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Parallel information transmission and circuit refinement of the corticostriatal system

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PARALLEL INFORMATION TRANSMISSION AND CIRCUIT REFINEMENT
OF THE CORTICOSTRIATAL SYSTEM

by

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ABSTRACT

The brain is a complex organ that not only gathers copious amounts of information, but also interprets and reacts to information gathered. In the present study we sought to understand how relevant information for a complex experience (cocaine) was transmitted from regions throughout the brain to cocaine-activated striatal cells producing an overall phenotype expected from mice under a strong cocaine experience. To accomplish this, Arc/Ai14 mice were first exposed to repeated prior intra-peritoneal cocaine injections, after which striatal injections of AAV-DIO-TVA adeno-associated virus (AAV) were performed. Post AAV injection, cocaine-activated cells were TRAPed, and finally (EnvA)SAD-ΔG-GFP rabies virus (RV) was injected into the striatum allowing for brain wide monosynaptic retrograde tracing of inputs (cocaine-activated inputs indicated by yellow fluorescence) onto cocaine-activated striatal MSNs. While data is still being tabulated, preliminary data suggests an increase in co-connectivity between cocaine-activated orbitofrontal and medial cortical neurons (retrosplenial/cingulate) and cocaine-activated striatal cells. Thus, preliminary data suggest that chronic cocaine pre-exposure lead to corticostriatal circuit refinement.
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LIST OF ABBREVIATIONS

AAV ................................................................. Adeno-Associated Virus
APLAC ............................................................ Administrative Panel on Laboratory Animal Care
Arc ................................................................. Actively-regulated cytoskeleton-associated protein
CreER\textsuperscript{T2} ........................................... Tamoxifen-Dependent Cre recombinase
CPu ..................................................................... Caudate & Putamen
D1 .................................................................. Direct Pathway Cell Receptors
D2 .................................................................. Indirect Pathway Cell Receptors
DA ...................................................................... Dopamine
GFP .................................................................... Green Fluorescence Protein
GPe ..................................................................... External Globus Pallidus
GPi ..................................................................... Internal Globus Pallidus
IACUC ................................................................. Institutional animal Care and Use Committee
IEG ..................................................................... Immediate Early Gene
MSN ................................................................ Medium Spiny Neuron
OFC .................................................................... Orbitofrontal Cortex
4-OHT .................................................................. 4-hydroxytamoxifen
PBS ..................................................................... Phosphate Buffered Saline
PFC .................................................................... Prefrontal Cortex
RG ..................................................................... Rabies Glycoprotein
RV ..................................................................... Rabies Virus
SARE ..............................Synaptic Activity-Responsive Element
SERMs ............................................ Selective Estrogen Receptor Modulators
SNCs .................................................. Substantia Nigra Pars compact
SNr ..................................................... Substantia Nigra Pars Reticulata
SRE ..................................................... Serum Response Element
SRF ..................................................... Serum Response Factor
SSR ..................................................... Site Specific Recombination
TRAP ................................................ Targeted Recombination in Active Populations
TM ........................................................ Tamoxifen
TVA ..................................................... Avian Tumor Virus Receptor A
INTRODUCTION

The brain is a complex sensory perceiving, processing, and motor controlling center allowing for both voluntary and involuntary control of actions. With over 86 billion neurons in the brain, each with a potentially large number of connections, scientists continue to struggle to map out neural connections between brain regions. These billions of neurons are housed within a finite number of brain regions, bringing up the important question of how it is that a seemingly infinite number of perceptions, decisions, and actions can be initiated from a limited number of brain regions. How are unique experiences transmitted and processed in the brain, culminating in physical changes in wiring in the brain that represent the unique experience?

Until recently, the field of neuroscience lacked the tools necessary to tackle the challenge of cataloging the complex amalgam of neurons throughout different regions of the brain and to unmask wiring diagrams revealing synapse-to-synapse neural connections. Specifically, the difficulty lay in imaging and reconstructing circuits with single synapse resolution within a vast volume of a complex tissue. Confounding this already daunting task was the need for precise spatiotemporal manipulation of neurons. With discoveries of new technologies in fields such as microscopy, virology, and genetic engineering, neuroscience is closer to fulfilling the goal of characterizing cellular constituents of the brain, describing how such constituents are organized into circuits, and how individual neurons and circuits process information ultimately guiding behavior.
An important structure of the brain for the present study was the dorsal striatum, a chief component of the basal ganglia (Figure 1). The dorsal striatum is one of the main input areas for the basal ganglia, receiving vast majority of its afferents from the cerebral cortex, and some afferents from the substantia nigra (SNr) and thalamus. Corticostriatal fibers can carry voluntary motor information, and importantly, cortical inputs come from many different cortical processing areas. Noteworthy is the role of the nigrostriatal pathway in movement, a pathway that is significantly disabled by certain neurodegenerative diseases, namely Parkinson’s Disease. A 2013 study by Wall et al. examined the process of information segregation in the striatum (Wall, La Parra, Callaway, & Kreitzer, 2013). In this study, it was demonstrated that information streams coming into the striatum preferentially targeted either direct or indirect pathway cells, hinting to a mechanism of differential information transmission to the two unique cell types. Specifically, it was determined that sensory cortical and limbic structures preferentially innervate direct pathway cells. In contrast, the motor cortex was found to preferentially innervate indirect pathway cells and thalamostriatal and dopaminergic inputs, which were found to have neither direct nor indirect pathway preference (Figure 2). Extending the effort by Wall et al., a 2015 study by Sippy et al. was able to parse out a specific function of direct pathway striatonigral neurons as contributing to what they called the “go” signal initiating goal-directed transformation of sensorimotor information into action (Sippy, Lapray, Crochet, & Petersen, 2015). Specifically, data in this study identified a sensory-specific connection onto direct-pathway cells that contribute to a
“go” signal resulting in action initiation. Together, these studies indicated differential input patterns onto heterogeneous populations of neurons that have rival effects on behavior.

Figure 1: Anatomