 5-hydroxytryptamine released from dopamine neurons in the caudate putamen of the rat. *Brain Research*, 674, 163-166.
- KEESE, C. R. & GIAEVER, I. 1994. A biosensor that monitors cell morphology with electrical fields. *IEEE Engineering in Medicine and Biology Magazine*, 13, 402-408.
- KILE, B. M., GUILLOT, T. S., VENTON, B. J., WETSEL, W. C., AUGUSTINE, G. J. & WIGHTMAN, R. M. 2010. Synapsins differentially control dopamine and serotonin release. *Journal of neuroscience*, 30, 9762-9770.
- KÖHLER-FORSBERG, O., N. LYDHOLM, C., HJORTHØJ, C., NORDENTOFT, M., MORS, O. & BENROS, M. E. 2019. Efficacy of anti-inflammatory treatment on major depressive disorder or depressive symptoms: meta-analysis of clinical trials. *Acta Psychiatrica Scandinavica*, 139, 404-419.
- LAZAREVIC, V., YANG, Y., FLAIS, I. & SVENNINGSSON, P. 2021. Ketamine decreases neuronally released glutamate via retrograde stimulation of presynaptic adenosine A1 receptors. *Molecular Psychiatry*, 26, 7425-7435.
- LI, J., QI, F., LI, C., TANG, L., LI, Y., LIU, Y., LI, Y. & ZHANG, G.-J. 2025. MXene-enhanced detachable transistor neuroprobe for real-time monitoring of serotonin dynamics in human organoids and living brains. *Chemical Engineering Journal*, 512, 162542.
- MENA, S., CRUIKSHANK, A., BEST, J., NIJHOUT, H., REED, M. C. & HASHEMI, P. 2024. Modulation of serotonin transporter expression by escitalopram under inflammation. *Communications Biology*, 7, 710.
- MOHANTY, S. P. & KOUZIANOS, E. 2006. Biosensors: A tutorial review. *Ieee Potentials*, 25, 35-40.
- MONCRIEFF, J., COOPER, R. E., STOCKMANN, T., AMENDOLA, S., HENGARTNER, M. P. & HOROWITZ, M. A. 2022. The serotonin theory of depression: a systematic umbrella review of the evidence. *Molecular Psychiatry*.
- MONTAÑEZ, S., OWENS, W. A., GOULD, G. G., MURPHY, D. L. & DAWS, L. C. 2003. Exaggerated effect of fluvoxamine in heterozygote serotonin transporter knockout mice. *Journal of neurochemistry*, 86, 210-219.
- NAKATSUKA, N. 2022. Aptamer–Field-Effect Transistors for Small-Molecule Sensing in Complex Environments. *Nucleic Acid Aptamers: Selection, Characterization, and Application*. Springer.
- NAKATSUKA, N., HEARD, K. J., FAILLÉTAZ, A., MOMOTENKO, D., VÖRÖS, J., GAGE, F. H. & VADODARIA, K. C. 2021. Sensing serotonin secreted from human serotonergic neurons using aptamer-modified nanopipettes. *Molecular Psychiatry*, 26, 2753-2763.
- NAKATSUKA, N., YANG, K.-A., ABENDROTH, J. M., CHEUNG, K. M., XU, X., YANG, H., ZHAO, C., ZHU, B., RIM, Y. S. & YANG, Y. 2018. Aptamer–field-effect transistors overcome Debye length limitations for small-molecule sensing. *Science*, 362, 319-324.
- NIIRANEN, A., LAAKSONEN, R., LIVANAINEN, M., MATTSON, K., FÄRKILÄ, M. & CANTELL, K. 1988. Behavioral assessment of patients treated with alpha-interferon. *Acta Psychiatrica Scandinavica*, 78, 622-626.

- NUTT, D., SPRIGGS, M. & ERRITZOE, D. 2023. Psychedelics therapeutics: What we know, what we think, and what we need to research. *Neuropharmacology*, 223, 109257.
- RECCHIA, F., LEUNG, C. K., CHIN, E. C., FONG, D. Y., MONTERO, D., CHENG, C. P., YAU, S. Y. & SIU, P. M. 2022. Comparative effectiveness of exercise, antidepressants and their combination in treating non-severe depression: a systematic review and network meta-analysis of randomised controlled trials. *British journal of sports medicine*, 56, 1375-1380.
- 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, 221-234.
- SAMARANAYAKE, S., ABDALLA, A., ROBKE, R., NIJHOUT, H. F., REED, M. C., BEST, J. & HASHEMI, P. 2016. A voltammetric and mathematical analysis of histaminergic modulation of serotonin in the mouse hypothalamus. *Journal of neurochemistry*, 138, 374-383.
- SAPOLSKY, R. M. 1996a. Stress, glucocorticoids, and damage to the nervous system: the current state of confusion. *Stress*, 1, 1-19.
- SAPOLSKY, R. M. 1996b. Why Stress Is Bad for Your Brain. *Science*, 273, 749-750.
- SAYLOR, R. A., HERSEY, M., WEST, A., BUCHANAN, A. M., BERGER, S. N., NIJHOUT, H. F., REED, M. C., BEST, J. & HASHEMI, P. 2019. In vivo hippocampal serotonin dynamics in male and female mice: Determining effects of acute escitalopram using fast scan cyclic voltammetry. *Frontiers in neuroscience*, 13, 362.
- SCHILDKRAUT, J. J. 1965. The catecholamine hypothesis of affective disorders: a review of supporting evidence. *American journal of Psychiatry*, 122, 509-522.
- SCHMITT, A., MÖSSNER, R., GOSSMANN, A., FISCHER, I., GORBOULEV, V., MURPHY, D., KOEPESELL, H. & LESCH, K. 2003. Organic cation transporter capable of transporting serotonin is up-regulated in serotonin transporter-deficient mice. *Journal of neuroscience research*, 71, 701-709.
- SCHÖMIG, E., LAZAR, A. & GRÜNDEMANN, D. 2006. Extraneuronal monoamine transporter and organic cation transporters 1 and 2: a review of transport efficiency. *Neurotransmitter transporters*, 151-180.
- 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, 404-418.
- SHAW, D. M., CAMPS, F. E. & ECCLESTON, E. G. 1967. 5-Hydroxytryptamine in the hind-brain of depressive suicides. *The British Journal of Psychiatry*, 113, 1407-1411.
- SHELIN, Y. I., WANG, P. W., GADO, M. H., CSERNANSKY, J. G. & VANNIER, M. W. 1996. Hippocampal atrophy in recurrent major depression. *Proceedings of the National Academy of Sciences*, 93, 3908-3913.
- SHRESTHA, K. & VENTON, B. J. 2024. Transient Adenosine Modulates Serotonin Release Indirectly in the Dorsal Raphe Nuclei. *ACS Chemical Neuroscience*, 15, 798-807.
- SMITH-APELDOORN, S. Y., VERAART, J. K., SPIJKER, J., KAMPHUIS, J. & SCHOEVEERS, R. A. 2022. Maintenance ketamine treatment for depression: a systematic review of efficacy, safety, and tolerability. *The Lancet Psychiatry*, 9, 907-921.
- 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, 173-180.

- STRAWBRIDGE, R., YOUNG, A. H. & CLEARE, A. J. 2017. Biomarkers for depression: recent insights, current challenges and future prospects. *Neuropsychiatric disease and treatment*, 1245-1262.
- THRELFELL, S., CRAGG, S. J., KALLÓ, I., TURI, G. F., COEN, C. W. & GREENFIELD, S. A. 2004. Histamine H3 receptors inhibit serotonin release in substantia nigra pars reticulata. *Journal of Neuroscience*, 24, 8704-8710.
- TRAVIS, E. R., WANG, Y.-M., MICHAEL, D. J., CARON, M. G. & WIGHTMAN, R. M. 2000. Differential quantal release of histamine and 5-hydroxytryptamine from mast cells of vesicular monoamine transporter 2 knockout mice. *Proceedings of the National Academy of Sciences*, 97, 162-167.
- TURNER, A. P. 2013. Biosensors: sense and sensibility. *Chemical Society Reviews*, 42, 3184-3196.
- VENTON, B. J. & CAO, Q. 2020. Fundamentals of fast-scan cyclic voltammetry for dopamine detection. *Analyst*, 145, 1158-1168.
- WEST, A., BEST, J., ABDALLA, A., NIJHOUT, H. F., REED, M. & HASHEMI, P. 2019. Voltammetric evidence for discrete serotonin circuits, linked to specific reuptake domains, in the mouse medial prefrontal cortex. *Neurochemistry international*, 123, 50-58.
- WEST, E. & DALLY, P. 1959. Effects of iproniazid in depressive syndromes. *British medical journal*, 1, 1491.
- WITT, C. E., MENA, S., HOLMES, J., HERSEY, M., BUCHANAN, A. M., PARKE, B., SAYLOR, R., HONAN, L. E., BERGER, S. N. & LUMBRERAS, S. 2023. Serotonin is a common thread linking different classes of antidepressants. *Cell chemical biology*, 30, 1557-1570. e6.
- WITTKAMPF, K., VAN RAVESTEIJN, H., BAAS, K., VAN DE HOOGEN, H., SCHENE, A., BINDELS, P., LUCASSEN, P., VAN DE LISDONK, E. & VAN WEERT, H. 2009. The accuracy of Patient Health Questionnaire-9 in detecting depression and measuring depression severity in high-risk groups in primary care. *General hospital psychiatry*, 31, 451-459.
- WOOD, K. M. & HASHEMI, P. 2013. Fast-Scan Cyclic Voltammetry Analysis of Dynamic Serotonin Responses to Acute Escitalopram. *ACS Chemical Neuroscience*, 4, 715-720.
- WOOD, K. M., ZEQA, 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, 351-359.
- XIAO, N., PRIVMAN, E. & VENTON, B. J. 2014. Optogenetic Control of Serotonin and Dopamine Release in Drosophila Larvae. *ACS Chemical Neuroscience*, 5, 666-673.
- ZHANG, Y. & BHAVNANI, B. R. 2006. Glutamate-induced apoptosis in neuronal cells is mediated via caspase-dependent and independent mechanisms involving calpain and caspase-3 proteases as well as apoptosis inducing factor (AIF) and this process is inhibited by equine estrogens. *BMC Neurosci*, 7, 49.
- ZHAO, C., CHEUNG, K. M., HUANG, I.-W., YANG, H., NAKATSUKA, N., LIU, W., CAO, Y., MAN, T., WEISS, P. S., MONBOUQUETTE, H. G. & ANDREWS, A. M. 2021. Implantable aptamer-field-effect transistor neuroprobes for in vivo neurotransmitter monitoring. *Science Advances*, 7, eabj7422.

ZHU, C.-B., LINDLER, 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, 2510-2520.

CHAPTER 5 - GENERAL METHODS

Solutions

Stock solutions were prepared at room temperature by dissolving histamine dihydrochloride, dopamine hydrochloride, serotonin hydrochloride, norepinephrine hydrochloride, adenosine, gamma-aminobutyric acid (GABA), glycine, l-glutamic acid, l-tyrosine, l-tryptophan, l-cysteine, l-lysine and l-aspartic acid into the buffer and diluting to the desired final concentration.

All buffers were prepared at room temperature and adjusted to physiological pH (7.4). PBS was composed of 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄ and 1.8 mM KH₂PO₄. TRIS was composed of 15 mM Trisma, 140 mM NaCl, 3.25 mM KCl, 1.2 mM CaCl₂, 1.25 mM NaH₂PO₄, 1.2 mM MgCl₂ and 2.0 mM Na₂SO₄. HEPES was composed of 20 mM HEPES, 140 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, 1.0 mM MgCl₂ and 10 mM glucose. Amino acid buffer was composed of 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, 2.5 μ M gamma-aminobutyric acid (GABA), 34 μ M glycine, 21.3 μ M glutamate, 7.2 μ M tyrosine, 11.1 μ M tryptophan, 1.1 μ M cysteine, 46 μ M lysine and 0.2 μ M aspartate.

Thiopentamide maleate (20 mg kg⁻¹) was dissolved in sterile saline (0.9% NaCl solution) at 5 mL kg⁻¹. Urethane was dissolved in sterile saline as a 25% w/v solution and administered at 7 μ L g⁻¹. Sleep mix was composed of 2 mL of 0.05 mg mL⁻¹ fentanyl, 2 mL of 5 mg mL⁻¹ medetomidine, 1 mL of 1 mg mL⁻¹ midazolam, and 15 mL sterile saline (0.9% NaCl solution) and administered at 0.05, 5 and 0.5 mg kg⁻¹, respectively. All solutions were made fresh at the time of injection.

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified.

Tips for solutions:

1. The amino acid buffer should be made fresh daily and not stored overnight. It should also be kept in the dark while using.
2. Solutions containing dopamine, serotonin, norepinephrine, adenosine, and histamine should be kept out of the light at all times as they are light sensitive.
3. Ideally, any experiment involving amino acids, dopamine, serotonin, norepinephrine, adenosine, or histamine should be performed in the dark.

Electrode Fabrication

Carbon fiber microelectrodes (CFMs) were made individually by aspirating a single T-650 carbon fiber (Goodfellow Corporation, PA, USA) into a 1.0 mm x 0.50 mm x 10 cm glass capillary (World Precision Instruments, Hertfordshire, UK). The capillary was then pulled using a PE-22 vertical puller (Narishige, Tokyo, Japan) to create a carbon-glass seal between 200 - 500 μm . The carbon fiber was trimmed by hand to $150 \pm 3 \mu\text{m}$ (serotonin and histamine experiments) or $50 \pm 3 \mu\text{m}$ (dopamine experiments) with a scalpel blade under a light microscope. See **Figure 5.1**.

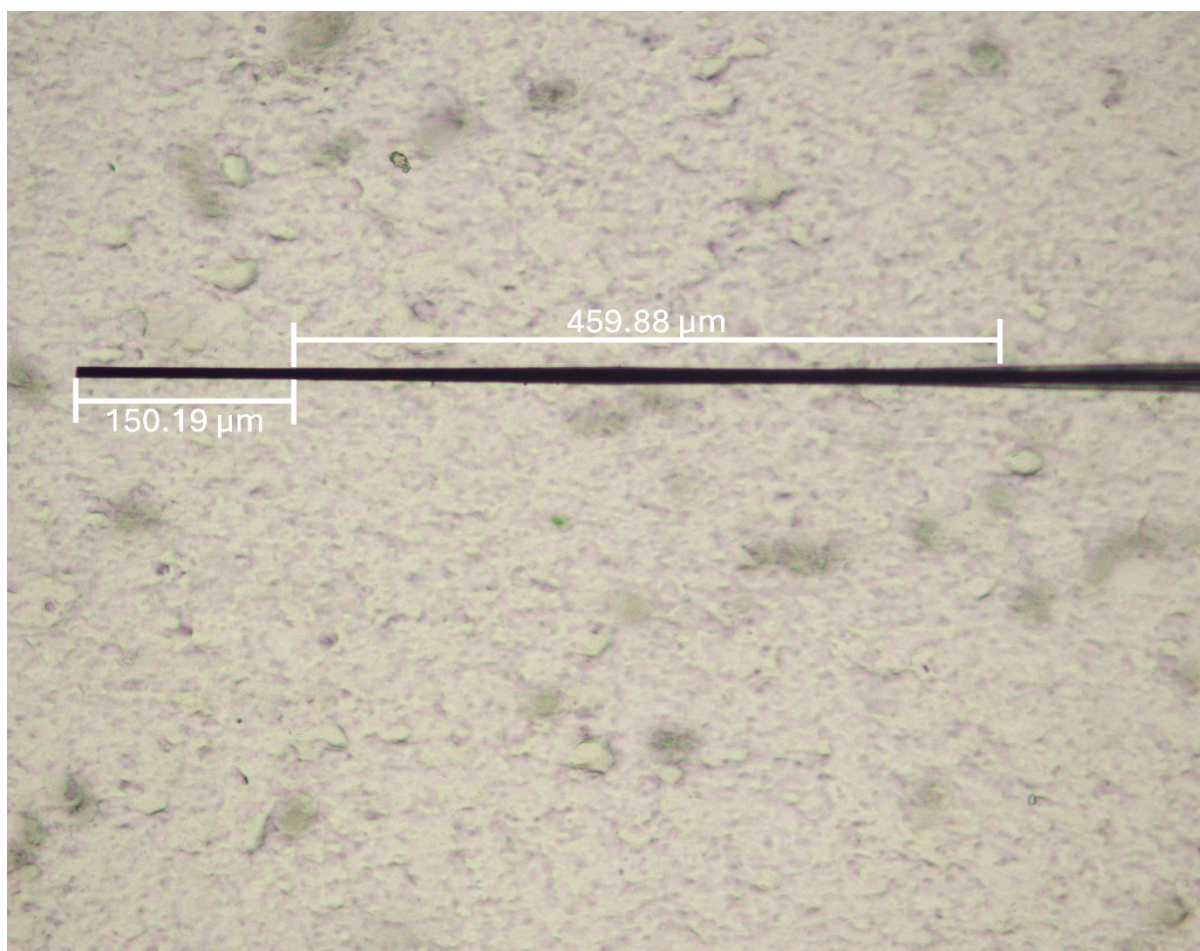


Figure 5.1. CFM under light microscope.

Next, a 1 cm long piece of heat shrink was placed on the middle of the capillary to protect the capillary from breaking while in the micromanipulator or flow cell depending on the experiment. Next, a stainless-steel wire was painted with silver paint and inserted into the back of the capillary to create a connection with the carbon fiber, and a heat shrink was placed half over the capillary and half over the stainless-steel wire's insulation. A connection pin was then attached to the wire and heat shrink was applied to the pin. A fully assembled electrode is shown in **Figure 5.2**.

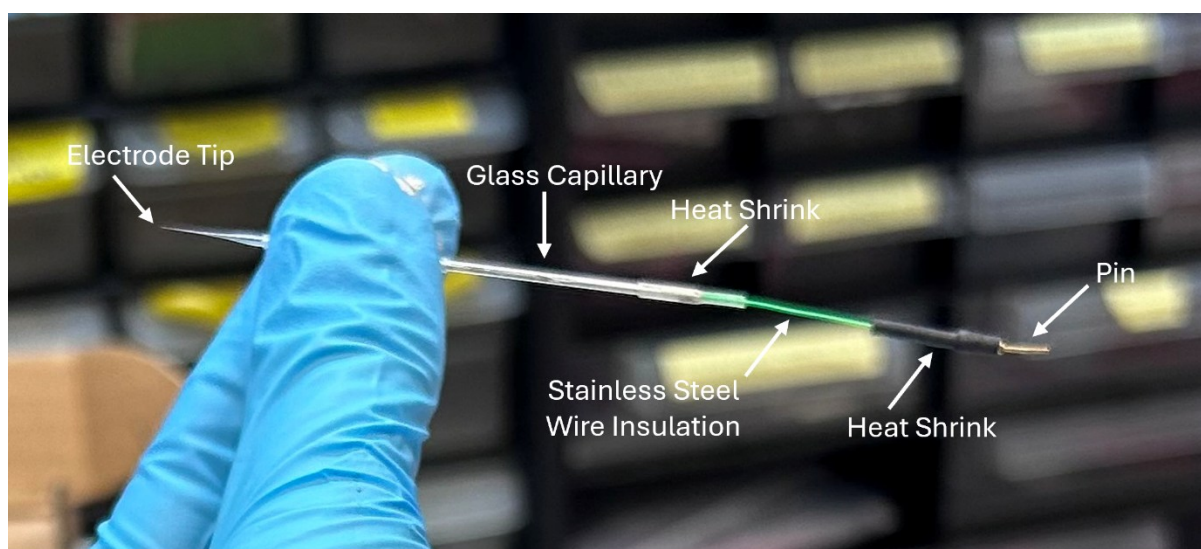


Figure 5.2. Picture of assembled CFM.

Electrode Modifications

Nafion Electrodeposition

Liquion (LQ-1105, 5% by weight Nafion) (Ion Power Solutions, New Castle, DE, USA) was electrodeposited onto the surface of the carbon fiber by dipping into the solution and applying a constant potential of 1.0 V vs. Ag/AgCl for 30 s. The electrode was then dried at 70°C for 10 min and allowed to set at room temperature overnight.

Amino Acid Modification

CFMs were placed into amino acid solutions at previously established physiological concentrations (in 1X PBS buffer: 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄ and 1.8 mM KH₂PO₄) and cycled using the triangular waveform (−0.4 V to 1.3 V to −0.4 V, 400 V s^{−1}) applied for 10 min at 60 Hz and 10 min at 10 Hz.

Lubricin Deposition

Lubricin aliquots at a concentration of 1.3 mg mL^{-1} were defrosted and diluted to a final working concentration of $150 \text{ } \mu\text{g mL}^{-1}$ and centrifuged and vortexed. Electrodes were lowered into solution (as shown in **Figure 5.3**) and left at room temperature for 15 min, removed from solution and rinsed with deionized water. Electrodes were stored between $2\text{-}8 \text{ }^{\circ}\text{C}$.

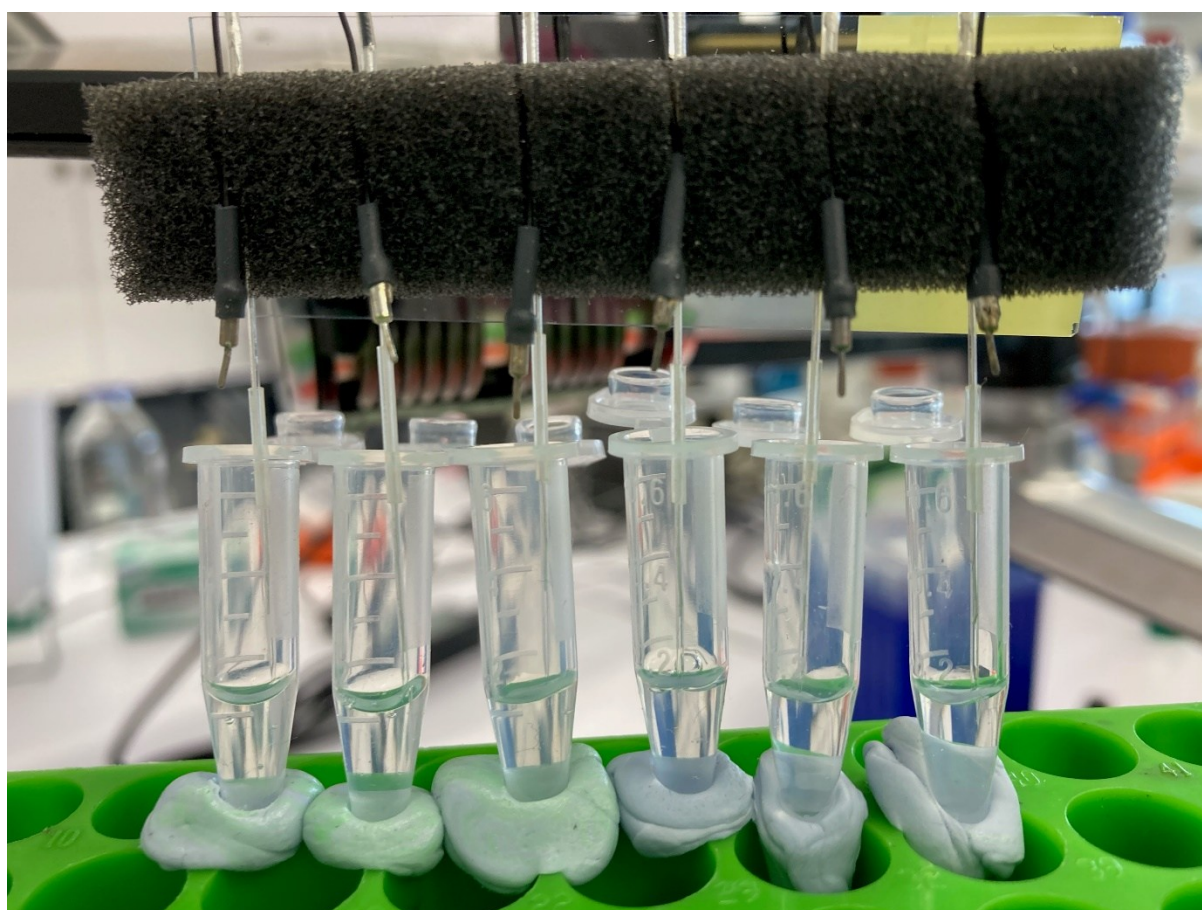


Figure 5.3. Lubricin Deposition onto CFMs. Image courtesy Marsilea Booth.

ECM Application

Geltrex (Invitrogen, Carlsbad, USA) was thawed on ice until it became a viscous liquid. A 1.00 μL drop of Geltrex was placed into a premade mold. The tip of a CFM was placed into the drop of Geltrex and held in place with blue tac. An additional 0.50 μL drop was placed onto the tip of the electrode to ensure electrode coverage. The CFM was baked at 37 °C for 20 minutes, then left to dry at room temperature overnight. The CFM was carefully lifted from the mold and placed in HEPES buffer for 20 minutes immediately before starting the experiment.

Data Collection and Analysis

FSCV and FSCAV were performed using a Dagan Potentiostat, (Dagan Corporation, Minneapolis, NM, United States), National Instruments multifunction device USB-6341 (National Instruments, Austin, TX), WCCV 4.0 software (Knowmad Technologies LLC, Tucson, AZ, United States) and a Pine Research headstage (Pine Research Instrumentation, Durham, NC, United States). Data filtering (zero phase, Butterworth, 2 kHz low-pass) and signal smoothing were achieved within the WCCV 4.0 software.

Analyte specific waveforms were applied to elicit the redox properties of each analyte (serotonin: 0.2 V to 1.0 V to -0.1 V to 0.2 V, 1000 V s^{-1} ; histamine: -0.5 V to -0.7 V to 1.1 V to -0.5 V, 600 V s^{-1} ; dopamine: -0.4 V to 1.3 V to -0.4 V, 400 V s^{-1}). For FSCV data collection, the waveform was applied at 10 Hz. For FSCAV data collection, the waveform was applied at 100 Hz. A more detailed account for FSCAV data acquisition can be found in Chapter 1.

Flow Injection Analysis (FIA)

FIA was performed in a system custom-built in-house (Hexter et al., 2023). A pseudo Ag/AgCl reference electrode was made by submerging a silver wire in a solution of 1 M HCl for 30 s at 5 V. A syringe infusion pump (Harvard Apparatus, Model Pump 11 Elite, Holliston, MA, USA) was used to maintain flow at 1.7 mL min^{-1} . The analyte was introduced into the flow stream via a six-port HPLC loop injector (Cheminert Valve, VICI, Houston, TX, USA) as a rectangular plug. **Figure 5.4** depicts the FIA setup. The syringe pump was connected to the valve at the 1 position, the outlet to the flow cell is at position 2, tubing for the sample loop is at positions 3 and 6, sample was injected at position 4, and a waste stream was at position 5. In the 'off' position, the buffer was pumped directly to the flow cell. The sample was injected and was held in the sample loop. When the valve was switched to the 'on' position, the buffer was then pushed through the sample loop and then to the flow cell delivering the bolus injection of sample to the electrode. The valve was then switched back to the 'off' position and the process was repeated.

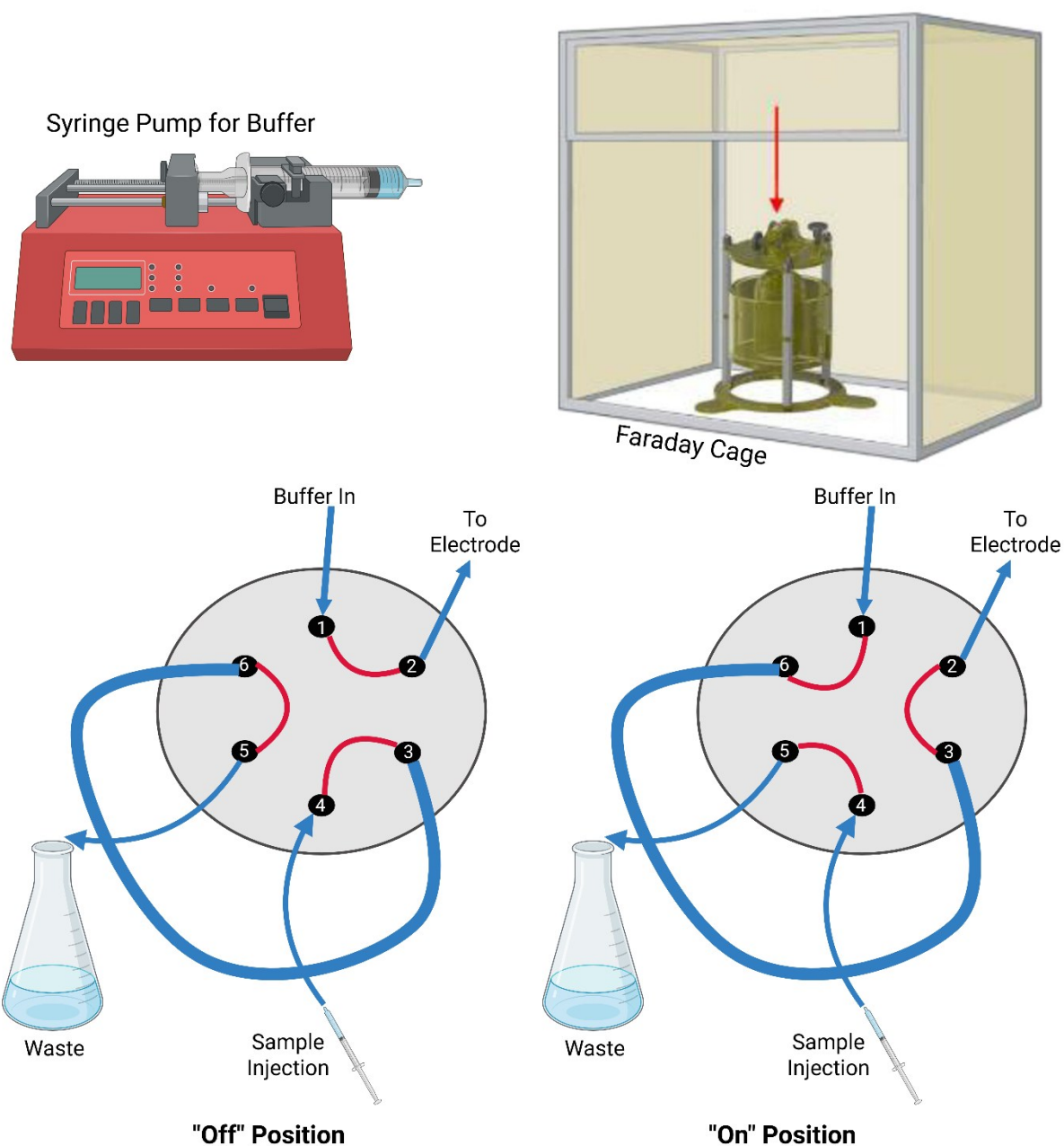


Figure 5.4. FIA setup. Image created using Biorender.

Tips for successful flow cell experiments:

1. Use a small amount of sticky tack to hold the reference electrode in place and seal the hole (make sure the outside of the hole is dry so the tack forms a seal). Movement of the reference electrode will disrupt measurements and leaking from the reference hole will cause flow abnormalities.

2. Use a 60 mL syringe for the buffer. This lessens the number of times you have to stop the pump and refill the buffer. Make sure the syringe pump settings are set to the correct inner diameter for the 60 mL syringe you are using. The flow rate should be set at 1.7 mL min^{-1} as per Hexter.
3. When cycling the electrode before taking measurements, turn off the buffer so as to not waste it but keep an eye on the oscilloscope. If the oscilloscope starts to shrink, the buffer level is falling below the electrode and needs to be topped up a bit. Turn the flow back on about 3 mins before the electrode is done cycling.
4. It helps the flow to slightly tip (10 degrees) the flow cell to help the buffer flow off/away from the electrode. This gives better injection profiles.
5. Make sure to completely flush the flow cell and all tubing with diH₂O after using. At least a full 60 mL should be flushed through with the valve set to the 'On' position to clean out the sample loop tubing. An additional 30-40 mL should be pushed through the 'sample in' tube to flush that tubing out as well (I like to open/close the valve a few times while water is running through to make sure everything gets plenty rinsed). If not adequately flushed, salt crystals will form and subsequent experiments will have flow rate inconsistencies when analyzing the data.
6. Always leave water in the flow cell tubing. If it dries out, air bubbles can form and cause pressure issues during injections.

Animal Surgery

All animal experiments were conducted in compliance with the Home Office license ([Scientific Procedures] Act 1986 and EU legislation). Male and female C57BL/6J mice (Charles River, Margate, UK), 6–12 weeks old and weighing 18–25 g, were group housed, had constant access to food and water, and were kept on a 12 h light/dark cycle.

A general schematic of animal experiments is shown in **Figure 5.5**. Two different experiment protocols were used after induction of anesthesia, (**A**) serotonin experiments or (**B**) histamine experiments.

A) Serotonin experiments: To induce and maintain anesthesia, 25% w/v urethane (Sigma–Aldrich Co), dissolved in 0.9% NaCl solution (Hospira) was given via i.p. injection ($7 \mu\text{L g}^{-1}$ body weight). Mouse body temperature was maintained using a heating pad (Braintree Scientific, Braintree, MA, United States). Stereotaxic surgery (David Kopf Instruments, Tujunga, CA, United States) was performed, and all coordinates were taken in reference to bregma. A small craniotomy was made using a dental drill and burr and a CFM was lowered into the CA2 region of the hippocampus (AP: -2.91, ML: +3.35, DV: -2.5 to -3.0) (Paxinos and Franklin, 2019) and adjusted in the dorsal/ventral plane until a serotonin signal was observed. A small craniotomy was made using a dental drill and burr and a stimulating electrode (insulated stainless steel, diameter: 0.2 mm, untwisted, Plastics One, Roanoke, VA, United States) was placed into the medial forebrain bundle (AP: -1.58, ML: +1.00, DV: -4.8) (Paxinos and Franklin, 2019). A pseudo Ag/AgCl reference

electrode, created by electroplating chloride (30 s in 1.0 M HCl at 5 V) onto a silver wire, was placed into the contralateral hemisphere.

FSCV measurements were taken until a serotonin signal was found. Electrical stimulation of serotonin was accomplished via linear constant current stimulus isolator (NL800A Neurolog, Medical Systems Corp, Great Neck, NY, USA) using the following parameters: frequency of 60 Hz, amplitude of 360 μ A each, 2 ms in width, and 2 s of total duration (120 pulses). Once the serotonin signal was established, FSCAV was then applied for a specified duration.

B) Histamine experiments: To induce and maintain anesthesia, a mixture of fentanyl, midazolam and medetomidine (0.05, 5 and 0.5 mg kg⁻¹, respectively) dissolved in 0.9% NaCl solution (Hospira) was given via i.p. injection and maintained via subcutaneous injection. Mouse body temperature was maintained using a heating pad (Braintree Scientific, Braintree, MA, United States). Stereotaxic surgery (David Kopf Instruments, Tujunga, CA, United States) was performed, and all coordinates were taken in reference to bregma. A small craniotomy was made using a dental drill and burr and a CFM was lowered into the cortex (AP: 0.00, ML: +2.00, DV: -0.50) (Paxinos and Franklin, 2019). A pseudo Ag/AgCl reference electrode, created by electroplating chloride (30 s in 1.0 M HCl at 5 V) onto a silver wire, was placed into the contralateral hemisphere. FSCAV measurements were taken until a histamine signal was found. FSCAV was then applied for a specified duration.

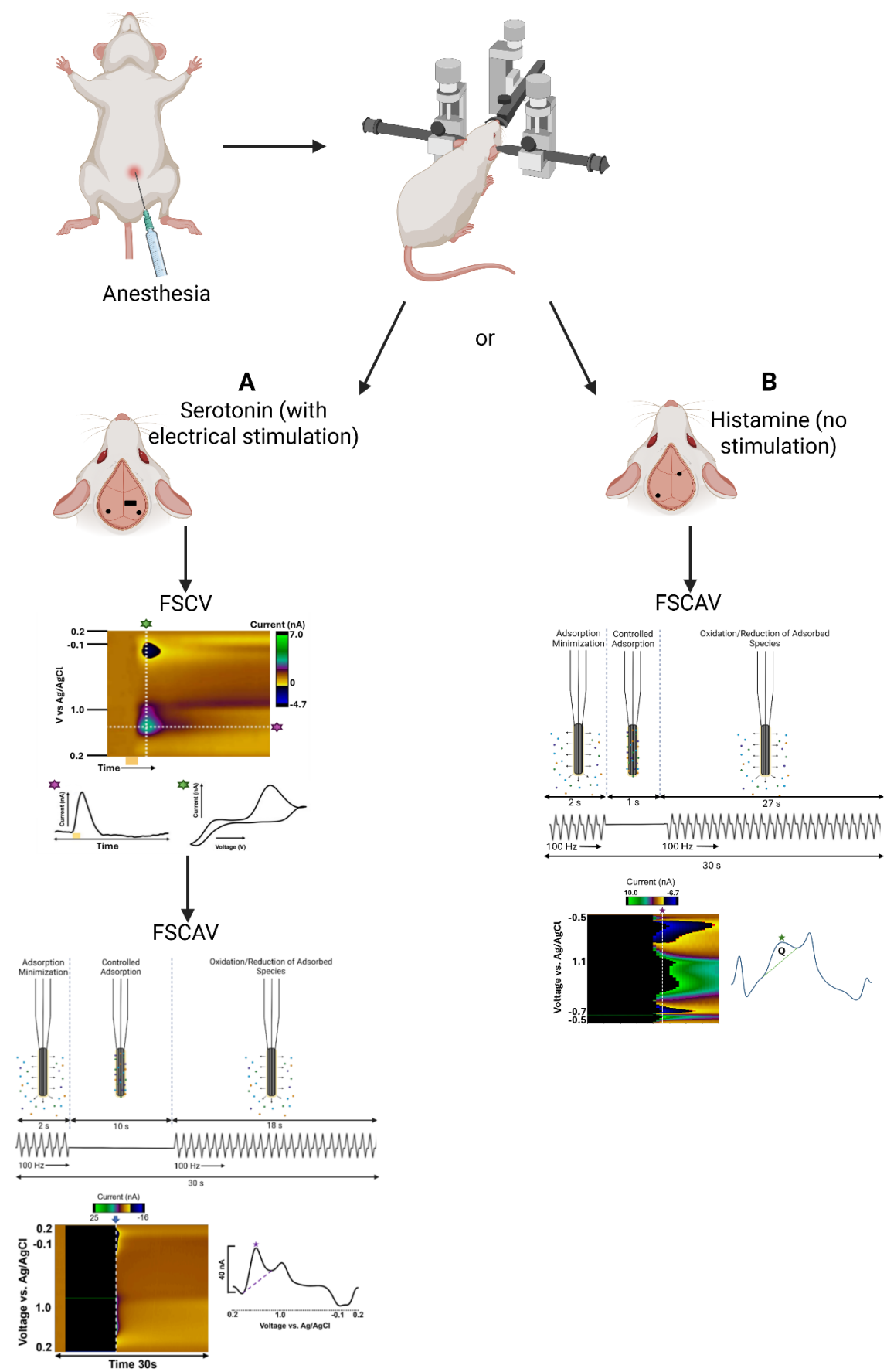


Figure 5.5. Schematic of animal experiment procedure. Image created using Biorender.

References

- HEXTER, M., VAN BATENBURG-SHERWOOD, J. & HASHEMI, P. 2023. Novel Experimental and Analysis Strategies for Fast Voltammetry: 2. A Troubleshoot-Free Flow Cell for FSCV Calibrations. *ACS Measurement Science Au*.
- PAXINOS, G. & FRANKLIN, K. B. 2019. *Paxinos and Franklin's the mouse brain in stereotaxic coordinates*, Academic press.

CHAPTER 6 – NOVEL EXPERIMENTAL STRATEGIES FOR FAST VOLTAMMETRY: LEVERAGING AMBIENT IN VIVO AMINO ACIDS TO AMELIORATE FSCAV STABILITY TOWARD SEROTONIN⁴

⁴ **Batey, L.**, Witt, CE., Mena, S., Honan, L., Hashemi, P. *In preparation.*

Abstract

Fast scan-controlled adsorption voltammetry (FSCAV) is an innovative method based on fast scan cyclic voltammetry (FSCV), that quantifies ambient levels of neurotransmitters at carbon fiber microelectrodes (CFMs). One ongoing challenge with FSCAV is the length of time (>1 hr) it takes the electrode to reach equilibration after in vivo implantation. We have previously mitigated this issue in two ways, by either waiting for the electrode to stabilize, or post-hoc analysis to remove any drift which risks eliminating pertinent data. In this work, we seek to understand why electrodes necessitate this lengthy equilibration time. Our first finding is that, in vivo, there is a positive drift in the signal upon insertion of the probe, whereas in a beaker, there is an exponential decay in signal over repeated measurements. Based on our and others' previous work, we ask if glutamate and/or other amino acids (structurally and functionally similar to glutamate), present at high basal concentrations in the brain, interact with CFMs. First, with FSCV we determine how the pre-modification of each amino acid onto CFMs changes the FSCV response to serotonin, allowing us to narrow down our scope to glutamate, GABA, glycine, and aspartate. Next, we use FSCAV to determine if pre-modification of these amino acids onto CFMs improves the signal, finding that pre-modifying the electrode with a combination of glutamate, GABA, and glycine increases the sensitivity to serotonin, and importantly, improves equilibration time in a complex matrix. Therefore, we show that by pre-modifying CFMs with these three amino acids, the equilibration time for in vivo FSCAV could be improved to around 20 minutes. Therefore, our investigations of this interesting phenomenon yield a new experimental strategy to improve FSCAV equilibration time and reduce experimental length.

Introduction

Fast scan cyclic voltammetry (FSCV) has been employed for many years to study the release and reuptake of neurotransmitters, including serotonin, in the brain. This technique utilizes redox signals at implanted carbon fiber microelectrodes (CFMs) to qualify and quantify analytes of interest in real time. To achieve real time measurements, electrochemical waveforms are applied at very fast ($>100\text{ s V s}^{-1}$) scan rates, however these fast scan rates induce a large capacitive current at the CFM which masks underlying Faradaic changes (Bard and Faulkner, 2001). Thus, to quantify only Faradic changes, FSCV employs background subtraction rendering the method only capable of quantifying changes in concentration, not the starting, basal or ambient analyte concentration. In response to this limitation, fast scan controlled adsorption voltammetry (FSCAV) was developed. This method is an offshoot of FSCV; data is collected during a short period of time at a high waveform application frequency (100 Hz) where adsorption of analytes to the CFM is minimized. A constant potential is then applied for 10 seconds, allowing the analyte to come into equilibrium with the electrode surface after which the waveform is reapplied, enabling quantification of the ambient analyte concentration around the electrode (Atcherley et al., 2013, Atcherley et al., 2015). We have used this method for close to a decade to report on basal serotonin concentrations in vivo (Abdalla et al., 2017, Saylor et al., 2019, Abdalla et al., 2020, Hersey et al., 2021b, Hersey et al., 2021a, Hersey et al., 2022, Witt et al., 2022, Berger et al., 2022).

FSCAV is a one-of-a-kind method in providing spatially and temporally resolved chemical information about steady state serotonin in vivo, however we have encountered several experimental and analysis issues that create time demands on the FSCAV experimenter. One issue we faced was a lengthy and technically difficult analysis procedure. We recently resolved this issue using a neural network analysis tool on our open-source online software that dramatically cuts analysis time and improves analysis reproducibility (Mena et al., 2022). A remaining issue with FSCAV is the length of time required for FSCAV to reach a steady state. This time period lasts over an hour where the signals gradually drift in a positive direction after CFM implantation into the brain. Until now, our only options were to either manually, or via data analysis, remove the drift we acquired from our data, or to wait over an hour for the electrode to stabilize. This latter issue is particularly problematic in vivo where adding additional hours for electrode stability significantly increases the risk of death to the animal before completion of the experiment. Additionally, post analysis is imperfect as it risks subtracting out real data. This occurs because the mathematical calculation of the drift, which is subtracted from the experimental data, is based on a combination of physiological variations and electrode drift.

In this work, we sought to understand the root of this length of time to reach stability to ultimately improve our protocols. Our first finding was that we could not replicate this stability profile in a beaker. While in vivo we found a positive drift in the signal upon insertion of the probe, in a beaker we found an exponential decay in signal over repeated measurements. To explain this phenomenon, we turned our attention toward the differences between the complex chemical environment of the brain and experiments in

a beaker, which are performed in simple buffers. We and others have previously found glutamate polymerizes onto carbon with a negative charge (Yu and Chen, 1997, Holmes et al., 2021). Therefore, we asked here if glutamate and/or other amino acids that are present at high basal concentrations in the brain matrix interact with CFMs to change FSCAV responses.

We studied an array of amino acids that are structurally similar to glutamate and are also present at high concentrations in the brain. We first used FSCV to determine how pre-modification of each amino acid onto CFMs changed the FSCV response to serotonin. This led us to narrow down our scope to glutamate, GABA, and glycine, which all improve the FSCV signal response. Next, we used FSCAV to determine if pre-modification of these amino acids onto CFMs also achieves signal increases towards serotonin. We found that pre-modifying the electrode with a combination of glutamate, GABA, and glycine not only increased the FSCAV sensitivity to serotonin, but significantly improved equilibration time of the electrode in a complex matrix of eight amino acids (designed to mimic the brain matrix). Therefore, as a final experiment, by pre-loading these three amino acids onto CFMs, we found that we could significantly decrease the equilibration time for in vivo FSCAV to around 20 minutes.

Thus, we present a new experimental strategy to improve FSCAV equilibration time and reduce experimental length.

Methods

Chemicals and Reagents

Electrodes were treated with solutions of gamma-aminobutyric acid (GABA) 2.5 μM , glycine 34 μM , glutamate 21.3 μM , tyrosine 7.2 μM , tryptophan 11.1 μM , cysteine 1.1 μM , lysine 46 μM and/or aspartate 0.2 μM individually or as a combination based on previously established physiological concentrations (Wang and Cynader, 2000, Ballini et al., 2008, Dolgodilina et al., 2016, Glykys and Mody, 2007, Ding et al., 1998, Oldenziel et al., 2006, Tagliamonte et al., 1973, Chaouloff et al., 1985). These amino acids (AAs) were dissolved into a 1X phosphate buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 1.0 mM KH_2PO_4) that was diluted from a 10X premixed buffer. Urethane was dissolved in sterile saline as a 25 % w/v solution. All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA).

Flow Injection Analysis (FIA)

FIA was performed in a system custom built in-house (Hexter et al., 2023). A pseudo-Ag/AgCl reference electrode was made by submerging in a solution of 1 M HCl for 15 s at 5 V. A syringe infusion pump (KD Scientific, Model KDS-410, Holliston, MA) was used to maintain flow at 1.7 mL min^{-1} . The analyte was introduced into the flow stream for 10 s via a six-port HPLC loop injector (Cheminert Valve, VICI, Houston, TX) as a rectangular plug.

Animals and Surgical Procedure

Mice (C57BL/6J) (Jackson Laboratory, Bar Harbor, ME, USA) were anesthetized via intraperitoneal (i.p.) injection with a 25% urethane solution at a volume of 7 $\mu\text{L g}^{-1}$ body weight. Following anesthetic administration, the mouse was placed into a stereotaxic system (David Kopf Instruments, Tujunga, CA, United States) where body temperature

was maintained via heating pad (Braintree Scientific, Braintree, MA, United States). Three holes were drilled into the skull of the mouse based on coordinates from the mouse brain atlas. All surgical coordinates were taken in reference to bregma (Paxinos and Franklin, 2019). The working electrode was placed in the CA2 region of the hippocampus (AP: -2.91, ML: +3.35, DV: -2.5), the stimulating electrode (insulated stainless-steel, diameter 0.2 mm, untwisted, Plastics One, Roanoke, VA) was placed in the medial forebrain bundle (AP: -1.58, ML: +1.00, DV: -4.80), and the pseudo Ag/AgCl reference electrode (made via the same specifications in the FIA section) was placed in the opposite hemisphere of the brain as the working and stimulating electrodes. Stimulation was accomplished via linear constant current stimulus isolator (NL800A Neurolog, Medical Systems Corp, Great Neck, NY, United States) with the following parameters: 60 Hz, 360 μ A each, 2 ms in width, and 2 s in length. Animal use followed NIH guidelines and complied with the University of South Carolina Institutional Animal Care and Use Committee under an approved protocol.

Microelectrode Fabrication

Carbon fiber microelectrodes (CFMs) were made individually by aspirating a single carbon fiber (Goodfellow Corporation, PA, USA) into a 0.6mm x 0.4mm glass capillary (A-M Systems, Inc., Sequim, WA). The capillary was then pulled by a PE-22 vertical puller (Narishige, Tokyo, Japan) to create a seal. The carbon fiber was then trimmed to either $100 \pm 5 \mu\text{m}$ (in vitro use) or $150 \pm 5 \mu\text{m}$ (in vivo use) under a light microscope.

Nafion Electrodeposition

Liquion (LQ-1105, 5% by weight Nafion) (New Castle, DE, United States) was electrodeposited onto the surface of the carbon fiber by dipping and applying a constant potential of 1.0 V for 30 s. The electrode was then dried in an oven at 70°C for 10 minutes and allowed to dry at room temperature overnight.

Amino Acid Electropolymerization

To deposit AAs, CFMs were placed into AA solutions (in 1X PBS buffer) and cycled using a triangular waveform (-0.4 V to 1.3 V to -0.4 V, 400 V s $^{-1}$) applied for 10 min at 60 Hz and 10 min at 10 Hz.

Data Collection and Analysis

FSCV and FSCAV were performed using a Dagan Potentiostat, (Dagan Corporation, Minneapolis, MN, United States), National Instruments multifunction device USB-6341 (National Instruments, Austin, TX), WCCV 4.0 software (Knowmad Technologies LLC, Tucson, AZ, United States) and a Pine Research headstage (Pine Research Instrumentation, Durham, NC, United States). Data filtering (zero phase, Butterworth, 2 kHz low pass) and signal smoothing were done within the WCCV 4.0 software. The “Jackson” waveform was applied to elicit the redox properties of serotonin (0.2 V to 1.0 V to -0.1 V to 0.2 V; 1000 V s $^{-1}$) (Jackson et al., 1995). For FSCV data collection, this was applied at 10 Hz. For FSCAV data collection, this was applied at 100 Hz. A more detailed account for FSCAV data acquisition can be found in Abdalla et al 2017 (Abdalla et al., 2017).

FSCAV Measurement Methods

Limits of integration to estimate the charge of the faradaic peak and maximum amplitude from FSCAV serotonin cyclic voltammograms are obtained using custom-designed automatic local minima and local maxima algorithms implemented in The Analysis Kid (Mena et al., 2021). Charge of the faradaic peak is calculated using the Simpson's rule. The first integration point is normalized to have a current value of zero to avoid subtraction of area between the negative and positive currents of the CVs. A linear regression is obtained between the two integration points to obtain the baseline used to measure the faradaic charge. This minimizes the interference from the capacitive peak. Linear regression models from post calibrations are obtained using linear least squares between concentration labels and estimated charge of the serotonin faradaic peak. The coefficient of determination (R^2) and the standard error of the estimate are used as parameters to assess the goodness of fit. FSCAV experimental drifts (vide infra) are modeled with a shifted negative exponential function shown in eq.1

$$Q(t) = -Q_0 e^{-kt} + Q_f \quad (1)$$

where $Q(t)$ is the charge of the faradaic serotonin peak at time t , Q_0 and Q_f determine the position of the exponential function in the Cartesian axes, and k is the exponential constant that determines the speed of growth. The time constant of the exponential function, $t_{63\%} = 1/k$, is used to characterize the speed of the sensor to achieve stability. The sensor response time (this was defined as the time it takes the sensor to reach 95% stability), was set as three times the time constant, since $t_{95\%} \approx 3t_{63\%}$. The nonlinear fitting is performed using the Levenberg-Marquardt algorithm (Ranganathan, 2004). Subtraction of exponential fit is performed by adding to the experimental trace an exponential decay function ($Q_0 e^{-kt}$) with the fitted parameters.

Statistical Analyses

Statistical significance was defined as $p < 0.05$. All statistical tests were performed using Python SciPy and Pingouin libraries. Distribution of the samples are shown as mean $\pm$ standard error of the mean (SEM). Differences in amplitude of the response and $t_{63\%}$ were tested to be significantly different using mixed design analysis of variance (ANOVA) with a between feature of amino acid applied to the solution and within feature of pre- and post-application of the polymerization waveform. After that, multiple paired and independent post-hoc comparisons with Tukey-Kramer correction of p values were performed to test for differences of specific groups.

Results and Discussion

In Vitro Responses to Serotonin Decay while In Vivo Responses Grow

FSCAV was developed to provide an index of steady-state, basal neurotransmitter levels at CFMs with a temporal resolution of approximately 1 minute (Atcherley et al., 2013, Atcherley et al., 2015, Abdalla et al., 2017). We originally applied this method in a similar way to FSCV where a 20-minute electrochemical cycling period was applied to the electrode in situ to prepare the surface before data acquisition (Rodeberg et al., 2017). This preparation is needed because application of an electrochemical waveform results in specific interactions of the matrix with the carbon surface, a process which typically takes some time to reach steady state, depending on the complexity of both the matrix and the waveform. Reliable data cannot be acquired during this period because the signals suffer significant baseline drift (Hsueh et al., 1997, Hermans et al., 2008). Once the surface is at equilibrium with the matrix, measurements can be taken more reliably. For FSCAV this ‘equilibration time’ is significantly prolonged. **Figure 6.1B** shows the

electrode response when measuring serotonin in the CA2 region of a mouse hippocampus once every minute on a Nafion-modified CFM. We have used Nafion-modified CFMs for serotonin measurements under the rationale that Nafion improves electrode integrity (Hashemi et al., 2009). The highlight feature of this *in vivo* signal is that the baseline can be fitted with a logarithmic growth function. Here, we define equilibration time as the time it takes the signal to come to 63% of equilibrium of a complex system. Based on this definition, this signal takes 43.93 min to come to stability. For data that has already been collected, this shift can be subtracted out using an exponential trace model (see methods). The result of this subtraction is shown inset in the red trace. However, this post hoc fix is not ideal.

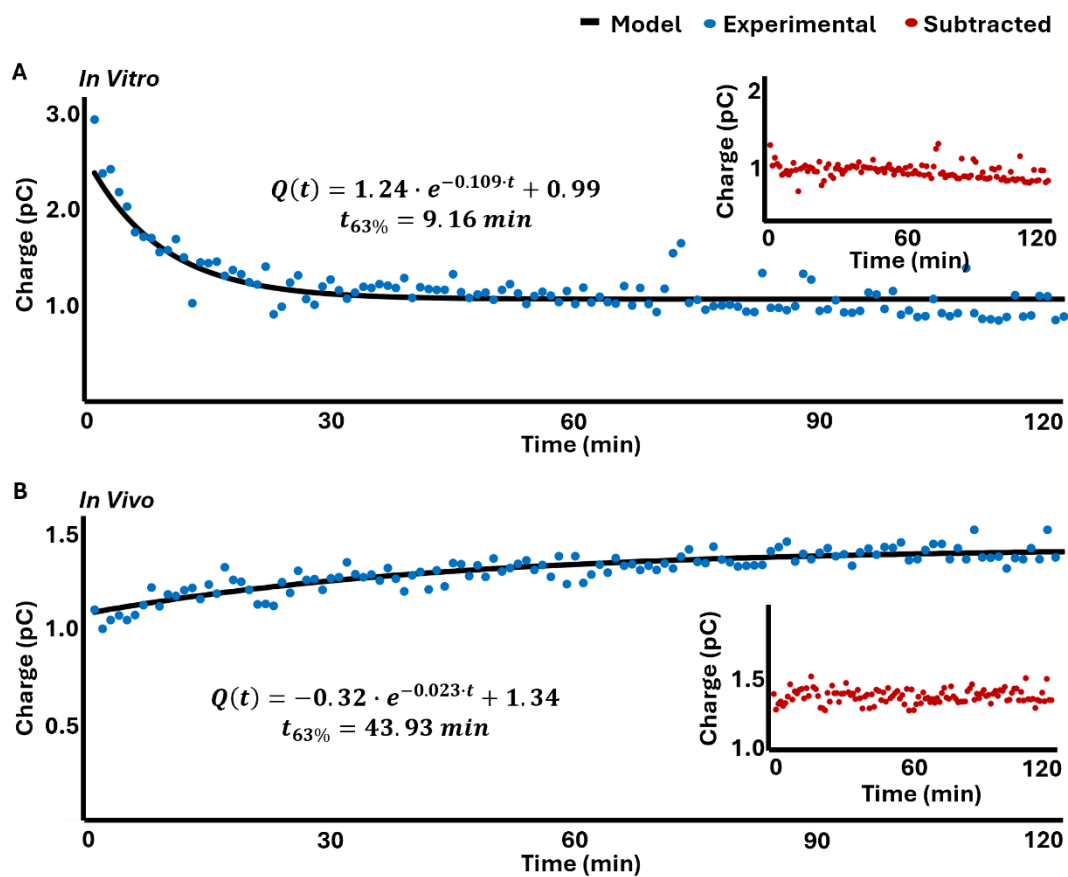


Figure 6.1: In vitro and in vivo representative examples of FSCAV acquisition for 120 min. (A) In vitro FSCAV acquisition in PBS-buffered 75 nM serotonin solution. From the fitted curve mode, the time constant is estimated to be 9.16 min. (B) In vivo FSCAV acquisition from the CA2 region of a mouse hippocampus. From the fitted curve model, the time constant is estimated to be 43.93 min. The experimental data is shown for both acquisitions after the exponential model subtraction (vide supra).

The estimation of the drift needs to occur on a section of the trace assumed physiologically stable (i.e., control state) to make sure the drift estimation is not influenced by physiological changes that would undermine the drift subtraction process. However, this assumption carries the inherent possibility of subtracting out biologically relevant changes. One could wait until the signal stabilizes, however the longer each in vivo experiment lasts, the higher the probability of failure due to complications with anesthesia. Thus, we asked what is causing the in vivo drift in a bid to lessen the equilibration time.

In seeking to replicate this drift in vitro, we encountered an immediate inconsistency. The in vitro trace in **Figure 6.1A** was obtained by measuring the electrode response to 75 nM serotonin (physiological range) in PBS for 120 minutes. This in vitro signal decays over time and reaches a steady state after about 9 minutes, this is in stark contrast to the in vivo signal that increases with time. As with the in vivo response, the drift can be modeled with a shifted negative exponential function shown inset in the figure.

The decline in sensitivity when using FSCV is what we expect due to fouling of the electrode surface by serotonin and its' oxidation products that has been demonstrated in a beaker in several studies (Jackson et al., 1995, Brazell et al., 1987, Hashemi et al.,

2009, Dunham and Venton, 2020, Shin et al., 2021). However, we have recently shown that this process is likely not the driver for electrode performance in vivo (Holmes et al., 2021). In this prior work, we found that the complex brain matrix is such that the processes at the electrode surface serve to increase, not decrease, the FSCV electrode response (Holmes et al., 2021). This important finding highlighted the inherent limitations of characterizing electrode sensitivity in a beaker. Nonetheless, in this prior work, we exploited this finding to confer improved stability to FSCV electrodes by showing that the amino acid, glutamate, could be pre-polymerized onto Nafion-modified CFMs to increase in vivo sensitivity (Holmes et al., 2021, Hashemi et al., 2009).

Therefore here, in application to FSCAV, we postulate that glutamate as well as other amino acids present in the brain matrix serve to slowly modify the electrode surface and increase the FSCAV response of the CFM to serotonin in vivo. We tested this hypothesis in the following sections.

Several Brain Amino Acids Improve FSCV Response Amplitude to Serotonin

Both in vivo and in vitro FSCAV signals necessitate equilibration time but the change in signal proceeds in opposite directions. Signal decay behavior for FSCV serotonin signals has been seen in vitro (Jackson et al., 1995, Hashemi et al., 2009, Brazell et al., 1987, Dunham and Venton, 2020, Shin et al., 2021). In this prior work, we showed that Nafion-coated electrodes exponentially lost their response to repeated flow injections of serotonin in vitro and showed that this decay was a direct result of stripping of the Nafion layer from the electrode surface. We found that this effect was curtailed in vivo because the Nafion layer was stabilized, in part, by high glutamate concentrations within the

extracellular environment that electropolymerize onto the electrode. Glutamate not only stabilizes the Nafion, but because it polymerizes with an overall negative charge, electrodes prepared with a pre-polymerized Nafion/glutamate layer are more sensitive and stable than Nafion alone (Holmes et al., 2021).

Given the high concentrations of not only glutamate, but other structurally and functionally similar amino acids in the extracellular matrix, we wondered if the growth behavior we observe here with FSCAV *in vivo* (**Figure 6.1B**) reflects a similar polymerization process. There is past precedence for the notion of modifying carbon electrodes with amino acids in the literature (Kordasht et al., 2021). For example, carbon electrodes modified with aspartate were used to distinguish dopamine and ascorbic acid measurements with high sensitivity (Juan and Xiaoli, 2012). Similarly, glycine electropolymerization has been used to improve catalytic activity of carbon electrodes (Narayana et al., 2014), to simultaneously measure catechol and hydroquinone (Harisha et al., 2018) and to measure L-Dopa (Palakollu et al., 2017). Lysine polymerization on carbon has enabled good peak separation between dopamine, ascorbic acid, and uric acid (Zhang et al., 2017) and poly-tryptophan carbon electrodes have been used to sense dopamine (Gong et al., 2020). Thus, in line with our previous FSCV experiments, we investigated a variety of amino acid pretreatments in **Figure 6.2**.

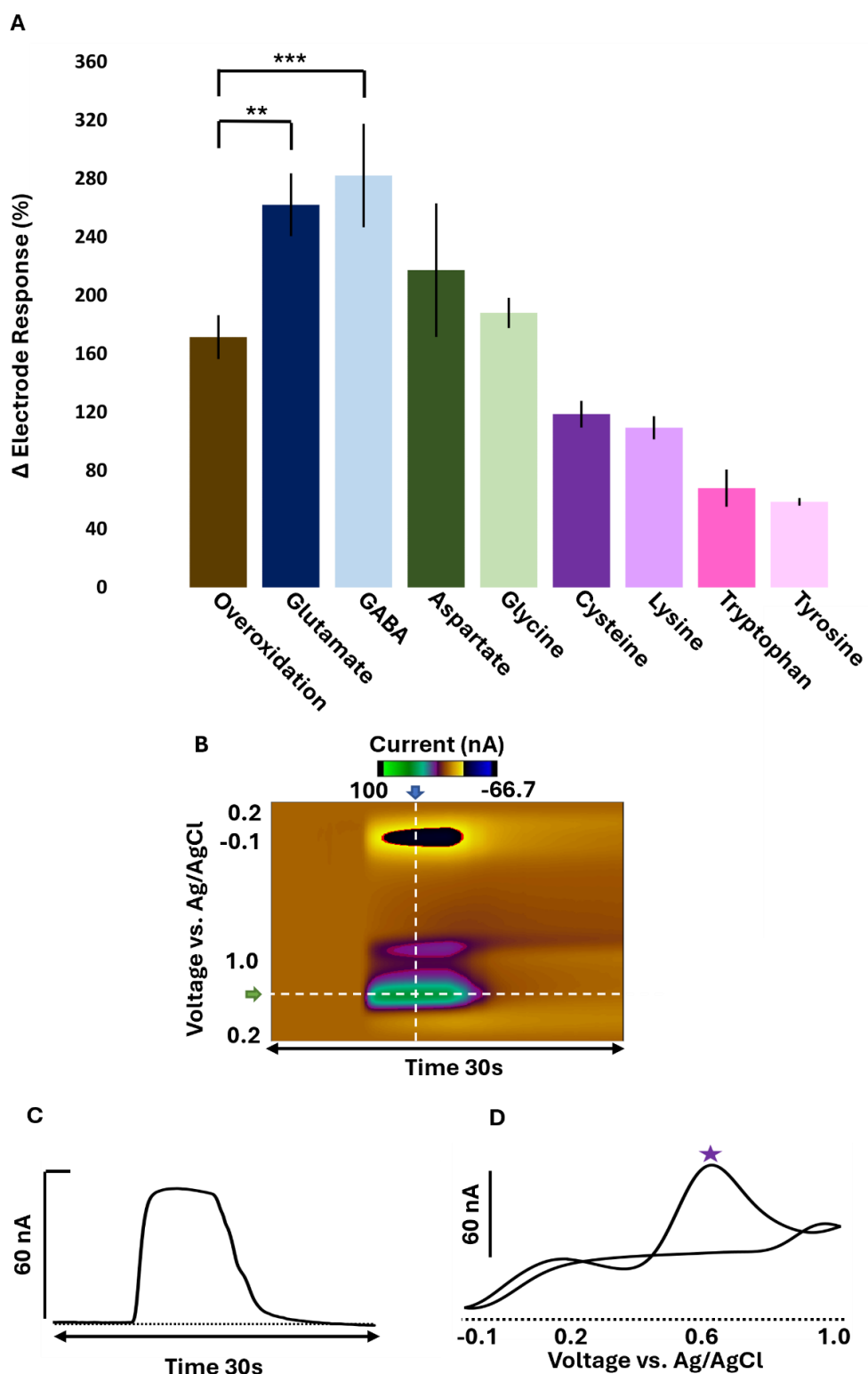


Figure 6.2: Surface modifications change FSCV response to serotonin. (A) Shows the percentage change in CFM response to a 1 μM injection of serotonin; each amino acid modification was statistically compared to the control (brown). Response and error bars show the mean $\pm$ SEM ($n = 3-9$) (** $p < 0.01$; *** $p < 0.001$). (B) Representative color plot of a 1 μM injection of serotonin. Green is indicative of oxidation and blue of

reduction. The horizontal line highlighted by the green arrow shows the extracted current vs time trace in C, and the vertical line highlighted by the blue arrow shows the cyclic voltammogram shown in D. The oxidation peak at approximately 0.6 V is indicated by the purple star.

Figure 6.2A shows the difference in response of the same CFMs before (bare) and after electrochemical cycling of an amino acid of interest to a 1 μ M flow injection of serotonin. **Figure 6.2B** is a representative color plot of such a 1 μ M injection of serotonin using the “Jackson” waveform (Jackson et al., 1995). These color plots are the raw data traces from FSCV and can be interpreted according to Michael et. al (Michael et al., 1998). Briefly they describe the acquisition of multiple cyclic voltammograms over time. The horizontal white line marked by the green arrow shows the current vs. time curve represented in **Figure 6.2C**, and the vertical line marked by the blue arrow shows the cyclic voltammogram (**Figure 6.2D**).

We focused on eight amino acids that are structurally similar to glutamate, although we appreciate that there are many others that might contribute to the process we are studying. Briefly, the treatment consisted of electrochemically cycling the CFM in a solution of each amino acid at concentrations we estimated to be physiologically relevant; a limitation here is that it is near impossible to ascertain true extracellular concentrations of these amino acids, thus for our purposes we chose numbers from the literature that were on the higher physiological end to maximize the effects we were studying (Wang and Cynader, 2000, Ballini et al., 2008, Dolgodilina et al., 2016, Glykys and Mody, 2007, Ding et al., 1998, Oldenziel et al., 2006, Tagliamonte et al., 1973,

Chaoulhoff et al., 1985). The amino acid pretreatment waveform was the same triangular waveform we previously used for glutamate polymerization (-1.2 V to 1.3 V at 400 V s⁻¹). The response of the Nafion-coated electrodes to 1 μM serotonin was 61.1 ± 3.38 nA (n=44; all injections on all electrodes). When treated with the waveform alone in PBS; ‘overoxidation’ (no amino acids present), this response changed by 171 ± 15.0%. This is because of the increased adsorption of serotonin to the electrode afforded by the negative surface at 1.3 V (Heien et al., 2003). Treatment with cysteine (119 ± 9.18%), lysine (109 ± 7.93%), tryptophan (67.9 ± 12.8%) and tyrosine (58.5 ± 2.59%) did not significantly increase the signal response more than overoxidation alone. Conversely, glycine (188 ± 10.3%), aspartate (217 ± 45.8%), glutamate (262 ± 21.5%) and GABA (282 ± 35.4%) increased electrode response to serotonin above overoxidation alone (glutamate and GABA were significant). We and others have previously found glutamate polymerizes onto carbon with a negative charge (Yu and Chen, 1997, Holmes et al., 2021), thus improving signal response to the positively charged serotonin cation. Given the structural similarities between glutamate, GABA, glycine and aspartate, it’s highly likely that the same occurs here (although the polymer structures are not studied in this paper).

Thus, it is clear a situation exists in vivo where a complex mixture of ambiently present amino acids polymerize onto and come to an equilibrium with the cycling carbon electrode. Next, we asked if we could utilize the amino acids that most increase the FSCV response amplitude to understand and even enhance in vivo FSCAV performance.

Amino Acids Improve Electrode Equilibration Time

We asked if glutamate, GABA, glycine, and aspartate would increase the FSCAV sensitivity to serotonin in a buffer that is more physiologically relevant than PBS. It is impossible to mimic the *in vivo* matrix in a beaker (Rauti et al., 2020). In our model, we decided to utilize a buffer containing all eight amino acids under the rationale that the instability we observe during FSCAV equilibration time (**Figure 6.1B**) is due to the slow interaction of amino acids over a period of a couple hours. Though sensitivity is not as much of an issue as equilibration time, we chose to study both parameters. We modified CFMs with glutamate, GABA, glycine, aspartate, or a combination and collected FSCAV files for 75 nM serotonin in a matrix containing all eight amino acids.

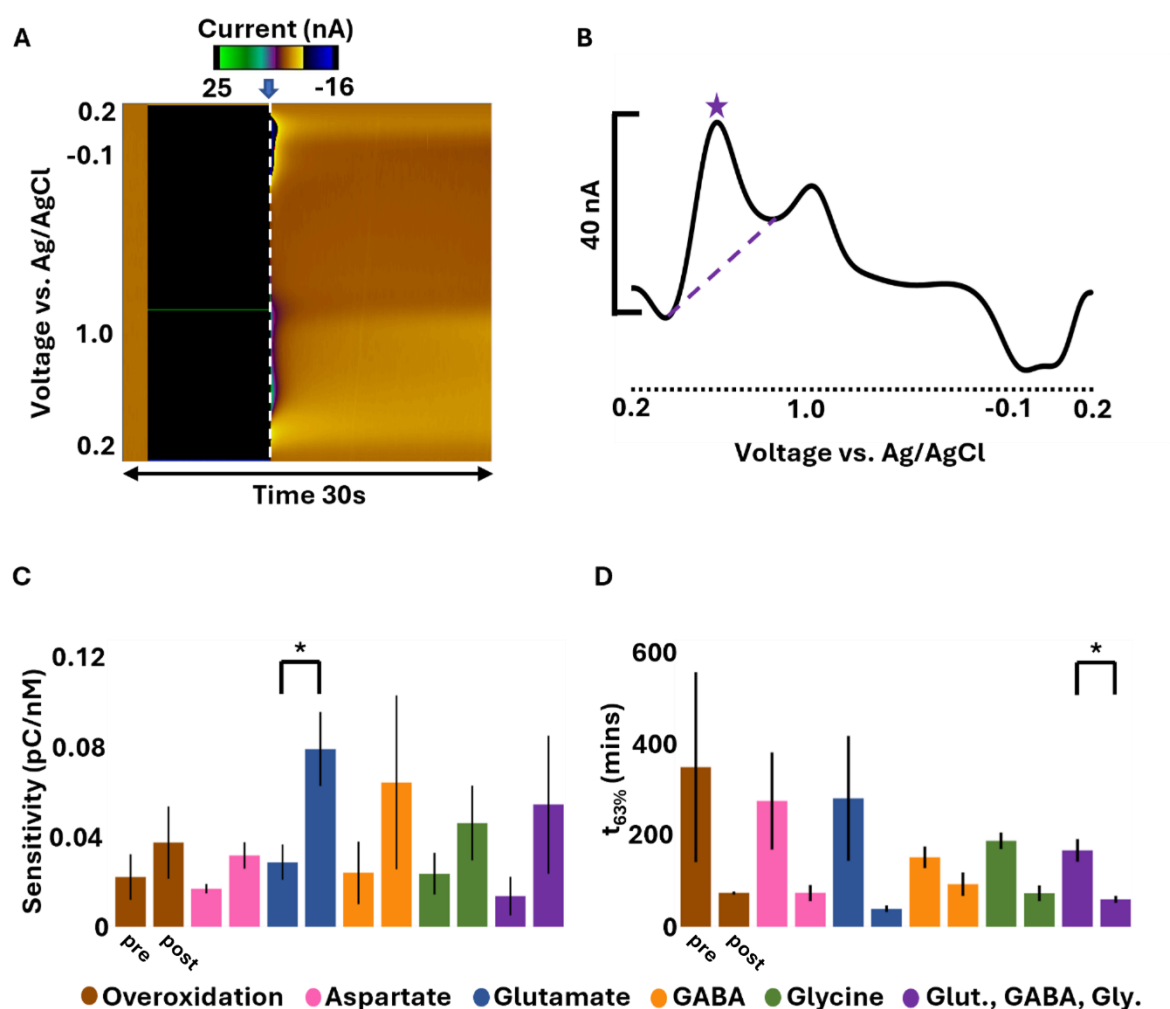


Figure 6.3: Surface treatments of carbon fibers with polymerized amino acids. (A) Representative FSCAV color plot for 75 nM serotonin. The vertical line marked by the blue arrow shows the extracted current vs. voltage trace shown in B. (B) CV extracted from white line in A. The purple star denotes the oxidation peak, and the dashed line indicates the integration area. (C) Shows the change in sensitivity in response to 25-100 nM serotonin pre (the left bar) vs. post (the right bar) polymerization; each modification was statistically compared to control (brown) (n = 3-4). (D) Shows the change in $t_{63\%}$ over 120 mins pre (the left bar) vs. post (the right bar) polymerization (n = 3-4). Response and error bars show the mean $\pm$ SEM. (* p < 0.05)

Figure 6.3A shows a representative FSCAV color plot. **Figure 6.3B** shows the third CV collected after reapplication of the waveform. The star represents the oxidation peak, and the dotted line denotes the area integrated to calculate charge. **Figure 6.3C** shows the increase in sensitivity after treatment with the four AAs, all improve sensitivity (overoxidation: pre 0.0225 ± 0.0101 pC/nM, post: 0.0378 ± 0.0160 pC/nM; aspartate: pre 0.0173 ± 0.00206 pC/nM, post 0.0321 ± 0.00582 pC/nM; glutamate: pre 0.0291 ± 0.00786 pC/nM, post 0.0794 ± 0.0165 pC/nM; GABA: pre 0.0244 ± 0.0139 pC/nM, post 0.0645 ± 0.0386 pC/nM; glycine: pre 0.0240 ± 0.00930 pC/nM, post 0.0465 ± 0.0166 pC/nM). Because glutamate, GABA and glycine had the more desirable effects, we next combined the three and found this combination to also increase sensitivity (pre 0.0140 ± 0.00854 pC/nM, post 0.0547 ± 0.0306 pC/nM). Next, we repeated the experiment in **Figure 6.1** by taking repeated FSCAV measurements on pretreated electrodes in the presence of eight amino acids and assessing the time it took for the signal to come to a stable baseline ($t_{63\%}$). **Figure 6.3D** shows all treatments (including overoxidation alone) improve equilibration time, with the combination of the three (glutamate, GABA and glycine) having the only significant effect. (Overoxidation: pre 350 ± 206 min, post: 76.2 ± 3.19

min; aspartate: pre 277 ± 105 min, post 76.2 ± 17.8 min; glutamate: pre 282 ± 136 min, post 42.2 ± 7.18 min; GABA: pre 153 ± 23.5 min, post 95.6 ± 25.6 min; glycine: pre 190 ± 17.8 min, post 75.6 ± 16.7 min; glut., GABA, gly: pre 169 ± 24.3 min, post 62.4 ± 7.34 min).

These experiments show that, in a beaker of a complex mixture of amino acids, we can utilize the phenomenon of these amino acids interacting with carbon surfaces to improve FSCAV sensitivity and equilibration time. We next asked if these phenomena hold in vivo.

Amino Acid Pretreatment Improves In Vivo Equilibration Time

Next, we investigated whether the modified electrodes could reduce equilibration time in vivo. We first verified that the eight amino acid combination matrix is a good mimic of the in vivo environment. **Figure 6.4A** shows the pooled in vivo Nafion-only modified electrodes' responses with time (green) plotted against Nafion and amino acid-modified electrodes with time (purple) in the eight amino acid matrix. The growth behavior in the in vitro amino acid matrix is strikingly similar to the in vivo increase in response, providing proof of principle that the complex amino acid matrix contributes to the growth for the in vivo response. Finally, the blue trace on **Figure 6.4A** shows the in vivo response to electrodes with the pre-modified three amino acid layer (glutamate, GABA and glycine). These electrodes have a significantly improved equilibration time as shown in **Figure 6.4B**. (38.8 ± 5.85 min Nafion only in vivo compared to 20.3 ± 3.04 min Nafion and amino acid modified in vivo; 40.33 ± 1.88 min Nafion and amino acid-modified in vitro), however it is likely that simply cycling in the eight amino acids would have similar benefits (as opposed to pre-treating).

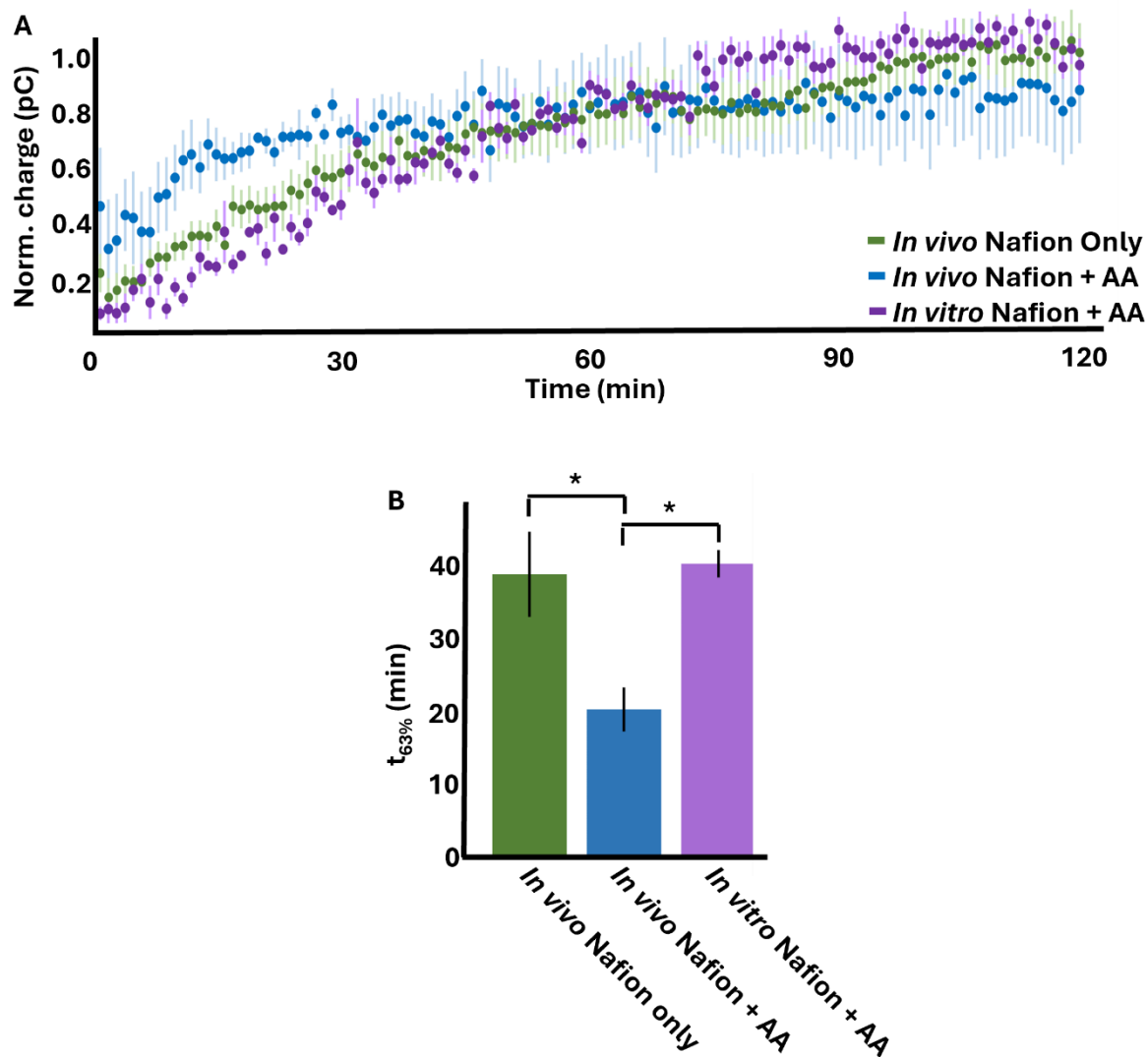


Figure 6.4: Amino acid-modified CFM equilibration times in vivo (A) Normalized in vivo traces of the Nafion-only electrode acquisitions (green, $n = 8$ animals), amino acid-modified electrodes (blue, $n = 5$ animals) and amino acid-modified electrodes in vitro (purple, $n = 6$). (B) Average $t_{63\%}$ for each data set. Bars represent mean $\pm$ SEM. (* $p < 0.05$)

We have thus not only explained the phenomenon we observed (increasing signal in vivo vs. decreasing in a beaker) but have exploited the findings to significantly improve upon it and better facilitate experiments.

Conclusions

Ambient levels of neurotransmitters can be quantified using a novel technique, FSCAV, which as an emerging method, carries some technical challenges. The first challenge is that the electrode may take over an hour after implantation to reach equilibrium, which we have dealt with in two ways. One option was to wait for the electrode to stabilize, increasing the likelihood of experimental loss. The other option was to remove the drift after the experiment using post hoc analysis, increasing the risk of eliminating important data. Here, we investigated the underlying cause of the long electrode equilibration time. We found that the FSCAV signal increased after in vivo implantation, while decaying in a beaker. We explored the differences between the complex brain environment and the simple buffer solutions to explain this discrepancy between in vivo and beaker experiments. We asked if an interaction between CFMs and amino acids found in the brain, specifically glutamate and/or amino acids similar to glutamate, could explain this discrepancy. Using FSCV, we determined that pre-modifying each individual amino acid onto CFMs would change the response to serotonin, focusing our efforts on glutamate, GABA, glycine, and aspartate. Subsequently, we used FSCAV to conclude that pre-modification with glutamate, GABA and glycine not only increased sensitivity to serotonin but also improved electrode equilibration time in a beaker with a complex matrix. Finally, we demonstrated that pre-modification of a CFM with these three amino acids before in vivo implantation significantly reduced equilibration time to around 20 minutes. We thus present a novel experimental strategy to improve FSCAV in vivo equilibration time, thereby minimizing experiment length.

References

- ABDALLA, A., ATCHERLEY, C. W., PATHIRATHNA, P., SAMARANAYAKE, S., QIANG, B., PEÑA, E., MORGAN, S. L., HEIEN, M. L. & HASHEMI, P. 2017. In Vivo Ambient Serotonin Measurements at Carbon-Fiber Microelectrodes. *Analytical Chemistry*, 89, 9703-9711.
- ABDALLA, A., WEST, A., JIN, Y., SAYLOR, R. A., QIANG, B., PEÑA, E., LINDEN, D. J., NIJHOUT, H. F., REED, M. C. & BEST, J. 2020. Fast serotonin voltammetry as a versatile tool for mapping dynamic tissue architecture: I. Responses at carbon fibers describe local tissue physiology. *Journal of neurochemistry*, 153, 33-50.
- 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, 14885-14892.
- 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, 2235-2238.
- BALLINI, C., CORTE, L. D., PAZZAGLI, M., COLIVICCHI, M. A., PEPEU, G., TIPTON, K. F. & GIOVANNINI, M. G. 2008. Extracellular levels of brain aspartate, glutamate and GABA during an inhibitory avoidance response in the rat. *Journal of neurochemistry*, 106, 1035-1043.
- BARD, A. J. & FAULKNER, L. R. 2001. *Electrochemical Methods: Fundamentals and Applications*, Wiley.
- BERGER, S. N., BAUMBERGER, B., SAMARANAYAKE, S., HERSEY, M., MENA, S., BAIN, I., DUNCAN, W., REED, M. C., NIJHOUT, H. F. & BEST, J. 2022. An In Vivo Definition of Brain Histamine Dynamics Reveals Critical Neuromodulatory Roles for This Elusive Messenger. *International journal of molecular sciences*, 23, 14862.
- BRAZELL, M., KASSER, R., RENNER, K., FENG, J., MOGHADDAM, B. & ADAMS, R. 1987. Electrocoating carbon fiber microelectrodes with Nafion improves selectivity for electroactive neurotransmitters. *Journal of neuroscience methods*, 22, 167-172.
- CHAOULOFF, F., ELGHOZI, J., GUEZENNEC, Y. & LAUDE, D. 1985. Effects of conditioned running on plasma, liver and brain tryptophan and on brain 5-hydroxytryptamine metabolism of the rat. *British journal of pharmacology*, 86, 33.
- DING, R.-G., ASADA, H. & OBATA, K. 1998. Changes in extracellular glutamate and GABA levels in the hippocampal CA3 and CA1 areas and the induction of glutamic acid decarboxylase-67 in dentate granule cells of rats treated with kainic acid. *Brain research*, 800, 105-113.
- DOLGODILINA, E., IMOBERSTEG, S., LACZKO, E., WELT, T., VERREY, F. & MAKRIDES, V. 2016. Brain interstitial fluid glutamine homeostasis is controlled by blood-brain barrier SLC7A5/LAT1 amino acid transporter. *Journal of Cerebral Blood Flow & Metabolism*, 36, 1929-1941.
- DUNHAM, K. E. & VENTON, B. J. 2020. Improving serotonin fast-scan cyclic voltammetry detection: new waveforms to reduce electrode fouling. *Analyst*, 145, 7437-7446.
- GLYKYS, J. & MODY, I. 2007. The main source of ambient GABA responsible for tonic inhibition in the mouse hippocampus. *The Journal of physiology*, 582, 1163-1178.
- GONG, Q.-J., HAN, H.-X., WANG, Y.-D., YAO, C.-Z., YANG, H.-Y. & QIAO, J.-L. 2020. An electrochemical sensor for dopamine detection based on the electrode of a poly-

- tryptophan-functionalized graphene composite. *New Carbon Materials*, 35, 34-41.
- HARISHA, K. V., KUMARA SWAMY, B. E. & EBENSO, E. E. 2018. Poly (glycine) modified carbon paste electrode for simultaneous determination of catechol and hydroquinone: A voltammetric study. *Journal of Electroanalytical Chemistry*, 823, 730-736.
- HASHEMI, P., DANKOSKI, E. C., PETROVIC, J., KEITHLEY, R. B. & WIGHTMAN, R. 2009. Voltammetric detection of 5-hydroxytryptamine release in the rat brain. *Analytical chemistry*, 81, 9462-9471.
- 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 increases sensitivity. *Analyst*, 128, 1413-1419.
- HERMANS, A., KEITHLEY, R. B., KITA, J. M., SOMBERS, L. A. & WIGHTMAN, R. M. 2008. Dopamine detection with fast-scan cyclic voltammetry used with analog background subtraction. *Analytical chemistry*, 80, 4040-4048.
- HERSEY, M., RENEAU, M., BERGER, S. N., MENA, S., BUCHANAN, A. M., OU, Y., TAVAKOLI, N., REAGAN, L. P., CLOPATH, C. & HASHEMI, P. 2022. A tale of two transmitters: serotonin and histamine as in vivo biomarkers of chronic stress in mice. *Journal of Neuroinflammation*, 19, 1-20.
- HERSEY, M., SAMARANAYAKE, S., BERGER, S. N., TAVAKOLI, N., MENA, S., NIJHOUT, H. F., REED, M. C., BEST, J., BLAKELY, R. D. & REAGAN, L. P. 2021a. Inflammation-induced histamine impairs the capacity of escitalopram to increase hippocampal extracellular serotonin. *Journal of Neuroscience*, 41, 6564-6577.
- HERSEY, M., WOODRUFF, J. L., MAXWELL, N., SADEK, A. T., BYKALO, M. K., BAIN, I., GRILLO, C. A., PIROLI, G. G., HASHEMI, P. & REAGAN, L. P. 2021b. High-fat diet induces neuroinflammation and reduces the serotonergic response to escitalopram in the hippocampus of obese rats. *Brain, behavior, and immunity*, 96, 63-72.
- HEXTER, M., VAN BATENBURG-SHERWOOD, J. & HASHEMI, P. 2023. Novel Experimental and Analysis Strategies for Fast Voltammetry: 2. A Troubleshoot-Free Flow Cell for FSCV Calibrations. *ACS Measurement Science Au*.
- HOLMES, J., WITT, C. E., KEEN, D., BUCHANAN, A. M., BATEY, L., HERSEY, M. & HASHEMI, P. 2021. Glutamate Electropolymerization on Carbon Increases Analytical Sensitivity to Dopamine and Serotonin: An Auspicious In Vivo Phenomenon in Mice? *Analytical Chemistry*, 93, 10762-10771.
- HSUEH, C., BRAVO, R., JARAMILLO, A. J. & BRAJTER-TOTH, A. 1997. Surface and kinetic enhancement of selectivity and sensitivity in analysis with fast scan voltammetry at scan rates above 1000 V/s. *Analytica Chimica Acta*, 349, 67-76.
- JACKSON, B. P., DIETZ, S. M. & WIGHTMAN, R. M. 1995. Fast-scan cyclic voltammetry of 5-hydroxytryptamine. *Analytical chemistry*, 67, 1115-1120.
- JUAN, L. & XIAOLI, Z. 2012. Fabrication of poly (aspartic acid)-nanogold modified electrode and its application for simultaneous determination of dopamine, ascorbic acid, and uric acid. *American Journal of Analytical Chemistry*, 2012.
- KORDASHT, H. K., HASANZADEH, M., SEIDI, F. & ALIZADEH, P. M. 2021. Poly (amino acids) towards sensing: Recent progress and challenges. *TrAC Trends in Analytical Chemistry*, 140, 116279.

- MENA, S., DIETSCH, S., BERGER, S. N., WITT, C. E. & HASHEMI, P. 2021. Novel, user-friendly experimental and analysis strategies for fast voltammetry: 1. The Analysis Kid for FSCV. *ACS Measurement Science Au*, 1, 11-19.
- MENA, S., VISENTIN, M., WITT, C. E., HONAN, L. E., ROBINS, N. & HASHEMI, P. 2022. Novel, User-Friendly Experimental and Analysis Strategies for Fast Voltammetry: Next Generation FSCAV with Artificial Neural Networks. *ACS Measurement Science Au*, 2, 241-250.
- MICHAEL, D., TRAVIS, E. R. & WIGHTMAN, R. M. 1998. Peer reviewed: color images for fast-scan CV measurements in biological systems. *Analytical chemistry*, 70, 586A-592A.
- NARAYANA, P., REDDY, T. M., GOPAL, P. & NAIDU, G. R. 2014. Electrochemical sensing of paracetamol and its simultaneous resolution in the presence of dopamine and folic acid at a multi-walled carbon nanotubes/poly (glycine) composite modified electrode. *Analytical Methods*, 6, 9459-9468.
- OLDENZIEL, W. H., DIJKSTRA, G., CREMERS, T. I. F. H. & WESTERINK, B. H. C. 2006. In vivo monitoring of extracellular glutamate in the brain with a microsensor. *Brain Research*, 1118, 34-42.
- PALAKOLLU, V. N., THAPLIYAL, N., CHIWUNZE, T. E., KARPOORMATH, R., KARUNANIDHI, S. & CHERUKUPALLI, S. 2017. Electrochemically reduced graphene oxide/Poly-Glycine composite modified electrode for sensitive determination of l-dopa. *Materials Science and Engineering: C*, 77, 394-404.
- PAXINOS, G. & FRANKLIN, K. B. 2019. *Paxinos and Franklin's the mouse brain in stereotaxic coordinates*, Academic press.
- RANGANATHAN, A. 2004. The levenberg-marquardt algorithm. *Tutorial on LM algorithm*, 11, 101-110.
- RAUTI, R., RENOUS, N. & MAOZ, B. M. 2020. Mimicking the brain extracellular matrix in vitro: a review of current methodologies and challenges. *Israel Journal of Chemistry*, 60, 1141-1151.
- 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, 221-234.
- SAYLOR, R. A., HERSEY, M., WEST, A., BUCHANAN, A. M., BERGER, S. N., NIJHOUT, H. F., REED, M. C., BEST, J. & HASHEMI, P. 2019. In vivo hippocampal serotonin dynamics in male and female mice: Determining effects of acute escitalopram using fast scan cyclic voltammetry. *Frontiers in neuroscience*, 13, 362.
- SHIN, H., GOYAL, A., BARNETT, J. H., RUSHEEN, A. E., YUEN, J., JHA, R., HWANG, S. M., KANG, Y., PARK, C., CHO, H.-U., BLAHA, C. D., BENNET, K. E., OH, Y., HEIEN, M. L., JANG, D. P. & LEE, K. H. 2021. Tonic Serotonin Measurements In Vivo Using N-Shaped Multiple Cyclic Square Wave Voltammetry. *Analytical Chemistry*, 93, 16987-16994.
- TAGLIAMONTE, A., BIGGIO, G., VARGIU, L. & GESSA, G. L. 1973. Free tryptophan in serum controls brain tryptophan level and serotonin synthesis. *Life Sciences*, 12, 277-287.
- WANG, X. F. & CYNADER, M. S. 2000. Astrocytes provide cysteine to neurons by releasing glutathione. *Journal of neurochemistry*, 74, 1434-1442.

- WITT, C. E., MENA, S., HONAN, L. E., BATEY, L., SALEM, V., OU, Y. & HASHEMI, P. 2022. Low-Frequency Oscillations of In Vivo Ambient Extracellular Brain Serotonin. *Cells*, 11, 1719.
- YU, A.-M. & CHEN, H.-Y. 1997. Electrocatalytic oxidation of hydrazine at the poly (glutamic acid) chemically modified electrode and its amperometric determination. *Synthesis and Reactivity in Inorganic and Metal-organic Chemistry*, 30, 599-607.
- ZHANG, D., LI, L., MA, W., CHEN, X. & ZHANG, Y. 2017. Electrodeposited reduced graphene oxide incorporating polymerization of l-lysine on electrode surface and its application in simultaneous electrochemical determination of ascorbic acid, dopamine and uric acid. *Materials Science and Engineering: C*, 70, 241-249.

CHAPTER 7 - FAST SCAN CONTROLLED ADSORPTION VOLTAMMETRY: A NEW METHOD FOR AMBIENT MEASUREMENTS OF HISTAMINE⁵

⁵ **Batey, L.**, Booth, M.A., Silva, S., Greene, W., Goodman, J., Kozlov, A., Hashemi, P. *In preparation*.

Abstract

Neuroinflammation is a physiological condition marked by elevated levels of brain histamine associated with various underlying causes. Additionally, peripheral inflammation can trigger neuroinflammation *via* vagal nerve innervation, leading to increased brain histamine levels from histaminergic neurons through neurotransmission, and mast cells and microglia through immune-related signaling. Fast scan cyclic voltammetry (FSCV) can analyze histamine release and reuptake but cannot quantify ambient levels. While microdialysis has typically been used to measure ambient neurotransmitter concentrations, the probe's large size and low temporal resolution present significant limitations for these measurements. Therefore, fast scan controlled adsorption voltammetry (FSCAV) was developed, offering improved temporal resolution and a significantly smaller probe size, which can also be used in conjunction with FSCV. In this chapter, we translate FSCAV to the measurement of histamine and show that histamine FSCV and FSCAV signals display significant similarity *in vitro*. We optimize the FSCAV parameters and determine that a coating of Lubricin on the electrode surface provides for sensitive and stable measurements over time and confirm the selectivity of the waveform toward other analytes. Finally, we apply this method *in vivo* in the mouse cortex. In this work, we present a novel tool for monitoring ambient histamine levels in the brain.

Introduction

Neuroinflammation is a physiological state characterized by an inflammatory response in the brain and spinal cord and has been implicated in many neurological disorders including Parkinson's disease, Alzheimer's disease, autism spectrum disorder, bipolar disorder and depression, just to name a few (Anichtchik et al., 2000, Rinne et al., 2002, Panula et al., 1997, Wright et al., 2017, Jin et al., 2009, Hersey et al., 2021a, Hersey et al., 2022, Yamada et al., 2020, Kano et al., 2004).

It is now known that peripheral inflammation can induce neuroinflammation through vagal nerve innervation (Jin et al., 2024). It has been shown that a peripheral injection of lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria known to elicit an immune response, increases phasic histamine levels in the brain demonstrating the link between peripheral inflammation and an increase in brain histamine (neuroinflammation) (Hersey et al., 2021b).

In the brain, histamine is derived from multiple sources, including neuronal, mast cell, and microglial populations. Neuronal histamine is primarily synthesized and released by histaminergic neurons in the tuberomammillary nucleus of the hypothalamus (Airaksinen and Panula, 1988, Panula et al., 1989, Inagaki et al., 1988), whereas mast cells and microglia contribute to histaminergic signaling through immune-related mechanisms (Tsai et al., 2011, Lull and Block, 2010).

To monitor these neurotransmitter levels in real-time, a technique known as fast scan cyclic voltammetry (FSCV) was developed (Millar et al., 1985, Millar et al., 1981, Kuhr and Wightman, 1986, Stamford et al., 1986, Stamford et al., 1984). FSCV utilizes carbon fiber microelectrodes (CFMs) which are biocompatible and have a diameter of approximately 7 μm which helps to reduce inflammation at the site of implantation (an important factor when monitoring histamine levels) (Hersey et al., 2019, Nesbitt et al., 2013, Nesbitt et al., 2015). FSCV provides high temporal and spatial resolution as well as high selectivity and sensitivity (Ou et al., 2019). However, the rapid scan rates generate a large background current which can obscure oxidation and reduction peaks during analysis. Therefore, background subtraction is necessary, allowing for the measurement of only evoked changes in neurotransmitter levels, while tonic (basal) concentrations cannot be directly quantified (Howell et al., 1986). Consequently, FSCV is limited to measuring fluctuations in evoked histamine concentrations, which originate exclusively from neuronal histamine release.

Historically, microdialysis has been the gold standard for measuring basal neurotransmitter levels. When coupled with mass spectrometry or high-performance liquid chromatography, it offers high chemical selectivity and the ability to quantify multiple analytes simultaneously. However, this technique also has notable limitations. The relatively large size of the microdialysis probe can cause significant tissue damage in the immediate vicinity, potentially altering the neurochemical environment being assessed (Nesbitt et al., 2013, Nesbitt et al., 2015). Furthermore, its low temporal resolution, with sampling occurring on the scale of minutes, limits its ability to capture rapid neurotransmitter fluctuations.

To overcome these limitations, fast scan controlled adsorption voltammetry (FSCAV) was developed as an alternative technique (Atcherley et al., 2013). Similar to FSCV, FSCAV employs CFMs, which minimize tissue damage due to their small diameter (<7 μm). Furthermore, FSCAV offers improved temporal resolution compared to microdialysis, enabling measurements at one-minute intervals (Abdalla et al., 2017, Atcherley et al., 2013, Atcherley et al., 2015). Additionally, because FSCAV utilizes a CFM-based detection system, both FSCV and FSCAV can be implemented using the same probe, facilitating multimodal neurochemical analysis.

In this chapter, we translate FSCAV to the measurement of ambient histamine. First, we demonstrate that histamine FSCV and FSCAV signals exhibit a high degree of similarity *in vitro*, with the oxidation and reduction peaks showing significant overlap. Second, we test three different FSCAV parameters to fine-tune the technique toward the measurement of histamine. Next, we investigate the impact of various electrode coatings on the sensitivity and stability of histamine FSCAV measurements and determine that Lubricin most effectively preserves optimal concentration ranges and electrode equilibration time. We then confirm that most interfering analytes are unlikely to affect histamine FSCAV signals. Finally, our method is applied to measure ambient *in vivo* histamine concentrations in the mouse cortex.

Herein, we introduce an innovative tool for monitoring ambient histamine levels in the brain.

Methods

Chemicals and Reagents

Histamine dihydrochloride, dopamine hydrochloride, serotonin hydrochloride, norepinephrine hydrochloride, adenosine, and thioperamide maleate (20 mg kg^{-1}) were purchased from Sigma-Aldrich (St. Louis, MO). 1X phosphate buffered saline ($0.01 \text{ M KH}_2\text{PO}_4$, $0.1 \text{ M Na}_2\text{HPO}_4$, 1.37 M NaCl , 0.027 M KCl) was prepared by diluting a 10X premixed solution purchased from Sigma-Aldrich (St. Louis, MO) with deionized water. Liquion (LQ-1105, 5% by weight Nafion) was purchased from Ion Power Solutions (New Castle, DE).

Carbon-Fiber Microelectrode Fabrication

The carbon-fiber microelectrodes were individually assembled by aspirating a single T-650 carbon fiber (diameter = $7 \text{ }\mu\text{m}$, Goodfellow Corporation, PA) into cylindrical glass capillaries: $1.0 \text{ mm} \times 0.50 \text{ mm} \times 10 \text{ cm}$ glass capillary (World Precision Instruments, Hertfordshire, UK). The carbon filled capillaries were then pulled using a P-22 vertical puller (Narishige Group, Setagaya-Ku, Tokyo, Japan) to form a carbon-glass seal under gravity. The carbon fibers were then cut by hand to $147 \pm 3 \text{ }\mu\text{m}$ in length with a scalpel blade under a microscope.

Fast-Scan Controlled-Adsorption Voltammetry (FSCAV)

Voltammetry was performed on a 2-electrode system. Waveforms were generated using a USB-6341DAC/ADC (National Instruments, TX, USA) device. The working electrode was controlled with a histamine specific waveform (HA: -0.5 V to -0.7 V to $+1.1 \text{ V}$ to -0.5 V ,

scan rate = 600 V/s). The electrode was cycled at 100 Hz for 20 min prior to measurements. FSCAV was performed as previously described using a CMOS precision analog switch, ADG419 (Analog Devices), to control the application of the waveform. The waveform (HA: -0.5 V to -0.7 V to $+1.1\text{ V}$ to -0.5 V , scan rate = 600 V/s) was applied at a frequency of 100 Hz for 2 s and then held at a constant potential of -0.5 V for 1 s using a Tenma bench power supply (Farnell, UK) followed by reapplication of the waveform for the remainder of the total file collection time of 30 s with a wait time of 30 s between subsequent files. Cyclic voltammograms (CVs) were used for HA identification. With FSCAV, the first CV after reapplication of the waveform with a signature HA peak was integrated to determine charge.

Nafion Electrodeposition

Nafion solution (Liquion-1105-MeOH, Ion Power, DE) was electrodeposited on the exposed carbon fibers by dipping in solution and holding at $+1\text{ V}$ vs. Ag/AgCl for 30 s. The microelectrodes were dried in an oven at 70°C for 10 min then allowed to dry at room temperature overnight.

Lubricin Deposition

Lubricin aliquots at a concentration of 1.3 mg/mL were defrosted and diluted to a final working concentration of $150\text{ }\mu\text{g/mL}$ and centrifuged and vortexed. Electrodes were lowered into solution and left at room temperature for 15 minutes, removed from solution and rinsed with deionized water. Electrodes were stored between $2\text{--}8^{\circ}\text{C}$.

Animals and Surgical Procedures

All animal experiments were conducted in compliance with the Home Office license ([Scientific Procedures] Act 1986 and EU legislation). Female C57BL/6J mice (Charles River, Margate, UK), 6–12 weeks old and weighing 18–25 g, were group housed, had constant access to food and water, and were kept on a 12 h light/dark cycle. To induce and maintain anesthesia, a mixture of fentanyl, midazolam and medetomidine (0.05, 5 and 0.5 mg kg⁻¹, respectively) dissolved in 0.9% NaCl solution (Hospira) was given via i.p. injection and maintained via subcutaneous injection. Mouse body temperature was maintained using a heating pad (Braintree Scientific, Braintree, MA, United States). Stereotaxic surgery (David Kopf Instruments, Tujunga, CA, United States) was performed, and all coordinates were taken in reference to bregma. A small craniotomy was made using a dental drill and burr and a CFM was lowered into the cortex (AP: 0.00, ML: +2.00, DV: -0.50) (Paxinos and Franklin, 2019). A pseudo Ag/AgCl reference electrode, created by electroplating chloride (30 s in 1.0 M HCl at 5 V) onto a silver wire, was placed into the contralateral hemisphere.

Statistical Analysis

All statistics were performed by GraphPad Prism 10 (GraphPad Software Inc., San Diego, USA), and all data are reported as the mean ± standard error of the mean (SEM). The “*n*” represents the number of electrodes tested. *P*-values were considered significant at the 95% confidence level ($p < 0.05$).

Results and Discussion

Histamine FSCV and FSCAV display significant alignment

In 2015, the Hashemi lab developed a novel histamine waveform (-0.5 V to -0.7 V to +1.1 V to -0.5 V; scan rate = 600 V s⁻¹) to measure evoked histamine in the mouse posterior hypothalamus (PH) with FSCV (Samaranayake, 2015). In addition to being able to measure evoked histamine release, this waveform allowed for the monitoring of changes in serotonin concentration (Samaranayake et al., 2016) which is beneficial since, along with histamine, serotonin has been implicated in the pathophysiology of depression (Woolley and Shaw, 1954). It was discovered that stimulated histamine release was followed by the inhibition of serotonin as a function of concentration and this inhibition was modulated by H₃ receptors (Samaranayake et al., 2016). Additionally, it was found that both a peripheral injection of LPS and the condition of chronic stress increased histamine levels in the mouse hypothalamus causing a decrease in ambient serotonin (Hersey et al., 2022, Hersey et al., 2021b).

Clearly, histamine plays an important role in the pathology of inflammation and depression. However, FSCV does not provide the full picture. Understanding the regulation of the tonic levels of histamine is critical for understanding its role in both inflammation and depression.

FSCAV has previously been used to quantify ambient, or tonic, dopamine and serotonin levels in the brain (Abdalla et al., 2017, Atcherley et al., 2013, Atcherley et al., 2015, Witt et al., 2022, Abdalla et al., 2020, West et al., 2019). FSCAV is a three-stage process which serves as a counterpart technique to FSCV, allowing for the quantification of ambient levels of neurotransmitters in addition to the evoked changes in neurotransmitter concentrations. In the first stage, the waveform is applied at a high frequency of 100 Hz

(FSCV is done at 10 Hz) which forces the adsorbed analyte off the electrode providing a 'clean' surface. In the second stage, the waveform is switched off and the electrode is held at a constant potential to allow for the adsorption of the analyte onto the electrode surface until it is in equilibrium with the surrounding environment. In the final stage, the waveform is reapplied at 100 Hz, inducing the desorption of the analyte from the electrode surface. The initial CV quantifies the analyte present on the electrode surface, which corresponds to the ambient concentration of the analyte.

Previously, FSCAV was applied to serotonin detection *in vivo*. This technique was used to quantify basal concentrations of serotonin across different brain regions (Abdalla et al., 2020) and to observe oscillations in ambient serotonin levels (Witt et al., 2022). Additionally, FSCAV was used to monitor ambient serotonin levels in mice that had been under chronic stress (Hersey et al., 2022) and in mice that had been exposed to LPS pre- and post- selective serotonin reuptake inhibitors (SSRI) (Hersey et al., 2021b).

Therefore, to develop a method to study tonic histamine, we started by combining the use of the histamine waveform and the FSCAV parameters used for dopamine and serotonin FSCAV (a 2 s initial desorption phase, a 10 s holding period, and an 18 s reapplication phase).

Figure 7.1Ai shows a representative FSCV color plot of 1 μ M histamine obtained by FIA using the histamine waveform applied at 10 Hz while **Figure 7.1Aii** shows the FSCAV counterpart with the waveform applied at 100 Hz. The dashed white lines denoted by the purple star signify the extracted cyclic voltammograms (CVs) from the FSCV and FSCAV

color plots shown together in **Figure 7.1B** with the FSCV CV shown as a dashed line and the FSCAV CV shown as a solid line. The vertical gray lines represent the integration bounds of the oxidation peaks which both occur at 0.4 V.

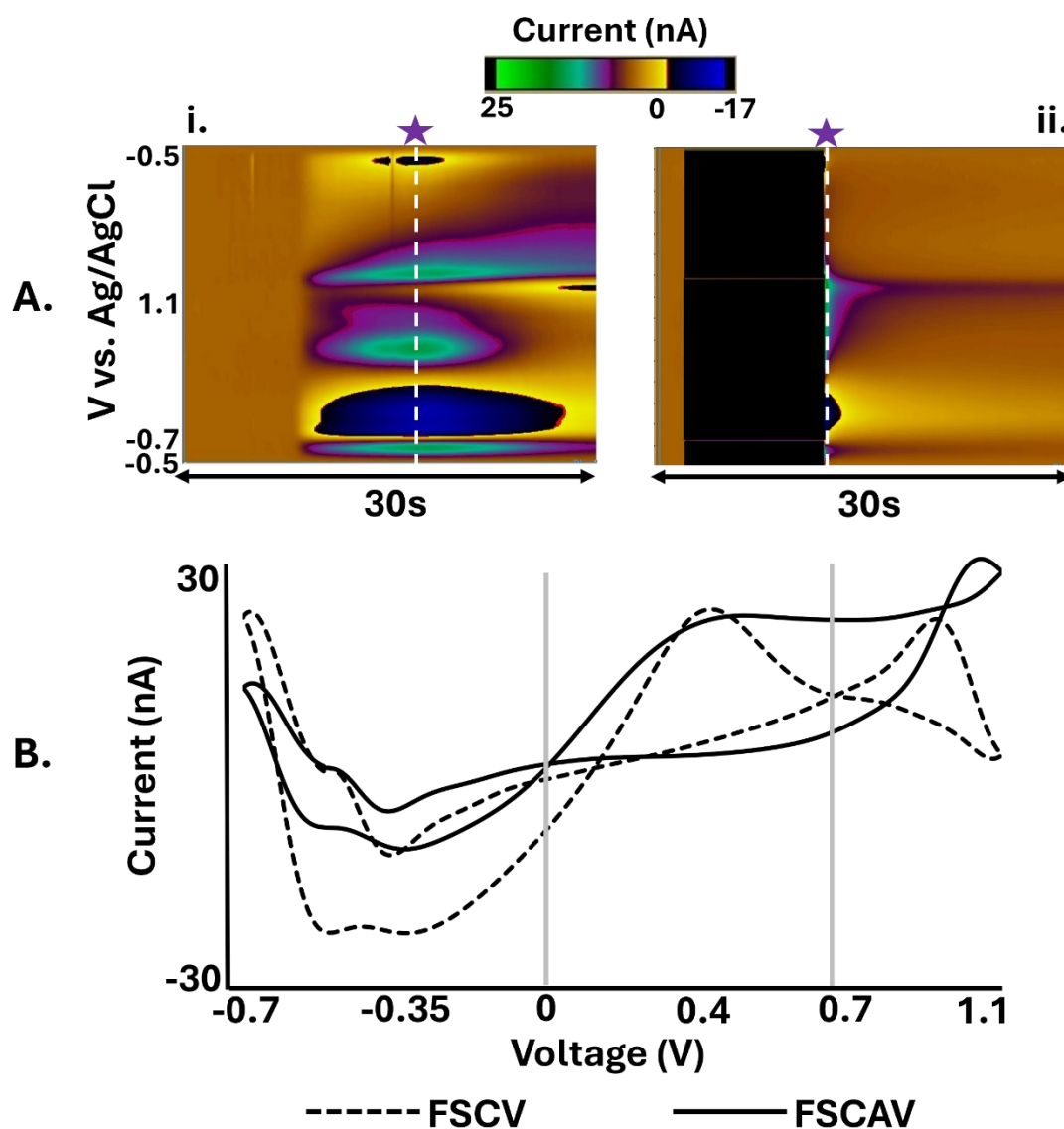


Figure 7.1: Histamine FSCV and FSCAV show good agreement. (A) Representative color plots of 1 μM histamine in vitro using i. FSCV and ii. FSCAV. (B) Cyclic voltammograms extracted from the white vertical lines indicated by the purple stars in Ai and Aii. FSCV trace is shown as a dotted line and FSCAV trace is a solid line. Gray vertical lines represent limits of integration.

The CVs exhibit a strong correlation with overlapping redox peaks. The peak boundaries for the FSCV CV at 0.01 V and 0.69 V and max peak height at 0.41 V, while the peak boundaries for the FSCAV peak were at 0.02 V and 0.70 V with a max peak height at 0.43 V. FSCAV produces a slightly broader oxidation peak, a characteristic previously seen with dopamine and serotonin FSCAV (Abdalla et al., 2017, Atcherley et al., 2013, Atcherley et al., 2015). We hypothesized that this was due to the higher frequency of waveform application. By applying the waveform at 100 Hz with FSCAV, versus 10 Hz with FSCV, the analyte has less time to reach the electrode surface and adsorb. This time delay results in a slower reaction rate, which produces a broader oxidation peak.

After confirming the similarity of the CVs obtained using FSCV and FSCAV, we proceeded to evaluate the FSCAV parameters.

Investigating FSCAV Parameters

Next, we examined how different FSCAV parameters might affect the electrode response to optimize the measurement. The parameters that would likely affect the histamine response are the length of the adsorption minimization period, the length of the adsorption period, and the scan rate. **Figure 7.2** shows the effects of these different parameters tested.

Dopamine and serotonin FSCAV use an adsorption minimization period of 2 s, thus we wanted to understand how changing this parameter would affect the histamine signal. As with dopamine and serotonin, increasing this time from 2 s (8.432 +/- 1.848 pC) to 5 s (9.419 +/- 2.600 pC) had no significant effect on the histamine signal as shown in **Figure**

176

7.2A. Therefore, as with dopamine and serotonin FSCAV, the adsorption minimization period for histamine FSCAV used was 2 s.

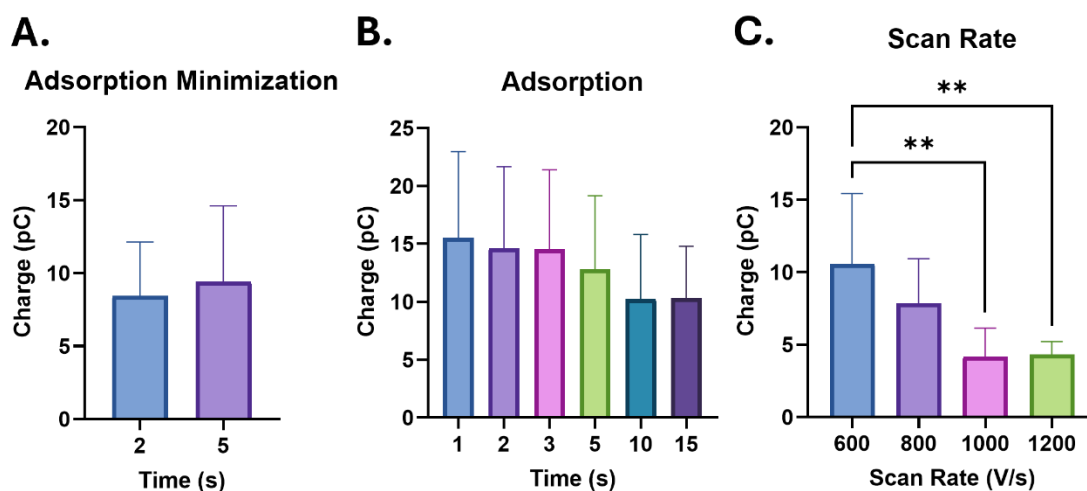


Figure 7.2: Bar graphs of histamine FSCAV parameters. The bars represent the charge from the integrated oxidation peak of the first CV after the waveform is reapplied (n=3-4). (A) Adsorption minimization period time, (B) adsorption time, and (C) scan rate of the applied waveform. Response and error bars show the mean $\pm$ SEM. (** p < 0.01)

The next parameter that could have a significant effect on the histamine signal is the adsorption period. The adsorption period is the time in which the applied voltage is held constant, allowing the analyte to adsorb onto the surface of the electrode until it reaches equilibrium with the surrounding environment. Histamine reaches this equilibrium rapidly. **Figure 7.2B** shows the effect that increasing the adsorption time has on the histamine signal. An adsorption period of 1 s produces a histamine signal of 15.526 $\pm$ 1.922 pC. Longer adsorption times do not produce a significant change in this response (2 s: 14.629 $\pm$ 1.818 pC; 3 s: 14.549 $\pm$ 1.766 pC; 5 s: 12.811 $\pm$ 1.640 pC; 10 s: 10.233

+/- 1.445 pC; 15 s: 10.333 +/- 1.153 pC); therefore, an adsorption time of 1 s was used as this was sufficient time for the electrode to reach equilibrium.

Additionally, we studied the effects of varying the scan rate of the histamine waveform. Increasing the scan rate did have a significant effect on the electrode. By increasing the scan rate, we observed a decrease in electrode response. (600 Vs⁻¹: 10.583 +/- 2.425 pC; 800 Vs⁻¹: 7.859 +/- 1.533 pC; 1000 Vs⁻¹: 4.167 +/- 0.992 pC; 1200 Vs⁻¹: 4.313 +/- 0.455 pC). This decrease in response is likely due to the limitations in the kinetics of the reaction. Therefore, we kept the scan rate at 600 Vs⁻¹.

FSCAV parameters must be analyte specific. For dopamine and serotonin, an adsorption minimization period of 2 s and adsorption period of 10 s is optimal though the scan rates differ with the scan rate for dopamine being 1200 Vs⁻¹ is optimal whereas for serotonin 1000 Vs⁻¹ is optimal. While for histamine an adsorption minimization period of 2 s and adsorption period of 1 s and a scan rate of 600 Vs⁻¹ is optimal. This is due to the differences in each analyte's adsorption kinetics and electrochemical properties. Because histamine's FSCV reaction is slower, it requires a slower scan rate than dopamine and serotonin. Additionally, histamine requires less time to reach equilibrium with the brain matrix. This is likely due to histamine being at a much higher concentration and therefore requiring less time to reach equilibrium.

In this section, we demonstrated that the optimal histamine FSCAV parameters are a waveform of -0.5 V to -0.7 V to +1.1 V to -0.5 V with a scan rate of 600 Vs⁻¹, an adsorption minimization period of 2 s, and an adsorption period of 1 s. Having established these

parameters, we next conducted calibrations using various electrode modifications to optimize measurement conditions for *in vivo* studies.

Lubricin Ideally Maintains Sensitivity and Equilibration Time

Subsequently, we turned our attention to calibrations and electrode coatings using the optimized FSCAV parameters. Based on previous mathematical modelling, we hypothesized the basal histamine concentration in the hypothalamus of healthy mice to be between 1-2 μM (Best et al., 2017, Samaranayake et al., 2016, Hersey et al., 2022) with these concentrations increasing upon inflammation.

We began by performing a calibration with Nafion-coated electrodes as shown in **Figure 7.3Ai**, as this is the coating used for histamine FSCV. Nafion produced a strong response; however, this coating substantially reduced the linear range of the calibration, with an upper limit of 1 μM . Nafion is a cation exchange polymer that works by pre-concentrating positively charged analytes, such as histamine, at the electrode surface while repelling negatively charged ions. While this preconcentration is good for analytes with much lower extracellular concentrations (such as 65 nM for serotonin (Abdalla et al., 2017)), at higher analyte concentrations in the micromolar range, the Nafion-coated electrode could be becoming saturated. When the Nafion is deposited onto the electrode surface, it is blocking some of the active sites while also pre-concentrating the analyte. Together, these combined effects cause the electrode to become saturated more quickly at lower concentrations.

Since Nafion proved to be nonoptimal for histamine FSCAV, we explored other electrode modifications and coatings.

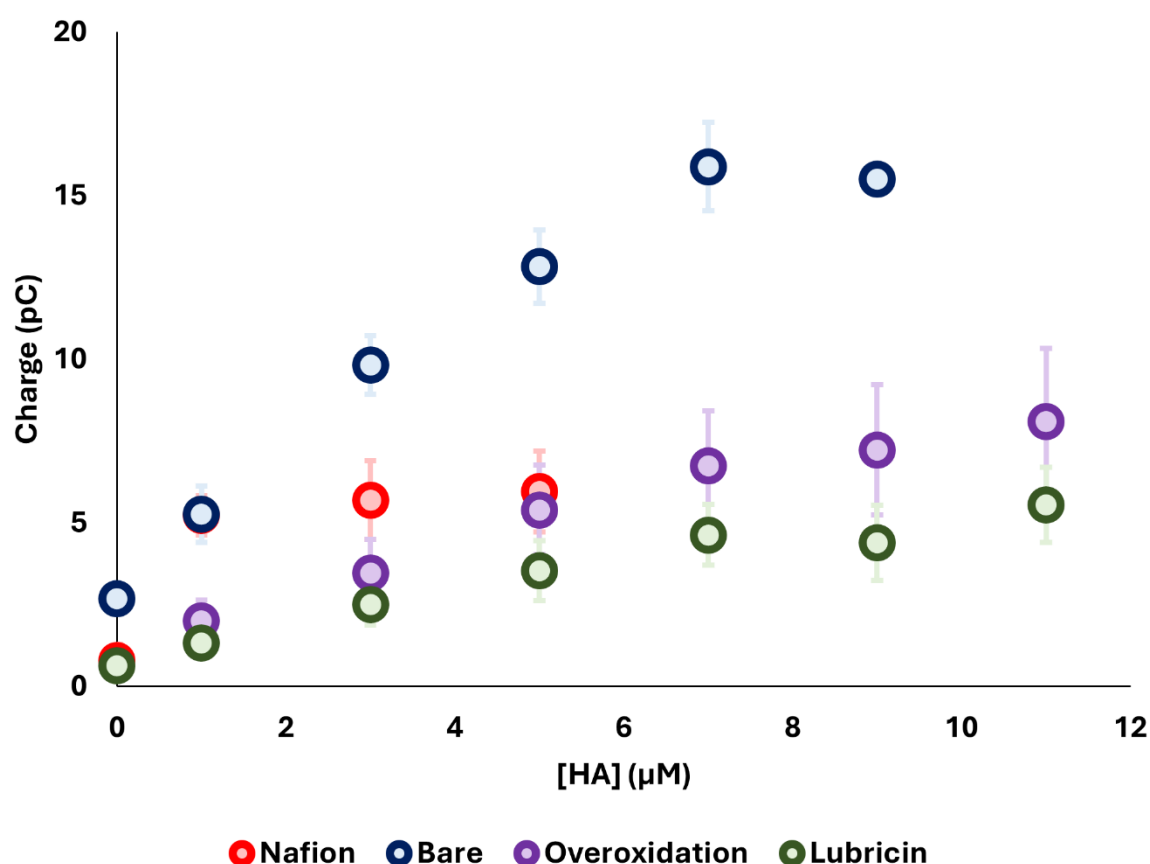


Figure 7.3: Histamine FSCAV Calibrations. Histamine FSCAV calibration curves for electrodes treated with Nafion (red), no treatment (bare (blue)), overoxidation waveform (purple), and iv. Lubricin (green). Response and error bars show the mean $\pm$ SEM.

We next evaluated the response of a bare electrode as shown in **Figure 7.3** to establish a baseline without any coatings or modifications. We hypothesized that a bare electrode may not generate a sufficient response to detect these basal levels; however, we were able to generate a response in 1 μ M histamine with a linear range up to 7 μ M. This extension of the linear range compared to the Nafion-coated electrodes is likely due to

the absence of a coating that blocks the electrode's active sites and the reduced preconcentration of the analyte on the electrode surface. Although this was an improvement compared to the Nafion-coated electrodes, we sought to expand the linear range further still and to address the issue of possible fouling of the electrode *in vivo*.

Another modification we tested was overoxidation of the electrode surface seen in **Figure 7.3**. This modification, which has been used previously with FSCV (Heien et al., 2003, Pathirathna et al., 2012, Dunham and Venton, 2020, Ross and Venton, 2014), is the process in which the anodic limit of the waveform is increased to over +1.0 V resulting in the etching and oxidation of the electrode surface increasing oxygen functional groups and preventing fouling (Takmakov et al., 2010, Heien et al., 2003, Stucky and Johnson, 2022, Castagnola et al., 2020). Overoxidation has been shown to increase the sensitivity of the electrode due to the addition of these active sites. The linear response reaches its maximum at 7 μ M.

We next turned our attention toward a compound called Proteoglycan-4, also known as Lubricin. Lubricin is a compound naturally produced in mammalian synovial joints which makes it biocompatible. It has previously been shown to possess anti-fouling properties on carbon-based electrodes (Russo et al., 2020). It self-assembles onto carbon surfaces producing 'loops' (**Figure 7.4**) that possess an overall negative charge (Han et al., 2019), thereby attracting positively charged cations such as histamine. The addition of lubricin onto the electrode surface gave a linear range up to 11 μ M seen in **Figure 7.3Aiv**.

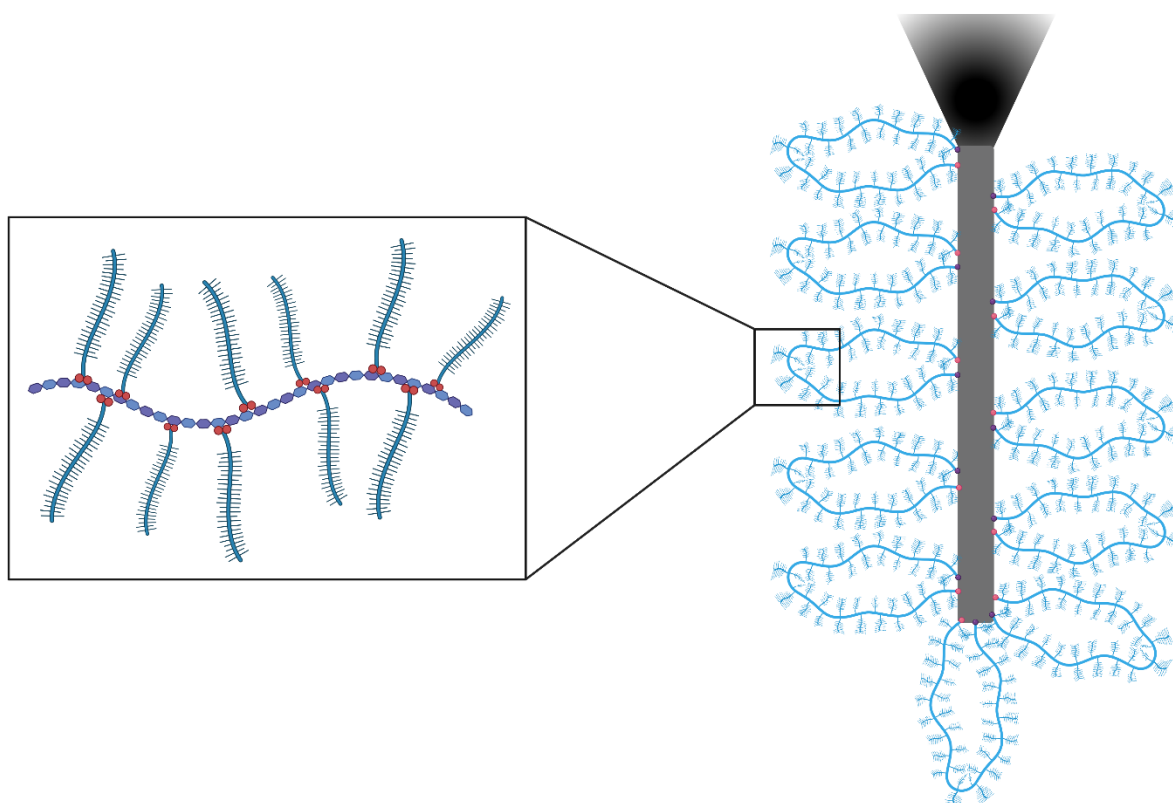


Figure 7.4: Lubricin schematic.

This extension of the linear calibration range is likely due to the Lubricin making the electrode less sensitive, unlike Nafion and overoxidation which make the electrode more sensitive.

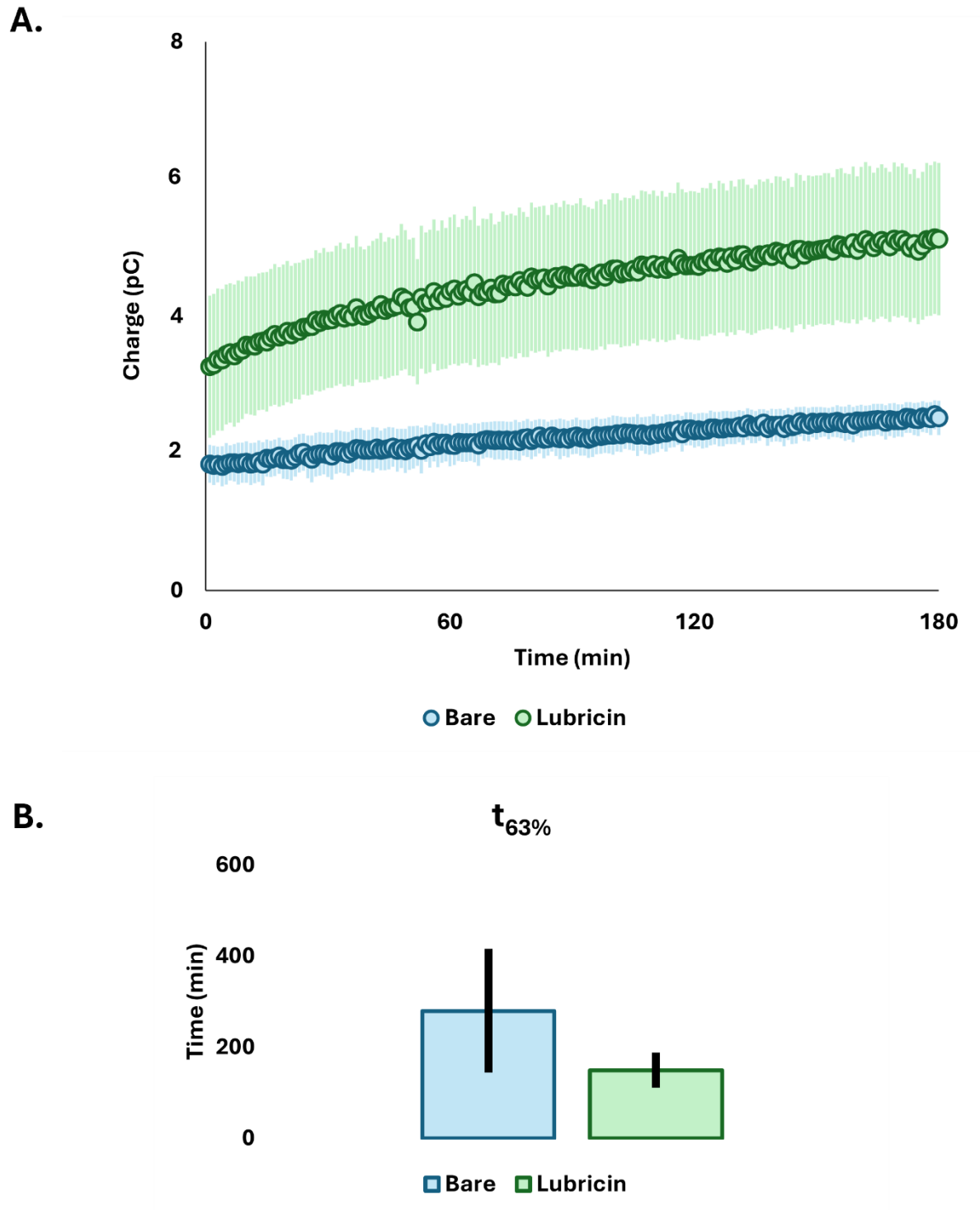


Figure 7.5: Histamine FSCAV coatings and stability (A) FSCAV traces of the bare electrodes (blue; $n=3$) and lubricin electrodes (green; $n=6$) in response to $1.5 \mu\text{M}$ histamine over 3 hours. (B) Average $t_{63\%}$ for each data set in A. Response and error bars show the mean $\pm$ SEM.

Having established that Lubricin is a suitable choice to provide a good linear range of a calibration, we sought to investigate how this coating would affect the time required for the electrode to reach equilibrium (stabilize). As discussed in Chapter 6, FSCAV measurements may take an extended amount of time to equilibrate. This extended equilibration time is due to the application of the electrochemical waveform resulting in specific interactions of the matrix with the carbon surface, a process which typically takes some time to reach steady state, depending on the complexity of both the matrix and the waveform.

Figure 7.5 shows the time it takes the FSCAV measurements to reach equilibrium. In **Figure 7.5A**, electrodes were placed in a beaker containing 1.5 μM histamine in PBS and FSCAV measurements were taken every minute for 3 hours. The response of the bare electrodes is shown in blue and the response of the Lubricin electrodes is shown in green. Not only did Lubricin produce a higher response than the bare electrodes to 1.5 μM histamine, the Lubricin electrodes also equilibrated in a shorter time as shown in **Figure 7.5B**. Here again, we define equilibration time ($t_{63\%}$) as the time it takes the signal to come to 63% of equilibrium of a complex system. The average $t_{63\%}$ for the bare electrodes was 279.7 $\pm$ 136.1 mins compared to the Lubricin electrodes which was 149.4 $\pm$ 39.0 mins.

Our explanation for this phenomenon is that the *in vivo* environment interacts with the chemical properties of the electrode surface causing the electrode to change over time. When applying a waveform that goes above the anodic limit of +1.0 V vs. Ag/AgCl, (as with the histamine waveform), surface oxide groups are introduced to the electrode's carbon

184

surface. This effect causes an increase in the redox current of the CVs (Rodeberg et al., 2017). For a bare electrode, the carbon surface is much more exposed to the environment allowing for a greater amount of oxide groups to be introduced to the carbon surface. However, when Lubricin is added to the electrode, this direct exposure of the carbon surface is much more limited, allowing for a smaller fraction of oxide groups to be produced. This smaller amount of oxide groups able to be produced results in a shorter equilibration time for the Lubricin electrodes.

In this section, we demonstrated that the application of Lubricin to the electrode surface produced an ideal linear range for calibrations of histamine and that it also decreased the equilibration time of the electrode. We next looked at any potential interferences of other neurotransmitters to our histamine signal.

Histamine FSCAV is Selective

We tested molecules that could potentially interfere with histamine measurements. Serotonin, dopamine, and norepinephrine are typically located in brain regions also containing histamine (Flik et al., 2015). **Figure 7.6** shows CVs of the possible interfering agents collected *in vitro* at concentrations believed to be equal to or greater than *in vivo* concentrations. The blue dotted line indicates the location of the histamine oxidation peak while the red dotted line indicates the location of the interfering agents' oxidation peaks. Based on the peak locations, these substances should not interfere with *in vivo* measurements as histamine should be readily identifiable versus the other substances whose oxidation peak is shifted towards a much more positive potential.

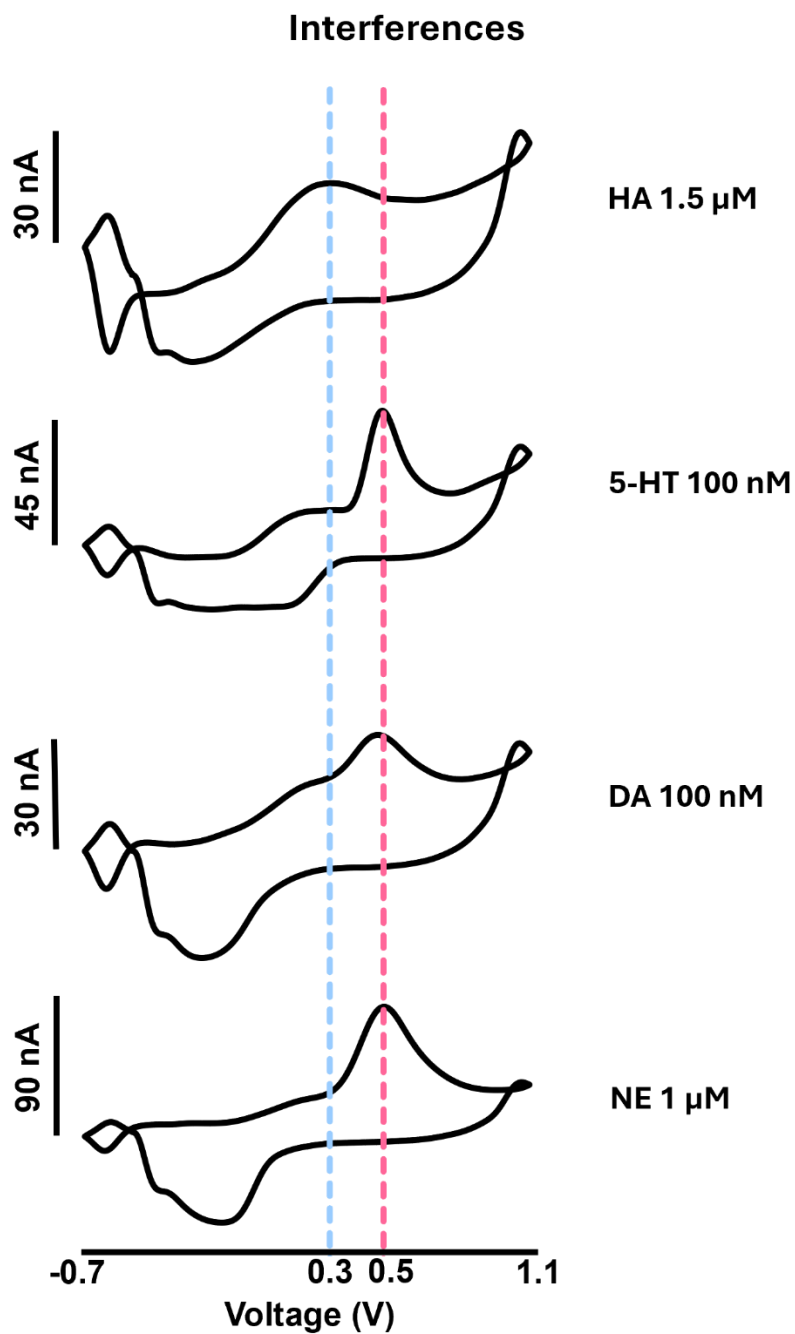


Figure 7.6: Histamine FSCAV interferences. FSCAV CVs for HA (1.5 μM), serotonin (5-HT 100 nM), dopamine (DA 100 nM) and norepinephrine (NE 1 μM). Vertical dashed lines represent the position of oxidation peaks.

Having established that these potential interferences will not be detrimental to identifying a histamine signal, we moved our experiment *in vivo*.

In vivo Histamine FSCAV

Upon confirming that these interferences would not pose a problem *in vivo*, we proceeded to implant a Lubricin-coated electrode into the mouse cortex and applied the optimized waveform and parameters. The cortex was chosen as histaminergic axons projecting from the tuberomammillary nucleus innervate the cortex and histamine levels in the cortex have been implicated in the state of wakefulness (Scammell et al., 2018).

Once the electrode was implanted into the brain, we took 60 minutes of FSCAV files to allow for the electrode to stabilize in the brain matrix as shown in **Figure 7.7C** (n=2 animals +/- SEM). We then co-administered an *i.p.* injection of saline (vehicle) and anesthetic indicated by the blue vertical line. The anesthetic used in this study was ‘sleep mix’, a combination of fentanyl (an opioid), midazolam (GABA-A receptor agonist), and medetomidine (α_2 adrenoceptor agonist). Upon administration of the sleep mix, we witnessed an immediate decrease in the histamine signal (5.469 +/- 0.429 pC to 4.676 +/- 0.095 pC) which we attribute to midazolam.

Histamine plays a crucial role in the brain’s arousal system, influencing states of sleep and wakefulness, being a wake promoter. Histaminergic neurons in the cortex are highly active during wakefulness and drop drastically during sleep (Lin et al., 2008, Parmentier et al., 2016). The H₁ histamine receptor is implicated in the sleep wake cycle as evidenced by the sedative effects of drugs such as diphenhydramine (an H₁ receptor antagonist) (Krystal et al., 2013, Monti et al., 1986). Additionally, drugs that target the histamine H₃ receptor and increase histamine levels have been explored to treat sleep disorders (Passani et al., 2004).

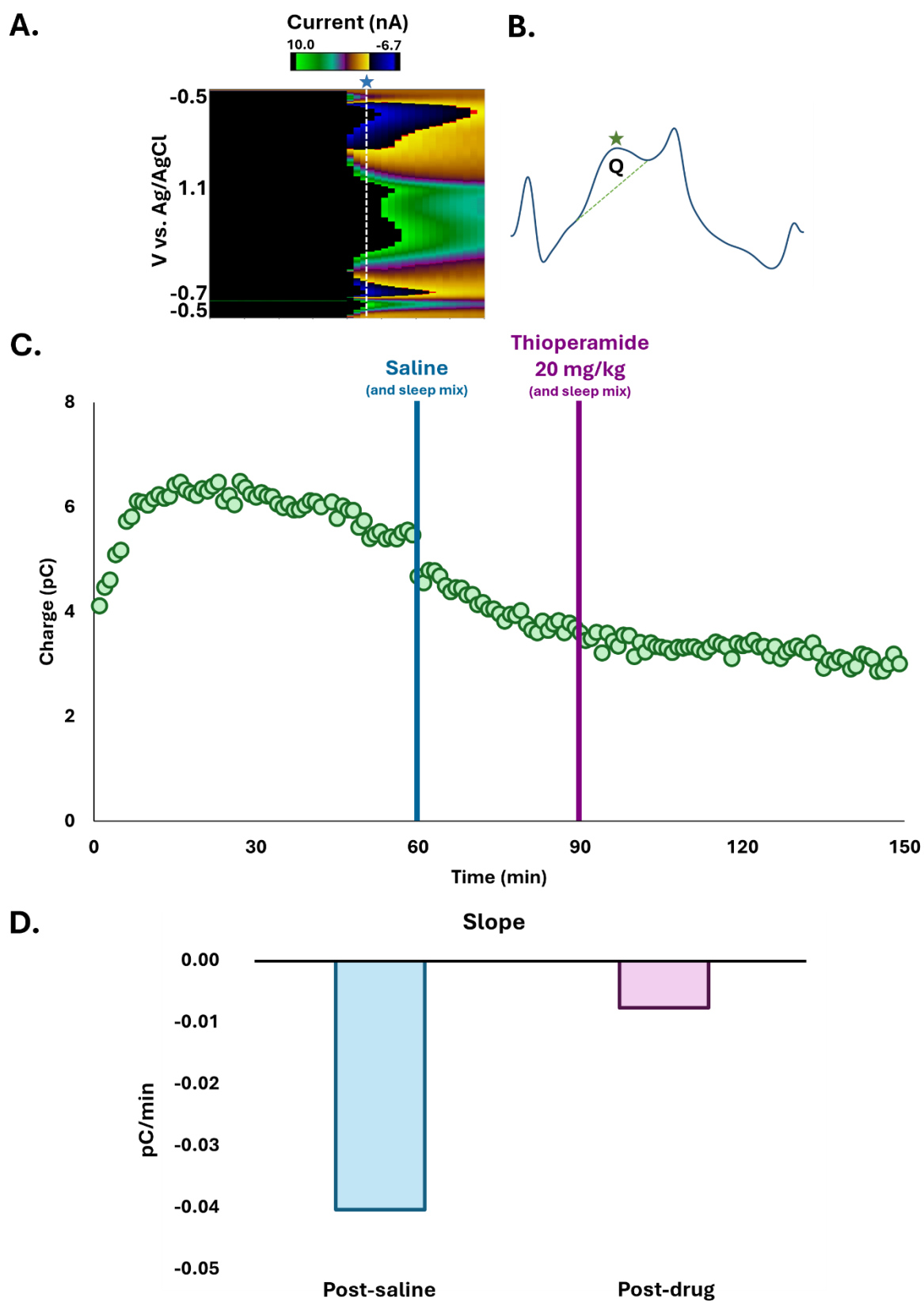


Figure 7.7: Extracellular histamine before and after drug administration. (A) An example histamine FSCAV color plot. Vertical white line indicated by the blue star shows the extracted CV in B. (B) CV extracted from

white line in A. The green star denotes the oxidation peak, and the dashed line indicates the integration region. (C) Extracellular histamine FSCAV measurements after administration of vehicle control (saline, *i.p.*; $n = 2$) and thioperamide (20 mg/kg, *i.p.*; $n = 2$) shown in green ($n=2 \pm$ SEM). The blue line denotes the time point at which saline was administered. The purple line denotes the time point at which thioperamide was administered. Sleep mix was topped up during administration of both vehicle and drug. (D) Bar graph showing the slope of the fitted line post-saline (blue) and post-drug (purple) ($n=2 \pm$ SEM). (* $p < 0.05$)

When the sleep mix was co-administered with thioperamide, an H_3 receptor antagonist, it greatly reduced the effect of the fall in histamine as shown by the slope analysis in **Figure 7.7D**. Post sleep mix and saline administration, extracellular histamine levels decreased by -0.0379 ± 0.0026 pC/min. Conversely, upon co-administration of sleep mix and thioperamide, the decrease in histamine reduced to -0.0108 ± 0.0031 pC/min. We believe this to be due to the competing mechanisms targeting the histaminergic system.

Midazolam is a GABA receptor agonist, therefore leading to an increase in GABA signaling. GABA binds to GABA receptors on histaminergic neurons, leading to reduced excitability, leading to a decrease in histamine and increase in sleepiness (Yu et al., 2015). Conversely, thioperamide is an H_3 autoreceptor antagonist. This agent blocks histamine from binding to the H_3 autoreceptor, thereby preventing the negative feedback loop which would normally lead to histamine inhibition and promoting wakefulness (Lin et al., 1990). When given together, these two drugs have opposing effects on one another, causing them to cancel out each other's effects.

Therefore, we have shown that not only can we measure extracellular histamine levels, but we can also observe the effects of anesthesia on them and demonstrate that these effects are mediated through H₃ receptors.

Conclusion

Neuroinflammation, characterized by elevated brain histamine levels, arises from various underlying causes and can be further exacerbated by peripheral inflammation via vagal nerve innervation, which stimulates histaminergic neurons through neurotransmission. While FSCV can quantify this stimulated release, its limitations prevent the measurement of ambient histamine levels. Although microdialysis has traditionally been used to measure ambient neurotransmitter concentrations, its large probe size and low temporal resolution pose significant limitations; thus, fast scan controlled adsorption voltammetry (FSCAV) was developed to provide enhanced temporal resolution and a miniaturized probe design, while also allowing for integration with FSCV. In this chapter, we applied FSCAV to the measurement of histamine and demonstrated that histamine FSCV and FSCAV signals exhibited significant similarity *in vitro*. We optimized the FSCAV parameters and determined that applying a Lubricin coating to the electrode surface enhanced sensitivity and stability over time. Additionally, we confirmed the waveform's selectivity for histamine against potential interfering analytes. Finally, we applied this method *in vivo* in the mouse cortex. Thus, in this research, we introduced a novel tool for analyzing ambient histamine levels in the brain.

References

- ABDALLA, A., ATCHERLEY, C. W., PATHIRATHNA, P., SAMARANAYAKE, S., QIANG, B., PEÑA, E., MORGAN, S. L., HEIEN, M. L. & HASHEMI, P. 2017. In Vivo Ambient Serotonin Measurements at Carbon-Fiber Microelectrodes. *Analytical Chemistry*, 89, 9703-9711.
- ABDALLA, A., WEST, A., JIN, Y., SAYLOR, R. A., QIANG, B., PEÑA, E., LINDEN, D. J., NIJHOUT, H. F., REED, M. C. & BEST, J. 2020. Fast serotonin voltammetry as a versatile tool for mapping dynamic tissue architecture: I. Responses at carbon fibers describe local tissue physiology. *Journal of neurochemistry*, 153, 33-50.
- AIRAKSINEN, M. S. & PANULA, P. 1988. The histaminergic system in the guinea pig central nervous system: an immunocytochemical mapping study using an antiserum against histamine. *Journal of comparative Neurology*, 273, 163-186.
- ANICHTCHIK, O. V., RINNE, J. O., KALIMO, H. & PANULA, P. 2000. An altered histaminergic innervation of the substantia nigra in Parkinson's disease. *Experimental neurology*, 163, 20-30.
- 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, 14885-14892.
- 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, 2235-2238.
- BEST, J., NIJHOUT, H., SAMARANAYAKE, S., HASHEMI, P. & REED, M. 2017. A mathematical model for histamine synthesis, release, and control in varicosities. *Theoretical Biology and Medical Modelling*, 14, 1-19.
- CASTAGNOLA, E., ROBBINS, E. M., WOEPPEL, K. M., MCGUIER, M., GOLABCHI, A., TAYLOR, I. M., MICHAEL, A. C. & CUI, X. T. 2020. Real-time fast scan cyclic voltammetry detection and quantification of exogenously administered melatonin in mice brain. *Frontiers in bioengineering and biotechnology*, 8, 602216.
- DUNHAM, K. E. & VENTON, B. J. 2020. Improving serotonin fast-scan cyclic voltammetry detection: new waveforms to reduce electrode fouling. *Analyst*, 145, 7437-7446.
- FLIK, G., FOLGERING, J. H. A., CREMERS, T. I. H. F., WESTERINK, B. H. C. & DREMENCOV, E. 2015. Interaction Between Brain Histamine and Serotonin, Norepinephrine, and Dopamine Systems: In Vivo Microdialysis and Electrophysiology Study. *Journal of Molecular Neuroscience*, 56, 320-328.
- HAN, M., SILVA, S. M., LEI, W., QUIGLEY, A., KAPSA, R. M., MOULTON, S. E. & GREENE, G. W. 2019. Adhesion and self-assembly of lubricin (PRG4) brush layers on different substrate surfaces. *Langmuir*, 35, 15834-15848.
- 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 increases sensitivity. *Analyst*, 128, 1413-1419.
- HERSEY, M., BERGER, S. N., HOLMES, J., WEST, A. & HASHEMI, P. 2019. Recent Developments in Carbon Sensors for At-Source Electroanalysis. *Analytical Chemistry*, 91, 27-43.

- HERSEY, M., HASHEMI, P. & REAGAN, L. P. 2021a. Integrating the monoamine and cytokine hypotheses of depression: Is histamine the missing link? *European Journal of Neuroscience*.
- HERSEY, M., RENEAU, M., BERGER, S. N., MENA, S., BUCHANAN, A. M., OU, Y., TAVAKOLI, N., REAGAN, L. P., CLOPATH, C. & HASHEMI, P. 2022. A tale of two transmitters: serotonin and histamine as in vivo biomarkers of chronic stress in mice. *Journal of Neuroinflammation*, 19, 1-20.
- HERSEY, M., SAMARANAYAKE, S., BERGER, S. N., TAVAKOLI, N., MENA, S., NIJHOUT, H. F., REED, M. C., BEST, J., BLAKELY, R. D. & REAGAN, L. P. 2021b. Inflammation-induced histamine impairs the capacity of escitalopram to increase hippocampal extracellular serotonin. *Journal of Neuroscience*, 41, 6564-6577.
- HOWELL, J. O., KUHR, W. G., ENSMAN, R. E. & MARK WIGHTMAN, R. 1986. Background subtraction for rapid scan voltammetry. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, 209, 77-90.
- INAGAKI, N., YAMATODANI, A., ANDO-YAMAMOTO, M., TOHYAMA, M., WATANABE, T. & WADA, H. 1988. Organization of histaminergic fibers in the rat brain. *Journal of Comparative Neurology*, 273, 283-300.
- JIN, C.-Y., ANICHTCHIK, O. & PANULA, P. 2009. Altered histamine H3 receptor radioligand binding in post-mortem brain samples from subjects with psychiatric diseases. *British journal of pharmacology*, 157, 118-129.
- JIN, H., LI, M., JEONG, E., CASTRO-MARTINEZ, F. & ZUKER, C. S. 2024. A body-brain circuit that regulates body inflammatory responses. *Nature*, 1-3.
- KANO, M., FUKUDO, S., TASHIRO, A., UTSUMI, A., TAMURA, D., ITOH, M., IWATA, R., TASHIRO, M., MOCHIZUKI, H. & FUNAKI, Y. 2004. Decreased histamine H1 receptor binding in the brain of depressed patients. *European Journal of Neuroscience*, 20, 803-810.
- KRYSTAL, A. D., RICHELSON, E. & ROTH, T. 2013. Review of the histamine system and the clinical effects of H1 antagonists: basis for a new model for understanding the effects of insomnia medications. *Sleep medicine reviews*, 17, 263-272.
- KUHR, W. G. & WIGHTMAN, R. M. 1986. Real-time measurement of dopamine release in rat brain. *Brain research*, 381, 168-171.
- LIN, J.-S., DAUVILLIERS, Y., ARNULF, I., BASTUJI, H., ANACLET, C., PARMENTIER, R., KOCHER, L., YANAGISAWA, M., LEHERT, P. & LIGNEAU, X. 2008. An inverse agonist of the histamine H3 receptor improves wakefulness in narcolepsy: studies in orexin-/- mice and patients. *Neurobiology of disease*, 30, 74-83.
- LIN, J.-S., SAKAI, K., VANNI-MERCIER, G., ARRANG, J.-M., GARBARG, M., SCHWARTZ, J.-C. & JOUVET, M. 1990. Involvement of histaminergic neurons in arousal mechanisms demonstrated with H3-receptor ligands in the cat. *Brain research*, 523, 325-330.
- LULL, M. E. & BLOCK, M. L. 2010. Microglial Activation and Chronic Neurodegeneration. *Neurotherapeutics*, 7, 354-365.
- MILLAR, J., ARMSTRONG-JAMES, M. & KRUK, Z. L. 1981. Polarographic assay of iontophoretically applied dopamine and low-noise unit recording using a multibarrel carbon fibre microelectrode. *Brain Research*, 205, 419-424.
- MILLAR, J., STAMFORD, J. A., KRUK, Z. L. & WIGHTMAN, R. M. 1985. Electrochemical, pharmacological and electrophysiological evidence of rapid dopamine release

- and removal in the rat caudate nucleus following electrical stimulation of the median forebrain bundle. *European Journal of Pharmacology*, 109, 341-348.
- MONTI, J., PELLEJERO, T. & JANTOS, H. 1986. Effects of H1-and H2-histamine receptor agonists and antagonists on sleep and wakefulness in the rat. *Journal of neural transmission*, 66, 1-11.
- NESBITT, K. M., JAQUINS-GERSTL, A., SKODA, E. M., WIPF, P. & MICHAEL, A. C. 2013. Pharmacological Mitigation of Tissue Damage during Brain Microdialysis. *Analytical Chemistry*, 85, 8173-8179.
- NESBITT, K. M., VARNER, E. L., JAQUINS-GERSTL, A. & MICHAEL, A. C. 2015. Microdialysis in the Rat Striatum: Effects of 24 h Dexamethasone Retrodialysis on Evoked Dopamine Release and Penetration Injury. *ACS Chemical Neuroscience*, 6, 163-173.
- OU, Y., BUCHANAN, A. M., WITT, C. E. & HASHEMI, P. 2019. Frontiers in electrochemical sensors for neurotransmitter detection: towards measuring neurotransmitters as chemical diagnostics for brain disorders. *Analytical Methods*, 11, 2738-2755.
- PANULA, P., PIRVOLA, U., AUVINEN, S. & AIRAKSINEN, M. 1989. Histamine-immunoreactive nerve fibers in the rat brain. *Neuroscience*, 28, 585-610.
- PANULA, P., RINNE, J., KUOKKANEN, K., ERIKSSON, K. S., SALLMEN, T., KALIMO, H. & RELJA, M. 1997. Neuronal histamine deficit in Alzheimer's disease. *Neuroscience*, 82, 993-997.
- PARMENTIER, R., ZHAO, Y., PERIER, M., AKAOKA, H., LINTUNEN, M., HOU, Y., PANULA, P., WATANABE, T., FRANCO, P. & LIN, J.-S. 2016. Role of histamine H1-receptor on behavioral states and wake maintenance during deficiency of a brain activating system: a study using a knockout mouse model. *Neuropharmacology*, 106, 20-34.
- PASSANI, M. B., LIN, J.-S., HANCOCK, A., CROCHET, S. & BLANDINA, P. 2004. The histamine H3 receptor as a novel therapeutic target for cognitive and sleep disorders. *Trends in pharmacological sciences*, 25, 618-625.
- PATHIRATHNA, P., YANG, Y., FORZLEY, K., MCELMURRY, S. P. & HASHEMI, P. 2012. Fast-Scan Deposition-Stripping Voltammetry at Carbon-Fiber Microelectrodes: Real-Time, Subsecond, Mercury Free Measurements of Copper. *Analytical Chemistry*, 84, 6298-6302.
- PAXINOS, G. & FRANKLIN, K. B. 2019. *Paxinos and Franklin's the mouse brain in stereotaxic coordinates*, Academic press.
- RINNE, J., ANICHTCHIK, O., ERIKSSON, K., KASLIN, J., TUOMISTO, L., KALIMO, H., RÖYTTÄ, M. & PANULA, P. 2002. Increased brain histamine levels in Parkinson's disease but not in multiple system atrophy. *Journal of neurochemistry*, 81, 954-960.
- 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, 221-234.
- ROSS, A. E. & VENTON, B. J. 2014. Sawhorse Waveform Voltammetry for Selective Detection of Adenosine, ATP, and Hydrogen Peroxide. *Analytical Chemistry*, 86, 7486-7493.
- RUSSO, M. J., HAN, M., QUIGLEY, A. F., KAPSA, R. M. I., MOULTON, S. E., DOEVEN, E., GUIJT, R., SILVA, S. M. & GREENE, G. W. 2020. Lubricin (PRG4) reduces fouling susceptibility and improves sensitivity of carbon-based electrodes. *Electrochimica Acta*, 333, 135574.

- SAMARANAYAKE, S., ABDALLA, A., ROBKE, R., NIJHOUT, H. F., REED, M. C., BEST, J. & HASHEMI, P. 2016. A voltammetric and mathematical analysis of histaminergic modulation of serotonin in the mouse hypothalamus. *Journal of neurochemistry*, 138, 374-383.
- SAMARANAYAKE, S. A., A.; ROBKE, R.; WOOD, K. M.; ZEQA, A.; HASHEMI, P. 2015. In Vivo Histamine Voltammetry in the Mouse Premammillary Nucleus. *Analyst*, 140, 3759-3765.
- SCAMMELL, T. E., JACKSON, A. C., FRANKS, N. P., WISDEN, W. & DAUVILLIERS, Y. 2018. Histamine: neural circuits and new medications. *Sleep*, 42.
- STAMFORD, J. A., KRUK, Z. L. & MILLAR, J. 1984. Regional differences in extracellular ascorbic acid levels in the rat brain determined by high speed cyclic voltammetry. *Brain research*, 299, 289-295.
- STAMFORD, J. A., KRUK, Z. L. & MILLAR, J. 1986. In vivo voltammetric characterization of low affinity striatal dopamine uptake: drug inhibition profile and relation to dopaminergic innervation density. *Brain research*, 373, 85-91.
- STUCKY, C. & JOHNSON, M. A. 2022. Improved serotonin measurement with fast-scan cyclic voltammetry: Mitigating fouling by SSRIs. *Journal of the Electrochemical Society*, 169, 045501.
- TAKMAKOV, P., ZACHEK, M. K., KEITHLEY, R. B., WALSH, P. L., DONLEY, C., MCCARTY, G. S. & WIGHTMAN, R. M. 2010. Carbon Microelectrodes with a Renewable Surface. *Analytical Chemistry*, 82, 2020-2028.
- TSAI, M., GRIMBALDESTON, M. & GALLI, S. J. 2011. *Mast cells and immunoregulation/immunomodulation*, Springer.
- WEST, A., BEST, J., ABDALLA, A., NIJHOUT, H. F., REED, M. & HASHEMI, P. 2019. Voltammetric evidence for discrete serotonin circuits, linked to specific reuptake domains, in the mouse medial prefrontal cortex. *Neurochemistry international*, 123, 50-58.
- WITT, C. E., MENA, S., HONAN, L. E., BATEY, L., SALEM, V., OU, Y. & HASHEMI, P. 2022. Low-Frequency Oscillations of In Vivo Ambient Extracellular Brain Serotonin. *Cells*, 11, 1719.
- WOOLLEY, D. W. & SHAW, E. 1954. A biochemical and pharmacological suggestion about certain mental disorders. *Proceedings of the National Academy of Sciences*, 40, 228-231.
- WRIGHT, C., SHIN, J. H., RAJPUROHIT, A., DEEP-SOBOSLAY, A., COLLADO-TORRES, L., BRANDON, N. J., HYDE, T. M., KLEINMAN, J. E., JAFFE, A. E., CROSS, A. J. & WEINBERGER, D. R. 2017. Altered expression of histamine signaling genes in autism spectrum disorder. *Translational Psychiatry*, 7, e1126-e1126.
- YAMADA, Y., YOSHIKAWA, T., NAGANUMA, F., KIKKAWA, T., OSUMI, N. & YANAI, K. 2020. Chronic brain histamine depletion in adult mice induced depression-like behaviours and impaired sleep-wake cycle. *Neuropharmacology*, 175, 108179.
- YU, X., YE, Z., HOUSTON, C. M., ZECHARIA, A. Y., MA, Y., ZHANG, Z., UYGUN, D. S., PARKER, S., VYSSOTSKI, A. L., YUSTOS, R., FRANKS, N. P., BRICKLEY, S. G. & WISDEN, W. 2015. Wakefulness Is Governed by GABA and Histamine Cotransmission. *Neuron*, 87, 164-78.

CHAPTER 8 – OPTIMIZING MEDIA AND ELECTRODE COATINGS FOR FAST SCAN CYCLIC VOLTAMMETRY CALIBRATIONS⁶

⁶ **Batey, L.**, Yao, G., Prieto, P., Booth, M.A., Silva, S., Greene, W., Hashemi, P. *In preparation*.

Abstract

Fast scan cyclic voltammetry (FSCV) is an effective technique for measuring neurotransmission in the brain. To analyze and quantify in vivo signals, in vitro calibrations in buffers that simulate the in vivo environment are required. An effective strategy to perform these in vitro calibrations is to utilize a flow cell apparatus. In this work, we sought to leverage electrode coatings and a variety of buffer solutions to more accurately replicate in vivo signals in vitro using a flow cell. As a basis for justification, we assess histamine FSCV signals in vivo and compare them to in vitro signals in an assortment of biological media, and deduce HEPES buffer most accurately depicts in vivo cyclic voltammograms. We illustrate that an application of an extracellular matrix onto the electrode produces histamine signals that strongly resemble those seen in vivo. Lastly, we show that a coating of Lubricin onto the electrode has a positive impact on serotonin measurements and decreases fouling effects for repeated histamine measurements. Here, we propose that these two novel electrode coatings, Lubricin and extracellular matrix, are beneficial for in vitro FSCV measurements and that bespoke surface treatments and physiological buffers are the most effective strategy to replicate in vivo conditions in vitro.

Introduction

Fast scan cyclic voltammetry (FSCV) is a powerful method for in vivo or in situ measurements of neurotransmitters. This method has been widely used to study the roles of several key neurotransmitters in the brain, including dopamine (Shin et al., 2017, Robinson et al., 2003, Wightman et al., 1988, Hashemi et al., 2012, Millar et al., 1985, Hettiarachchi et al., 2024), serotonin (Hashemi et al., 2012, Hashemi et al., 2009, Hashemi et al., 2011, Jackson and Mark Wightman, 1995, Hashemi et al., 2015, Saylor et al., 2019, Wood and Hashemi, 2013, Buchanan et al., 2024, Holmes et al., 2021, Witt et al., 2023, Hersey et al., 2022), norepinephrine (Park et al., 2011, Park et al., 2015, Park et al., 2013, Fox et al., 2016), and histamine (Berger et al., 2022, Hersey et al., 2021a, Samaranayake et al., 2016, Samaranayake, 2015, Hashemi et al., 2011, Hersey et al., 2022, Hersey et al., 2021b).

To quantify and characterize in vivo signals, they are typically replicated in vitro using a flow cell apparatus (shown in **Figure 8.1**). A bolus of known concentration is injected into the inlet of a flow cell which interacts with a carbon fiber microelectrode (CFM) in the flow stream. FSCV signals are collected before, during, and after the injection, which illustrate how the electrode responds to a given analyte. These experiments are typically performed in buffers such as Trizma (Tris), phosphate buffered saline (PBS) or HEPES buffer solution (HEPES). Calibrations are essential, and a flow cell is an effective tool for performing them.

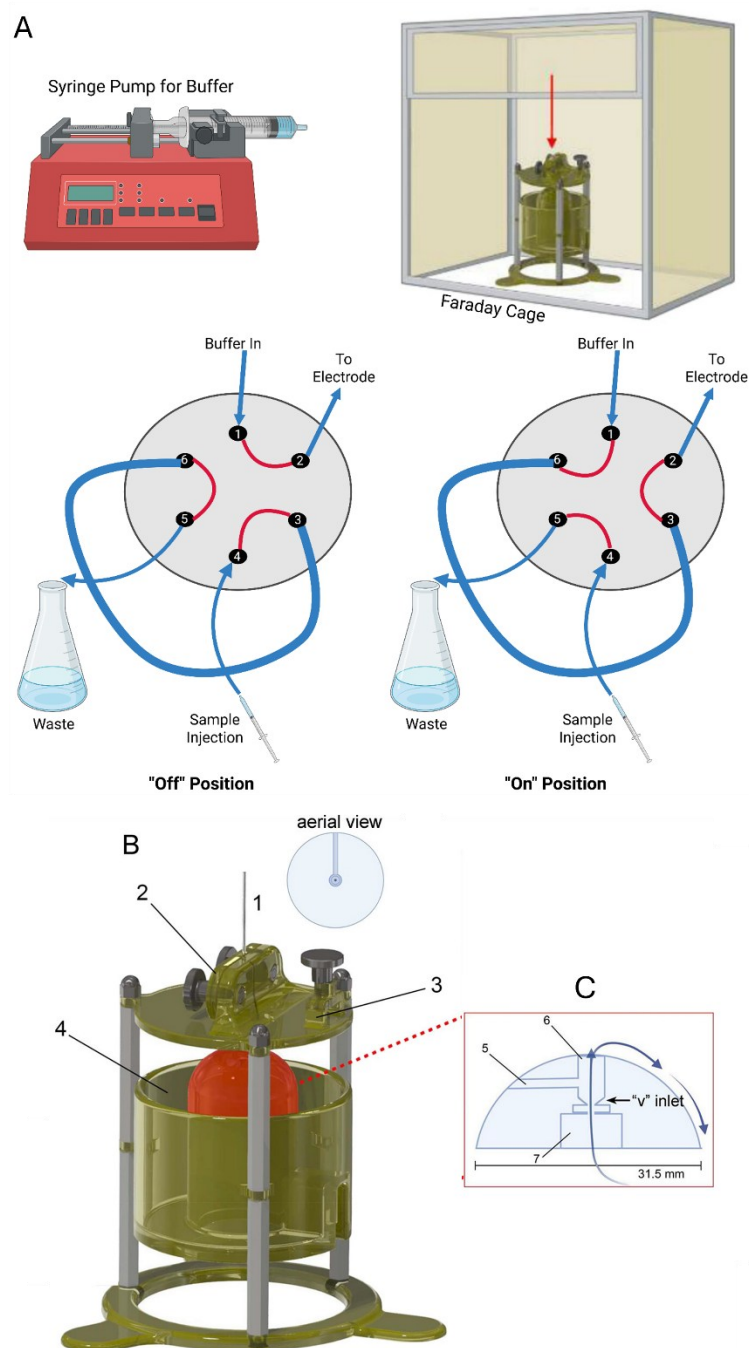


Figure 8.1. Flow cell apparatus setup. (A) The syringe pump flows buffer into the valve. When valve is in the 'off' position, buffer bypasses the sample loop and flows to the electrode. When valve is in the 'on' position, buffer flows through the sample loop then to the electrode. (B) Depiction of flow cell. 1 – electrode; 2 – electrode holder; 3 - headstage holder; 4 – waste reservoir. Inset shows aerial view of red

dome. (C) Side view of red dome. 5 – reference channel; 6 – channel opening; 7 – buffer inlet. (A) created using Biorender. (B and C) adapted from Hexter et al. (Hexter et al., 2023).

However, flow cell measurements often do not fully replicate *in vivo* signals with respect to the precise position of the redox peaks of analytes. This is not surprising since the *in vivo* environment is near impossible to replicate *in vitro*. As a result, additional methods are often needed to validate the accuracy of *in vivo* signals, such as pharmacology. Different analysis media can change the position of the redox peaks (Meunier et al., 2017), therefore investigating context specific media for FSCV experiments (e.g. which medium best replicates a certain analyte's signal *in vivo*) is important.

Another potential direction towards better replicating *in vivo* signals is electrode coatings. It is generally thought that the *in vivo* environment is detrimental to the electrode surface (although this view is being updated in recent work, see Holmes et. al. 2021 and Chapter 6). Therefore, to address *in vivo* performance of electrodes, researchers have used coatings to preserve the integrity of signals during *in vivo* experiments. Such coatings, which include Nafion and PEDOT, are applied to the CFM surface via different methods and help with FSCV signal intensity and stability. Nafion and polyglutamate have been shown to increase the sensitivity to serotonin over serotonin's metabolite, 5-HIAA, *in vivo* and reduce electrode fouling (Hashemi et al., 2009, Holmes et al., 2021); PEDOT and polypyrrole have been demonstrated to increase sensitivity and selectivity to dopamine while also decrease electrode fouling *in vivo* (Vreeland et al., 2015, Pihel et al., 1996b); carbon nanotube-modified CFMs were shown to increase sensitivity to serotonin while decreasing electrode fouling *in vivo* (Swamy and

Venton, 2007) and to enhance the sensitivity for adenosine while decreasing the interference by ATP (Ross and Venton, 2012).

In this paper, we aim to combine analysis media and electrode coatings to better mimic in vivo signals in vitro and improve general electrode performance in the flow cell. First, as proof of justification, we compare histamine signals collected in vivo with signals collected using a flow cell in different established physiological buffers and find differences in shape of the cyclic voltammograms, concluding that HEPES best represents the in vivo signal. Second, by modifying our electrodes with extracellular matrix (ECM), we found that the in vitro histamine FSCV signals bore a strong resemblance to in vivo signals. Finally, we investigate the effects of a novel electrode coating, Lubricin (Lub), on histamine and serotonin signals, finding that Lub has beneficial effects for measurements of serotonin and antifouling properties for histamine.

In sum, we present two novel electrode coatings, Lub and ECM, that improve various aspects of the FSCV response. Our work shows that tailored coatings and buffers may be the best approach to distinct experimental setups (i.e. different analytes, different biological systems).

Methods

Chemicals

Buffers were prepared at room temperature and adjusted to physiological pH (7.4). PBS was composed of 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄ and 1.8 mM KH₂PO₄.

TRIS was composed of 15 mM Trisma, 140 mM NaCl, 3.25 mM KCl, 1.2 mM CaCl₂, 1.25 mM NaH₂PO₄, 1.2 mM MgCl₂ and 2.0 mM Na₂SO₄. HEPES was composed of 20 mM HEPES, 140 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, 1.0 mM MgCl₂ and 10 mM glucose. Stock solutions were prepared at room temperature by dissolving histamine dihydrochloride and serotonin hydrochloride into the buffer and diluting to the desired final concentration. Urethane was dissolved in sterile saline as a 25% w/v solution and administered at 7 μ L g⁻¹. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified.

Flow Injection Analysis (FIA)

FIA was performed in a system custom-built in-house (Hexter et al., 2023). A pseudo Ag/AgCl reference electrode was made by submerging a silver wire in a solution of 1 M HCl for 30 s at 5 V. A syringe infusion pump (Harvard Apparatus, Model Pump 11 Elite, Holliston, MA, USA) was used to maintain flow at 1.7 mL min⁻¹. The analyte was introduced into the flow stream via a six-port HPLC loop injector (Cheminert Valve, VICI, Houston, TX, USA) as a rectangular plug.

Microelectrode Fabrication

Carbon fiber microelectrodes (CFMs) were made individually by aspirating a single T-650 carbon fiber (Goodfellow Corporation, PA, USA) into a 1.0 mm x 0.50 mm x 10 cm glass capillary (World Precision Instruments, Hertfordshire, UK). The capillary was then pulled by a PE-22 vertical puller (Narishige, Tokyo, Japan) to create a seal. The carbon fiber was then trimmed to 150 $\pm$ 5 μ m with a scalpel blade under a light microscope.

Nafion Electrodeposition

Liquion (LQ-1105, 5% by weight Nafion) (Ion Power Solutions, New Castle, DE, USA) was electrodeposited onto the surface of the carbon fiber by dipping into the solution and applying a constant potential of 1.0 V for 30 s. The electrode was then dried at 70 °C for 10 minutes and allowed to set at room temperature overnight.

Lubricin Deposition

Lubricin aliquots at a concentration of 1.3 mg/mL were defrosted and diluted to a final working concentration of 150 µg/mL and centrifuged and vortexed. Electrodes were lowered into solution and left at room temperature for 15 minutes, removed from solution and rinsed with deionized water. Electrodes were stored between 2-8 °C.

Extracellular Matrix Coating

Geltrex (Invitrogen, Carlsbad, USA) was thawed on ice until it became a viscous liquid. A 1.00 µL drop of Geltrex was placed into a premade mold. The tip of a CFM was placed into the drop of Geltrex and held in place with blue tac. An additional 0.5 µL drop was placed onto the tip of the electrode to ensure electrode coverage. The CFM was baked at 37 °C for 20 minutes, then left to dry overnight at room temperature. The CFM was carefully lifted from the mold and placed in HEPES buffer for 20 minutes immediately before starting the experiment.

Data Collection and Analysis

FSCV was performed using a Dagan Potentiostat, (Dagan Corporation, Minneapolis, NM, USA), National Instruments multifunction device USB-6341 (National Instruments, 202

Austin, TX, USA), WCCV 4.0 software (Knowmad Technologies LLC, Tucson, AZ, USA) and a Pine Research headstage (Pine Research Instrumentation, Durham, NC, USA). Data filtering (zero phase, Butterworth, 3 kHz low-pass) and signal smoothing were done within the WCCV 4.0 software. The histamine waveform (-0.5 to -0.7 to $+1.1$ to -0.5 V at 600 V s^{-1}) or serotonin waveform ($+0.2$ to $+1.0$ to -0.1 to $+0.2$ V at 1000 V s^{-1}) was applied at 60 Hz for 10 min, then at 10 Hz for 10 min prior to data collection at 10 Hz. For in vivo histamine data, histamine was evoked via biphasic stimulation applied through a linear constant current stimulus isolator (NL800A Neurolog, Digitimer North America LLC, Fort Lauderdale, FL, USA) with stimulation parameters at 60 Hz, $360\text{ }\mu\text{A}$, 2 ms in width, and 2 s in length.

Animals and Surgical Procedures

All animal procedures were done in accordance with the regulations of the Institutional Animal Care and Use Committee (IACUC) at the University of South Carolina. C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) aged 6–12 weeks were used. Animals were group housed with ad libitum access to food and water and kept on a 12 h light/dark cycle. Stereotaxic surgery (David Kopf Instruments, Tujunga, CA, USA) was done following induction of deep and sustained anesthesia from an intraperitoneal (i.p.) injection of urethane (described above). Mouse body temperature was maintained using a thermal heating pad (Braintree Scientific, Braintree, MA, USA). All surgical coordinates were taken in reference to bregma (Paxinos and Franklin, 2019). A Nafion modified CFM was lowered into the posterior hypothalamus (AP: -2.5 , ML: 0.5 , DV: -5.5 to -5.6) and a stimulating electrode (insulated stainless-steel, $d = 0.2\text{ mm}$, untwisted; Plastics One, Roanoke, VA, USA) was placed into the medial forebrain bundle (AP: -1.1 , ML: $+1.1$, DV: -2.0 to -2.3)

-5.0) (Paxinos and Franklin, 2019). A pseudo Ag/AgCl reference electrode was inserted into the contralateral hemisphere.

Statistical Analysis

All statistics were performed by GraphPad Prism 10 (GraphPad Software Inc., San Diego, USA), and all data are reported as the mean $\pm$ standard error of the mean (SEM). The “n” represents the number of electrodes tested. P-values were considered significant at the 95% confidence level ($p < 0.05$).

Results and Discussion

A Comparison of FSCV Histamine Signals Collected In vivo vs. In vitro in Different Media

Previously in 2015, the Hashemi lab developed a novel histamine selective electrochemical waveform (-0.5 V to -0.7 V to +1.1 V to -0.5 V; scan rate = 600 V s⁻¹) to measure evoked histamine release in the mouse posterior hypothalamus using FSCV via stimulation of the medial forebrain bundle using a Nafion-modified carbon fiber microelectrode (CFM) (Samaranayake, 2015), which is shown in **Figure 8.2Ai**. A detailed description of how to interpret color plots can be found elsewhere (Michael et al., 1998); briefly, voltage is shown on the y-axis, time on the x-axis and current is shown in false color. The vertical white dashed line represents the cyclic voltammogram (CV) shown in the right corner. To convert charge into concentration, the CFMs required post-calibration which was performed in TRIS buffer. TRIS was used because it is thought to mimic the ionic concentration of the brain matrix (Hermans et al., 2008, Brown et al., 2004), theoretically allowing for more accurate post-calibrations. We compared this in vivo CV with CVs of histamine (10 μ M: approximate concentration of evoked histamine in

204

a mouse (Samaranayake, 2015, Berger et al., 2022)) on bare and Nafion-modified electrodes in a flow cell using TRIS buffer and other physiological media that we thought would also closely resemble the brain matrix. **Figure 8.2B** shows the histamine CVs, normalized to the maximum current of the forward scan (-0.7 V to +1.1 V) in each buffer.

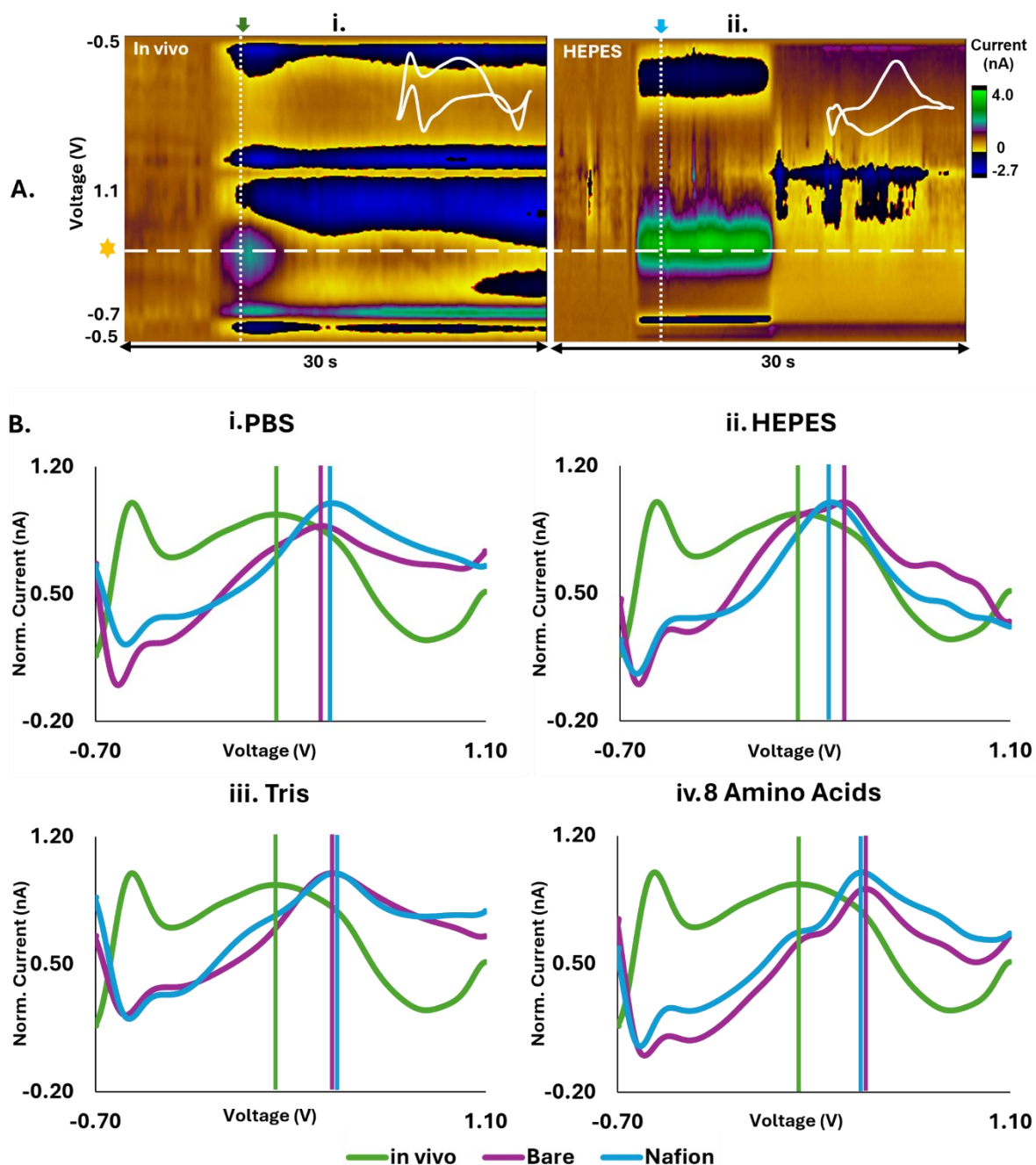


Figure 8.2. Histamine signals in vivo versus in vitro (10 μ M). (A) shows representative color plots for (i) in vivo histamine FSCV signal and (ii) in vitro FIA of 10 μ M histamine in HEPES buffer on a Nafion-modified electrode. The vertical dashed white lines indicated by arrows represent the CV extracted for analysis shown inset in white. The horizontal white dashed line indicated by the yellow star represents the max height of the oxidation peak. (B) shows the normalized forward scan of histamine CVs in vivo (green) versus bare (purple) and Nafion-modified (blue) electrodes in (i) PBS, (ii) HEPES, (iii) TRIS, and (iv) eight amino acid buffer. Horizontal lines indicate peak maximum voltage potential.

Upon analysis of the CVs, we found differences in the shape and oxidation potential of the in vitro CVs compared to in vivo. In vivo, the major oxidation event occurs at 0.14 V, however, we do not see this same oxidation peak placement when measuring in vitro. In PBS and in TRIS, the main oxidation peak is shifted to a higher potential around 0.40 V. In HEPES buffer, the Nafion-modified and bare CFMs have peaks closer to the in vivo at 0.27 V and 0.35 V, respectively. In the buffer containing eight amino acids, there are two oxidation peaks: the larger oxidation peak occurs at 0.44 V, though there is a small peak present at 0.14 V, like the in vivo CV.

TRIS buffer was created to better mimic the ionic environment of the brain in the late 1990s. For years, many CFM calibrations were performed in a buffer containing only Tris/HCl and NaCl (Baur et al., 1988, Pihel et al., 1995, Pihel et al., 1996a). Eventually, other ions (K^+ , Mg^{2+} , Ca^{2+} , SO_4^{2-} , and PO_4^{3-}) were added to approximate the in vivo ionic conditions more precisely, to allow for better calibrations (Hermans et al., 2008, Brown et al., 2004). PBS buffer is a commonly used physiological buffer containing NaCl, KCl, Na_2HPO_4 , and KH_2PO_4 . We opted to examine this buffer as it is used for many FSCV and FSCAV calibrations. HEPES buffer contains HEPES acid (4-(2-hydroxyethyl)-1-

piperazineethanesulfonic acid), which acts as the main buffering agent, along with NaCl, KCl, CaCl₂, MgCl₂, and glucose. HEPES buffer is primarily used in cell culture because it does not require a CO₂ controlled environment, ensuring a stable pH value in the laboratory. We chose to investigate this buffer because since it is used for cell culture applications (i.e. neuronal cells) it may resemble the in vivo brain environment. The eight amino acid buffer was created by our lab (see Chapter 6) to better mimic the brain in vivo environment for fast scan controlled cyclic voltammetry. We used PBS as the base of our buffer and chose eight amino acids which are found in the brain interstitial fluid at relatively high concentrations and may be structurally similar to glutamate (which we previously found to improve electrode response to serotonin (Holmes et al., 2021)) to add to the buffer to better mimic the biological makeup of the brain.

The presence of double peaks (in the eight amino acids buffer) and matrix-based shifting of peaks led us to next ask about the mechanisms that give rise to these peaks. During FSCV of monoamines, unstable radicals are created during the fast scans that interact with each other forming polymers on the electrode surface. For serotonin one of these reactions occurs at 0.2 V (Jackson et al., 1995), this could also be the case for histamine. We have hypothesized for a long time that the in vivo oxidation of histamine is not classical two-electron oxidation and reduction (Samaranayake, 2015). Because the CVs more closely resemble a quasi-reversible or irreversible electrochemical reaction, it could be that the mechanism of the response involves a charge transfer of the histamine molecule during the scans that leads to a change in the double layer capacitance (See **Figure 8.2** and related text for full discussion of this). Considering charge transfer as the mechanism of the response, we would expect to see high sensitivity to the ionic

207

environment (i.e. the different ionic makeups of the media) and it would be expected that the peaks shift slightly in response. Given this, peak location in HEPES most closely correlates to the in vivo oxidation peak. Therefore, we conclude HEPES more closely resembles the in vivo matrix than PBS or TRIS to analyze histamine.

Modifying CFMs with Nafion and ECM better Models In Vivo System In Vitro

We have demonstrated that HEPES buffer more accurately captures the histamine CVs that we observe in vivo, however, the oxidation peaks are sharper in vitro than in vivo. Therefore, we next investigated the effects of modifying our electrodes to investigate whether we could further improve in vivo resemblance.

The electrochemical mechanisms of the histamine FSCV response have not fully been established. The electrochemical principles that govern the FSCV response are complicated because:

1. FSCV is an adsorption controlled process. Analytes will adsorb onto the CFM surface creating an analyte-CFM complex. This complex is then what undergoes a redox reaction and, therefore, the redox potentials are going to be different from the standard redox potentials used with regular (slow scan) cyclic voltammetry.
2. All FSCV reactions appear quasi- or non-reversible due to the high scan rates employed. Scan rates above 100 V s^{-1} can sometimes outrun the electron transfer kinetics of a reaction, especially for reactions with slower electron transfer rates. Therefore, the molecule may not fully change back to the original species during the reverse scan. This leads to the appearance of a quasi-reversible reaction.

The redox reaction of dopamine with FSCV is well understood. **Figure 8.3A** shows a representation of dopamine adsorbing onto the CFM surface creating a weak complex and undergoing a two-electron transfer to give a typical dopamine FSCV color plot and CV.

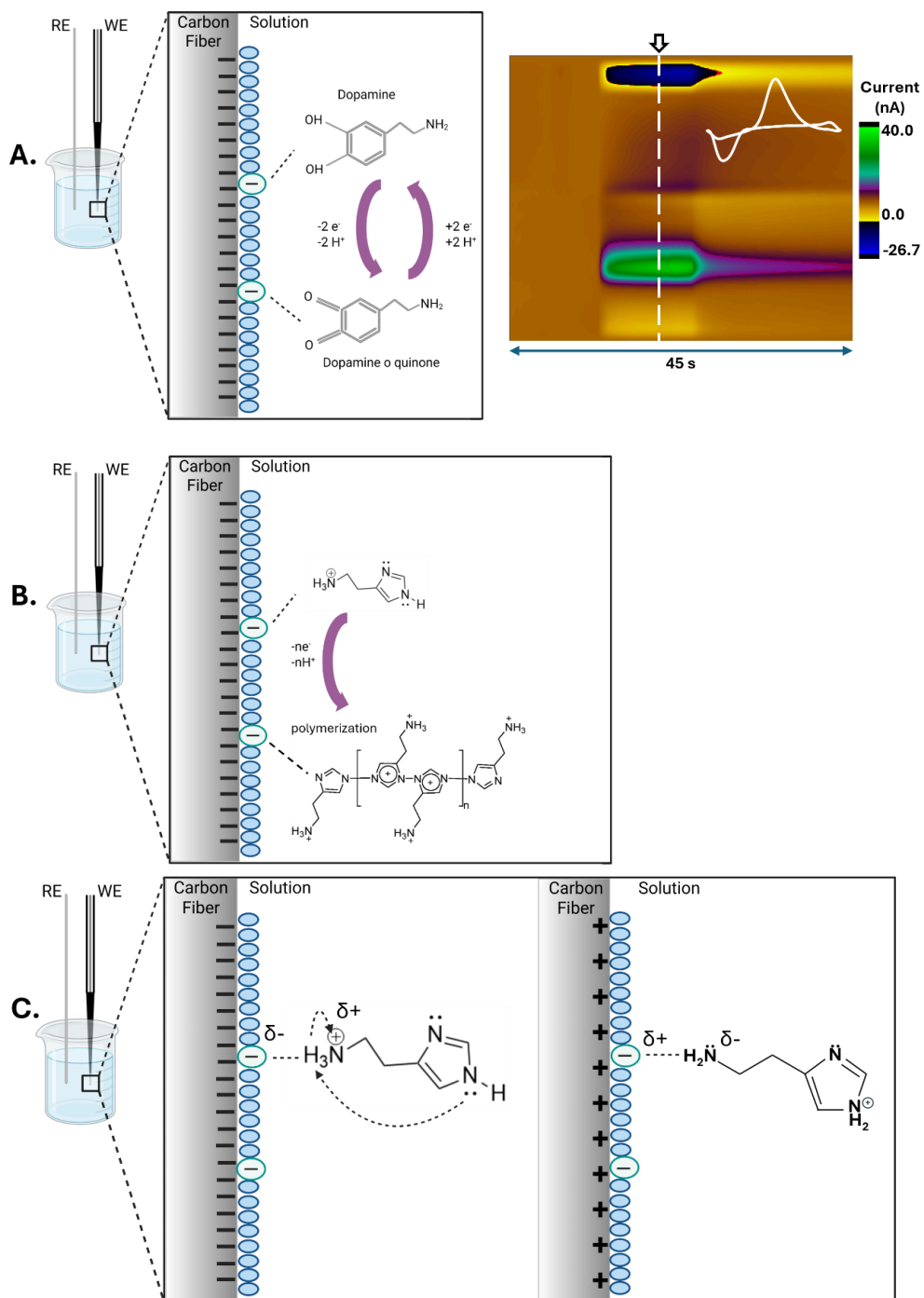


Figure 8.3. Dopamine and histamine oxidation mechanisms. (A) Electrochemical redox reaction for dopamine at a CFM. FSCV color plot for 1 μM dopamine is shown with CV inset in white. (B) Proposed mechanism for histamine oxidation and electropolymerization (Puthongkham et al., 2019). (C) Proposed mechanism for charge transfer of histamine. Image created using Biorender.

However, we do not believe that the process for histamine is the same as the process for dopamine (e.g. **Figure 8.3B**). The oxidation peak is much more broad and the oxidation potential will shift (depending on the media or CFM coating) as shown in **Figure 8.3B**. Additionally, amperometry does not give a current response until a potential of 1.0 V. A CFM was placed in a beaker containing PBS buffer and held at a constant potential and a sample of 10 μM histamine was injected into the beaker. The final current was compared to the initial current to generate **Figure 8.4**.

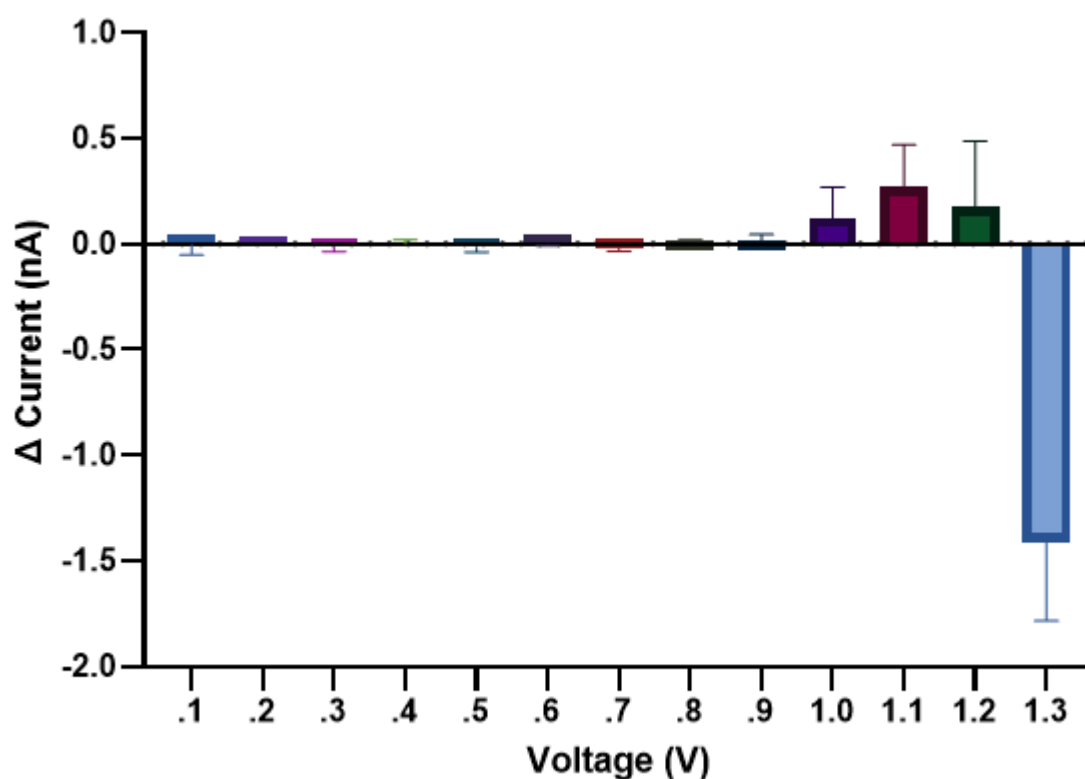


Figure 8.4. Histamine amperometry.

As histamine amperometry detects no change in current until 1.0 V, this means that electrons do not get transferred below this potential. Additionally, at 1.3 V, there is a large decrease in current as opposed to an increase. We believe this to be caused by severe etching of the carbon fiber by holding at a constant potential of 1.3 V for 30 s (Heien et al., 2003).

We hypothesize here that rather than classical electron transfer, the histamine forms a complex with the CFM, the waveform changes the conformation of the histamine molecule and its polarity at the CFM surface, and the molecule then undergoes charge transfer (as depicted in **Figure 8.3C**). While scanning positive through the waveform and the electrode gets more positive, a hydrogen from the protonated primary amine ($-\text{NH}_3^+$) is taken by the tau-nitrogen in the imidazole ring forming an imidazolium ion with a positive charge and a neutral primary amine. Shifting the molecule's charge causes a change in the electrochemical bilayer. Therefore, after background-subtraction, differences in the electrical double layer lead to corresponding changes in the histamine FSCV signal.

Given that we're assuming charge transfer as the mechanism of the FSCV response, we considered which aspects of the in vivo environment might cause this broadening to occur, and hypothesized it was most likely due to crowding (the accumulation of proteins and other biomolecules onto the electrode surface). The aggregation of these biological molecules onto the electrode surface could change the electronegativity of the histamine molecule, thus to which extent the charge is transferred across the molecules,

changing the shape of the peak. Therefore, we looked into a way to replicate the FSCV signal in a protein-rich in vivo environment.

Extracellular matrix (ECM) is obtained from the Engelbreth-Holm-Swarm mouse sarcoma line, a tumor which is dense in ECM proteins. Key components of the ECM, including laminin, collagen IV, entactin, and heparan sulfate proteoglycans, are commonly used for 3D cell culture (Koc et al., 2021). Many of the ECM components are charged, creating an ionic environment which represents biological environments. Since this medium is primarily designed to support cell growth, it will likely contain many key in vivo components (though there is no data showing the exact composition), as represented in **Figure 8.5A**. A drop of ECM was applied to a Nafion-modified CFM using a premade mold to form a spherical mass of ECM. **Figure 8.5B** shows a photograph of the Nafion- and ECM-modified CFM under a light microscope. It is worth noting the ECM mass completely covers the tip of the ECM, though it is very thick and irregular.

We tested this ECM coating using FIA and introduced a large histamine concentration (250 μ M) in HEPES buffer to the electrode. The large size of the ECM coating causes the histamine to take longer to reach the electrode surface (45 s) compared to a Nafion-modified CFM (9 s) and also delays histamine clearance from the electrode surface, as shown in the color plot in **Figure 8.5C**. However, the Nafion- and ECM-modified CFM CV more closely resembles the histamine in vivo CV with its peak position at 0.34 V, as shown in **Figure 8.5D**.

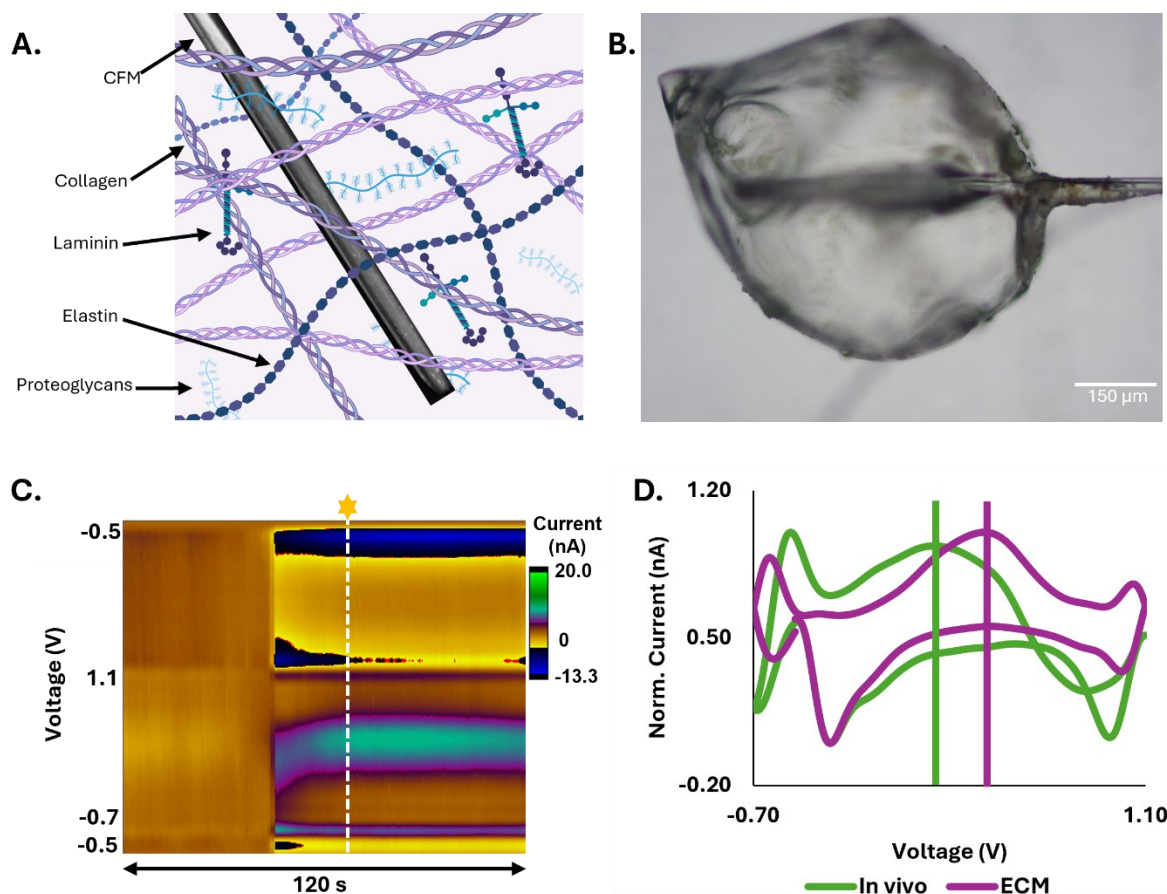


Figure 8.5. ECM coating. (A) Diagram of a CFM embedded in ECM. Image created using Biorender. (B) Image of a CFM coated in ECM under a light microscope. (C) Representative color plot of 250 μM histamine injected onto a Nafion- and ECM-coated CFM using FIA in HEPES buffer; the vertical white line denoted by the star represents the CV extracted for analysis in (D). (D) Normalized histamine CVs in vivo (green) and Nafion- and ECM-coated (purple). Horizontal lines indicate peak maximum voltage potential.

This demonstrates that the combination of HEPES buffer and an ECM coating creates a better model environment for in vitro studies to better mimic in vivo signals. This result bolsters our hypothesis above (charge transfer as mechanism of the FSCV histamine response) that crowding the electrode surface with other molecules changes the electronegativity and hence the charge transfer across the histamine molecule, fundamentally changing the shape of FSCV peak.

This result shows the potential power of using ECM to improve FSCV signal, however more work is needed in the future to create thin films that do not create large diffusional barriers as seen here. Previously, work has also shown utility in using ECM for sensors. Electrochemical sensors modified with ECM can monitor cell adhesion and proliferation (Fohlerová et al., 2007, Zhang et al., 2012) and thin coatings of ECM on micro electrocorticography sensors have been used to protect them from damage when inserted into the brain (Ceyssens et al., 2017).

Having found a method to infer more in vivo like characteristics to the histamine signal, we next turned towards stability and sensitivity of the response.

The Effect of Lubricin on CFM Response to Histamine and Serotonin

Having established that HEPES and ECM coated electrodes are excellent ways to mimic the in vivo environment for histamine calibrations, we next investigated a novel electrode coating to enhance electrode sensitivity and stability for histamine and serotonin FSCV.

Similar to in vivo measurements of other neurotransmitters, such as dopamine and serotonin, evoked histamine release occurs at relatively low concentrations (low micromolar) in vivo (Berger et al., 2022, Samaranayake, 2015). Therefore, it is important to investigate improving the sensitivity of CFMs toward histamine. Additionally, serotonin and its metabolites can cause fouling of the electrode surface (Brazell et al., 1987, Jackson et al., 1995, Hashemi et al., 2009, Dunham and Venton, 2020, Shin et al., 2021).

Therefore, it is necessary to also explore ways to improve the stability of the electrode

over several hours, as this is the amount of time in vivo experiments typically run. We turned our attention to a novel coating, Lubricin (Lub), to address these concerns.

Lub (also known as proteoglycan-4) is a relatively large glycoprotein (a protein molecule with one or more covalently attached carbohydrate groups) found in an array of tissues and fluids in mammals (Han et al., 2024). It possesses the ability to self-assemble into a “telechelic” polymer brush on a variety of surfaces, including carbon (Han et al., 2019). Lub possesses two globular end domains with a mucin-like domain in the center, which is negatively charged. When exposed to the carbon surface (dip coated), the two end domains adhere to the electrode surface while the middle mucin-domain forms a protruding loop-like brush structure (Greene et al., 2015, Russo et al., 2020). This makes it an ideal substance for our purposes, being both biocompatible and easy to apply. SEM imaging of Lub coated CFMS are shown in **Figure 8.6**.

We theorized the Lub would improve the stability of histamine FSCV since, as shown in Chapter 7, it improved the stability of histamine FSCAV (average $t_{63\%}$ (time it takes measurements to reach 63% stability) for the bare electrodes was 279.7 +/- 136.1 mins compared to the Lub electrodes which was 149.4 +/- 39.0 mins) and it has previously been shown to act as an antifouling agent (Han et al., 2024, Russo et al., 2020, Greene et al., 2015, Greene et al., 2018).

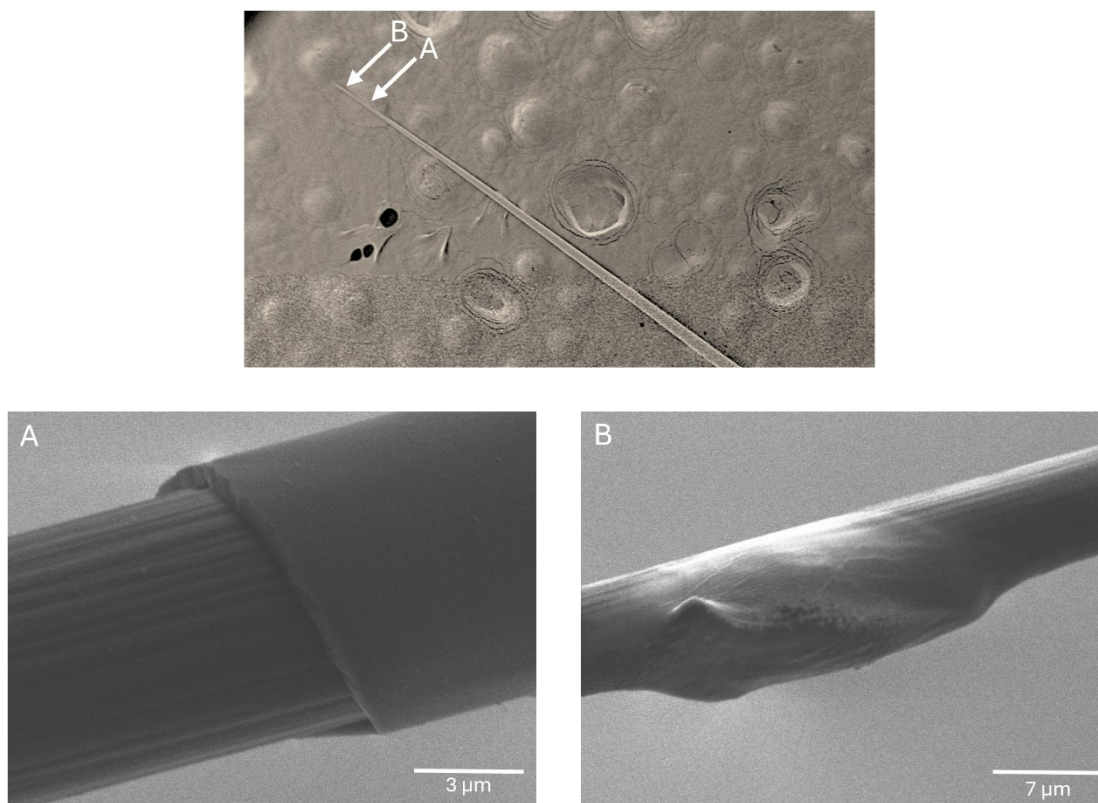


Figure 8.6. SEM imaging of Lub coated CFM. (Top) Scanning electron micrograph image of a CFM dip-coated in Lubricin with arrows pointing to (A) the glass-fiber junction and (B) Lubricin deposition on the fiber. Imaging done by Pablo Prieto.

To investigate the sensitivity and stability of Lub-modified CFMs, we performed calibrations and stability experiments as shown in **Figure 8.7**. **Figure 8.7A** and **8.7C** show calibration curves for histamine and serotonin FSCV, respectively. Nafion modified CFMs are shown in blue and Lub modified CFMs in green. For both histamine and serotonin, Lub modified CFMs resulted in an increase in sensitivity (Histamine: Lub = $0.414 \pm 0.079 \text{ nA } \mu\text{M}^{-1}$ vs Nafion = $0.354 \pm 0.008 \text{ nA } \mu\text{M}^{-1}$; Serotonin: Lub = $0.184 \pm 0.043 \text{ nA nM}^{-1}$ vs Nafion = $0.166 \pm 0.039 \text{ nA nM}^{-1}$), though not significant, over Nafion modified CFMs. In the previous chapter, Lub modification resulted in a decrease in

sensitivity for histamine FSCAV, and here it resulted in an increase for FSCV. We hypothesize this is due to adsorption time. For FSCAV, we give a much longer time for the analyte to adsorb onto the electrode surface (1 s) than we do for FSCV (4 ms). Lub applied to the electrode surface can block some of the active sites on the electrode surface, hence its antifouling properties, but means less sites are available for adsorption during the longer holding periods with FSCAV which allows for more accumulation of analyte on the electrode surface. For FSCV, this is not a problem because FSCV uses minimal holding periods (ms) to promote adsorption of the analyte which will therefore preconcentrate but not over-accumulate the analyte on the electrode surface. Lub also possesses an overall negative charge which facilitates the preconcentration of positively charged histamine (as Nafion also does).

For serotonin sensitivity, there is not much increase in sensitivity compared to Nafion coated CFMs, though there is an increase versus bare CFMs (Hashemi et al., 2009). This is likely because both Lub and Nafion cause serotonin preconcentration, as serotonin is positively charged, and therefore result in similar sensitivities.

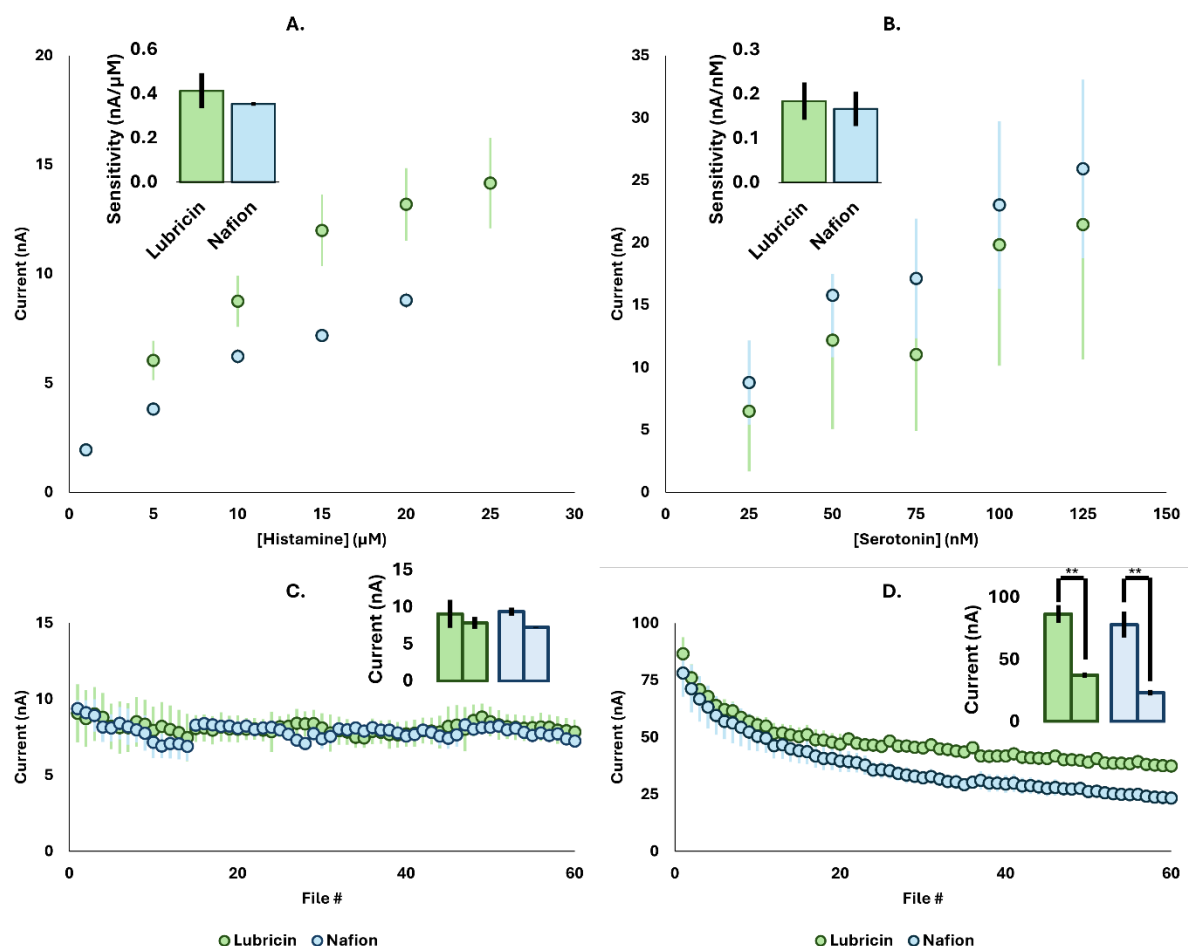


Figure 8.7. Sensitivity and stability of Lubricin and Nafion-modified CFMs. (A,B) Histamine and serotonin calibration curves (average $\pm$ SEM), respectively, on Lub CFMs (green; $n=5$) and Nafion-modified CFMs (blue; $n=4$), inset bar graphs depict the slope of the curve; (C,D) Histamine and serotonin stability (average $\pm$ SEM), respectively, on Lub CFMs (green; $n=3$) and Nafion-modified CFMs (blue; $n=3$), inset bar graphs depict the electrode response of File 1 versus File 60 (** $p < 0.01$).

We next turned our attention to electrode stability toward histamine and serotonin, as shown in **Figure 8.7B** and **8.7D** respectively. **Figure 8.7B** shows the electrode response to 10 μM histamine over 60 measurements. The inset bar chart shows the average current of File1 versus File60 for Lub in green and Nafion in blue. **Figure 8.7D** shows the same but in response to 1 μM serotonin. Looking at stability, a Lub coating had no significant effect on histamine stability compared to the Nafion coating (Lub: File1 = 218

9.065 +/- 1.900 nA, File60 = 7.844 +/- 0.795 nA; Nafion: File1 = 9.368 +/- 0.555 nA, File60 = 7.250 +/- 0.055 nA). We hypothesize that this is likely due to histamine oxidation not producing side products which foul the electrode surface. However, the same is not true for serotonin (Lub: File1 = 86.596 +/- 7.164 nA, File60 = 37.488 +/- 2.156 nA; Nafion: File1 = 78.125 +/- 10.365 nA, File60 = 23.339 +/- 2.214 nA). Serotonin is known to foul the electrode surface (Brazell et al., 1987, Jackson et al., 1995, Hashemi et al., 2009, Dunham and Venton, 2020, Shin et al., 2021) and therefore cause a decrease in signal over subsequent measurements. While the Lub coating does not completely prevent the fouling of the electrode from serotonin, the percent decrease in the signal at File1 versus File60 (Lub = 55.67 %_{decrease}, Nafion = 69.64 %_{decrease}) is less than the decrease for the Nafion coated CFMs, demonstrating Lub has slightly more antifouling capability than Nafion.

In summary, Lub improves the CFM sensitivity to histamine while also improving stability towards serotonin.

Conclusion

FSCV has proven to be effective when measuring neurotransmission in the brain. In vitro calibrations are required to accurately assess in vivo signals, however in vitro signals often look different from in vivo signals because of the differences in media. In this study, we compared in vivo and in vitro CVs of histamine in different media and concluded HEPES buffer and an application of ECM onto the electrode surface most closely resembled in vivo signals. We then showed that a coating of Lubricin onto the electrode provided enhanced serotonin measurements and decreased electrode fouling during

histamine measurements. We suggest two novel electrode coatings, Lubricin and ECM, are valuable when performing in vitro FSCV measurements and that customized surface treatments and physiological buffers offer the most effective approach to replicate in vivo conditions in vitro.

References

- BAUR, J. E., KRISTENSEN, E. W., MAY, L. J., WIEDEMANN, D. J. & WIGHTMAN, R. M. 1988. Fast-scan voltammetry of biogenic amines. *Analytical chemistry*, 60, 1268-1272.
- BERGER, S. N., BAUMBERGER, B., SAMARANAYAKE, S., HERSEY, M., MENA, S., BAIN, I., DUNCAN, W., REED, M. C., NIJHOUT, H. F. & BEST, J. 2022. An In Vivo Definition of Brain Histamine Dynamics Reveals Critical Neuromodulatory Roles for This Elusive Messenger. *International journal of molecular sciences*, 23, 14862.
- BRAZELL, M., KASSER, R., RENNER, K., FENG, J., MOGHADDAM, B. & ADAMS, R. 1987. Electrocoating carbon fiber microelectrodes with Nafion improves selectivity for electroactive neurotransmitters. *Journal of neuroscience methods*, 22, 167-172.
- BROWN, P. D., DAVIES, S. L., SPEAKE, T. & MILLAR, I. D. 2004. Molecular mechanisms of cerebrospinal fluid production. *Neuroscience*, 129, 957-70.
- BUCHANAN, A. M., MENA, S., CHOUKARI, I., VASA, A., CRAWFORD, J. N., FADEL, J., MAXWELL, N., REAGAN, L., CRUIKSHANK, A. & BEST, J. 2024. Serotonin as a biomarker of toxin-induced Parkinsonism. *Molecular Medicine*, 30, 33.
- CEYSSENS, F., DEPREZ, M., TURNER, N., KIL, D., VAN KUYCK, K., WELKENHUYSEN, M., NUTTIN, B., BADYLAK, S. & PUERS, R. 2017. Extracellular matrix proteins as temporary coating for thin-film neural implants. *Journal of neural engineering*, 14, 014001.
- DUNHAM, K. E. & VENTON, B. J. 2020. Improving serotonin fast-scan cyclic voltammetry detection: new waveforms to reduce electrode fouling. *Analyst*, 145, 7437-7446.
- FOHLEROVÁ, Z., SKLÁDAL, P. & TURÁNEK, J. 2007. Adhesion of eukaryotic cell lines on the gold surface modified with extracellular matrix proteins monitored by the piezoelectric sensor. *Biosensors and Bioelectronics*, 22, 1896-1901.
- FOX, M. E., BUCHER, E. S., JOHNSON, J. A. & WIGHTMAN, R. M. 2016. Medullary Norepinephrine Projections Release Norepinephrine into the Contralateral Bed Nucleus of the Stria Terminalis. *ACS Chemical Neuroscience*, 7, 1681-1689.
- GREENE, G. W., MARTIN, L. L., TABOR, R. F., MICHALCZYK, A., ACKLAND, L. M. & HORN, R. 2015. Lubricin: A versatile, biological anti-adhesive with properties comparable to polyethylene glycol. *Biomaterials*, 53, 127-136.
- GREENE, G. W., ORTIZ, V., POZO-GONZALO, C., MOULTON, S. E., WANG, X., MARTIN, L. L., MICHALCZYK, A. & HOWLETT, P. C. 2018. Lubricin antiadhesive coatings exhibit size-selective transport properties that inhibit biofouling of electrode surfaces with minimal loss in electrochemical activity. *Advanced Materials Interfaces*, 5, 1701296.
- HAN, M., SILVA, S. M., LEI, W., QUIGLEY, A., KAPSA, R. M., MOULTON, S. E. & GREENE, G. W. 2019. Adhesion and self-assembly of lubricin (PRG4) brush layers on different substrate surfaces. *Langmuir*, 35, 15834-15848.
- HAN, M., SILVA, S. M., RUSSO, M. J., DESROCHES, P. E., LEI, W., QUIGLEY, A. F., KAPSA, R. M., MOULTON, S. E., STODDART, P. R. & GREENE, G. W. 2024. Lubricin (PRG-4) anti-fouling coating for surface-enhanced Raman spectroscopy biosensing: towards a hierarchical separation system for analysis of biofluids. *Analyst*, 149, 63-75.
- HASHEMI, P., ABDALLA, A., JIN, Y., BEST, J., LINDEN, D. & REED, M. In Vivo Serotonin Chemistry and Local Cytoarchitecture: A Combined Voltammetric, Mathematical

- and Microscopy Study. NEUROPSYCHOPHARMACOLOGY, 2015. NATURE PUBLISHING GROUP MACMILLAN BUILDING, 4 CRINAN ST, LONDON N1 9XW, ENGLAND, S522-S523.
- HASHEMI, P., DANKOSKI, E. C., LAMA, R., WOOD, K. M., TAKMAKOV, P. & WIGHTMAN, R. M. 2012. Brain dopamine and serotonin differ in regulation and its consequences. *Proceedings of the National Academy of Sciences*, 109, 11510-11515.
- HASHEMI, P., DANKOSKI, E. C., PETROVIC, J., KEITHLEY, R. B. & WIGHTMAN, R. 2009. Voltammetric detection of 5-hydroxytryptamine release in the rat brain. *Analytical chemistry*, 81, 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, 749-759.
- 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 increases sensitivity. *Analyst*, 128, 1413-1419.
- HERMANS, A., KEITHLEY, R. B., KITA, J. M., SOMBERS, L. A. & WIGHTMAN, R. M. 2008. Dopamine detection with fast-scan cyclic voltammetry used with analog background subtraction. *Analytical chemistry*, 80, 4040-4048.
- HERSEY, M., RENEAU, M., BERGER, S. N., MENA, S., BUCHANAN, A. M., OU, Y., TAVAKOLI, N., REAGAN, L. P., CLOPATH, C. & HASHEMI, P. 2022. A tale of two transmitters: serotonin and histamine as in vivo biomarkers of chronic stress in mice. *Journal of Neuroinflammation*, 19, 1-20.
- HERSEY, M., SAMARANAYAKE, S., BERGER, S. N., TAVAKOLI, N., MENA, S., NIJHOUT, H. F., REED, M. C., BEST, J., BLAKELY, R. D. & REAGAN, L. P. 2021a. Inflammation-induced histamine impairs the capacity of escitalopram to increase hippocampal extracellular serotonin. *Journal of Neuroscience*, 41, 6564-6577.
- HERSEY, M., WOODRUFF, J. L., MAXWELL, N., SADEK, A. T., BYKALO, M. K., BAIN, I., GRILLO, C. A., PIROLI, G. G., HASHEMI, P. & REAGAN, L. P. 2021b. High-fat diet induces neuroinflammation and reduces the serotonergic response to escitalopram in the hippocampus of obese rats. *Brain, behavior, and immunity*, 96, 63-72.
- HETTIARACHCHI, P., NIYANGODA, S., SHIGEMOTO, A., SOLOWIEJ, I. J., BURDETTE, S. C. & JOHNSON, M. A. 2024. Caged Zn²⁺ Photolysis in Zebrafish Whole Brains Reveals Subsecond Modulation of Dopamine Uptake. *ACS Chemical Neuroscience*, 15, 772-782.
- HEXTER, M., VAN BATENBURG-SHERWOOD, J. & HASHEMI, P. 2023. Novel Experimental and Analysis Strategies for Fast Voltammetry: 2. A Troubleshoot-Free Flow Cell for FSCV Calibrations. *ACS Measurement Science Au*.
- HOLMES, J., WITT, C. E., KEEN, D., BUCHANAN, A. M., BATEY, L., HERSEY, M. & HASHEMI, P. 2021. Glutamate Electropolymerization on Carbon Increases Analytical Sensitivity to Dopamine and Serotonin: An Auspicious In Vivo Phenomenon in Mice? *Analytical Chemistry*, 93, 10762-10771.
- JACKSON, B. P., DIETZ, S. M. & WIGHTMAN, R. M. 1995. Fast-scan cyclic voltammetry of 5-hydroxytryptamine. *Analytical chemistry*, 67, 1115-1120.

- JACKSON, B. P. & MARK WIGHTMAN, R. 1995. Dynamics of 5-hydroxytryptamine released from dopamine neurons in the caudate putamen of the rat. *Brain Research*, 674, 163-166.
- KOC, A., SAHOGLU GOKTAS, S., AKGUL CAGLAR, T. & CAGAVI, E. 2021. Defining optimal enzyme and matrix combination for replating of human induced pluripotent stem cell-derived cardiomyocytes at different levels of maturity. *Experimental Cell Research*, 403, 112599.
- MEUNIER, C. J., ROBERTS, J. G., MCCARTY, G. S. & SOMBERS, L. A. 2017. Background Signal as an in Situ Predictor of Dopamine Oxidation Potential: Improving Interpretation of Fast-Scan Cyclic Voltammetry Data. *ACS Chemical Neuroscience*, 8, 411-419.
- MICHAEL, D., TRAVIS, E. R. & WIGHTMAN, R. M. 1998. Peer reviewed: color images for fast-scan CV measurements in biological systems. *Analytical chemistry*, 70, 586A-592A.
- MILLAR, J., STAMFORD, J. A., KRUK, Z. L. & WIGHTMAN, R. M. 1985. Electrochemical, pharmacological and electrophysiological evidence of rapid dopamine release and removal in the rat caudate nucleus following electrical stimulation of the median forebrain bundle. *European Journal of Pharmacology*, 109, 341-348.
- PARK, J., BUCHER, E. S., BUDYGIN, E. A. & WIGHTMAN, R. M. 2015. Norepinephrine and dopamine transmission in 2 limbic regions differentially respond to acute noxious stimulation. *Pain*, 156, 318-327.
- PARK, J., BUCHER, E. S., FONTILLAS, K., OWESSON-WHITE, C., ARIANSEN, J. L., CARELLI, R. M. & WIGHTMAN, R. M. 2013. Opposing Catecholamine Changes in the Bed Nucleus of the Stria Terminalis During Intracranial Self-Stimulation and Its Extinction. *Biological Psychiatry*, 74, 69-76.
- PARK, J., TAKMAKOV, P. & WIGHTMAN, R. M. 2011. In vivo comparison of norepinephrine and dopamine release in rat brain by simultaneous measurements with fast-scan cyclic voltammetry. *Journal of neurochemistry*, 119, 932-944.
- PAXINOS, G. & FRANKLIN, K. B. 2019. *Paxinos and Franklin's the mouse brain in stereotaxic coordinates*, Academic press.
- PIHEL, K., HSIEH, S., JORGENSON, J. W. & WIGHTMAN, R. M. 1995. Electrochemical detection of histamine and 5-hydroxytryptamine at isolated mast cells. *Analytical chemistry*, 67, 4514-4521.
- PIHEL, K., TRAVIS, E. R., BORGES, R. & WIGHTMAN, R. M. 1996a. Exocytotic release from individual granules exhibits similar properties at mast and chromaffin cells. *Biophysical journal*, 71, 1633-1640.
- PIHEL, K., WALKER, Q. D. & WIGHTMAN, R. M. 1996b. Overoxidized Polypyrrole-Coated Carbon Fiber Microelectrodes for Dopamine Measurements with Fast-Scan Cyclic Voltammetry. *Analytical Chemistry*, 68, 2084-2089.
- PUTHONGKHAM, P., LEE, S. T. & VENTON, B. J. 2019. Mechanism of Histamine Oxidation and Electropolymerization at Carbon Electrodes. *Analytical Chemistry*, 91, 8366-8373.
- 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, 1763-1773.
- ROSS, A. E. & VENTON, B. J. 2012. Nafion-CNT coated carbon-fiber microelectrodes for enhanced detection of adenosine. *Analyst*, 137, 3045-3051.

- RUSO, M. J., HAN, M., QUIGLEY, A. F., KAPSA, R. M. I., MOULTON, S. E., DOEVEN, E., GUIJT, R., SILVA, S. M. & GREENE, G. W. 2020. Lubricin (PRG4) reduces fouling susceptibility and improves sensitivity of carbon-based electrodes. *Electrochimica Acta*, 333, 135574.
- SAMARANAYAKE, S., ABDALLA, A., ROBKE, R., NIJHOUT, H. F., REED, M. C., BEST, J. & HASHEMI, P. 2016. A voltammetric and mathematical analysis of histaminergic modulation of serotonin in the mouse hypothalamus. *Journal of neurochemistry*, 138, 374-383.
- SAMARANAYAKE, S. A., A.; ROBKE, R.; WOOD, K. M.; ZEQA, A.; HASHEMI, P. 2015. In Vivo Histamine Voltammetry in the Mouse Premammillary Nucleus. *Analyst*, 140, 3759-3765.
- SAYLOR, R. A., HERSEY, M., WEST, A., BUCHANAN, A. M., BERGER, S. N., NIJHOUT, H. F., REED, M. C., BEST, J. & HASHEMI, P. 2019. In vivo hippocampal serotonin dynamics in male and female mice: Determining effects of acute escitalopram using fast scan cyclic voltammetry. *Frontiers in neuroscience*, 13, 362.
- SHIN, H., GOYAL, A., BARNETT, J. H., RUSHEEN, A. E., YUEN, J., JHA, R., HWANG, S. M., KANG, Y., PARK, C., CHO, H.-U., BLAHA, C. D., BENNET, K. E., OH, Y., HEIEN, M. L., JANG, D. P. & LEE, K. H. 2021. Tonic Serotonin Measurements In Vivo Using N-Shaped Multiple Cyclic Square Wave Voltammetry. *Analytical Chemistry*, 93, 16987-16994.
- SHIN, M., FIELD, T. M., STUCKY, C. S., FURGURSON, M. N. & JOHNSON, M. A. 2017. Ex Vivo Measurement of Electrically Evoked Dopamine Release in Zebrafish Whole Brain. *ACS Chemical Neuroscience*, 8, 1880-1888.
- SWAMY, B. K. & VENTON, B. J. 2007. Carbon nanotube-modified microelectrodes for simultaneous detection of dopamine and serotonin in vivo. *Analyst*, 132, 876-884.
- VREELAND, R. F., ATCHERLEY, C. W., RUSSELL, W. S., XIE, J. Y., LU, D., LAUDE, N. D., PORRECA, F. & HEIEN, M. L. 2015. Biocompatible PEDOT: Nafion composite electrode coatings for selective detection of neurotransmitters in vivo. *Analytical chemistry*, 87, 2600-2607.
- WIGHTMAN, R., AMATORH, C., ENGSTROM, R., HALE, P., KRISTENSEN, E., KUHR, W. & MAY, L. 1988. Real-time characterization of dopamine overflow and uptake in the rat striatum. *Neuroscience*, 25, 513-523.
- WITT, C. E., MENA, S., HOLMES, J., HERSEY, M., BUCHANAN, A. M., PARKE, B., SAYLOR, R., HONAN, L. E., BERGER, S. N. & LUMBRERAS, S. 2023. Serotonin is a common thread linking different classes of antidepressants. *Cell chemical biology*, 30, 1557-1570. e6.
- WOOD, K. M. & HASHEMI, P. 2013. Fast-Scan Cyclic Voltammetry Analysis of Dynamic Serotonin Responses to Acute Escitalopram. *ACS Chemical Neuroscience*, 4, 715-720.
- ZHANG, Y., WANG, L., ZHU, J., HU, Y., XING, W. & CHENG, J. 2012. Real-time monitoring of extracellular matrix-mediated PC12 cell attachment and proliferation using an electronic biosensing device. *Biotechnology letters*, 34, 397-404.

CHAPTER 9 – CONCLUSION AND FUTURE WORK

Depression is one of the leading causes of disability worldwide with diagnoses and treatments difficult due to the lack of understanding as to the root cause of depression. Multiple theories as to the basis of depression have been put forth, but no one theory has been proven conclusive. Serotonin and histamine are of particular interest regarding depression due to the comorbidity of depression and inflammation. Techniques that can accurately capture neurotransmitter signaling in the brain are in a constant state of development and optimization. Such techniques shown to accurately capture neurotransmitter dynamics are fast scan cyclic voltammetry (FSCV) and fast scan controlled adsorption voltammetry (FSCAV). The objective of this dissertation was to employ novel methodologies to improve FSCV and FSCAV measurements. In Chapter 1, I began this thesis by introducing neurotransmission, the serotonergic and histaminergic systems, serotonin and histamine co-modulation, and neurotransmission measurement techniques. In Chapter 2, I reviewed how inflammation via endotoxin affects the neurotransmitters dopamine, serotonin, and norepinephrine in the brain. In Chapter 3, I reviewed the three theories of depression and how they are all individual parts of one larger picture. In Chapter 4, I presented essential analytical techniques used to study depression. In Chapter 5, I explained the general methods used in this research. In Chapter 6, I described a new electrode surface modification of amino acids (glutamate, GABA, and glycine) for improved sensitivity and decreased electrode equilibration time for measurements of extracellular serotonin using FSCAV. In Chapter 7, I presented FSCAV for histamine detection. I demonstrated that the coating of Lubricin on the electrode surface allowed for sensitive and stable measurements over time and illustrated how anesthetics and thioperamide offset one another to influence extracellular histamine concentrations in the mouse cortex. Lastly in Chapter 8, I

described how custom buffers and electrode modifications are necessary for different analytes to reproduce in vivo signals in vitro for better electrode calibrations. I showed that a combination of modifying CFMs with Nafion and ECM and measuring in HEPES buffer provided in vitro signals more similar to in vivo ones.

The continuation of this work could include:

1. studies to improve FSCV and FSCAV measurements with additional surface modifications of the CFMs and applying these modifications to in vivo analyses.
2. further analysis of Lubricin modifications.
3. analysis and mathematical modeling of simultaneous histamine and serotonin FSCAV measurements on one electrode.

APPENDICES

Appendix A – Rights and Permissions to Reprint

Chapter 2 reproduced under a CC-BY-4.0 creative commons license

<https://creativecommons.org/licenses/by/4.0/>

 CC BY 4.0

ATTRIBUTION 4.0 INTERNATIONAL
Deed

Canonical URL: <https://creativecommons.org/licenses/by/4.0/>

[See the legal code](#)

You are free to:

Share — copy and redistribute the material in any medium or format for any purpose, even commercially.

Adapt — remix, transform, and build upon the material for any purpose, even commercially.

The licensor cannot revoke these freedoms as long as you follow the license terms.

Under the following terms:

 **Attribution** — You must give [appropriate credit](#), provide a link to the license, and [indicate if changes were made](#). You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.

No additional restrictions — You may not apply legal terms or [technological measures](#) that legally restrict others from doing anything the license permits.

Notices:

You do not have to comply with the license for elements of the material in the public domain or where your use is permitted by an applicable [exception or limitation](#).

No warranties are given. The license may not give you all of the permissions necessary for your intended use. For example, other rights such as [publicity, privacy, or moral rights](#) may limit how you use the material.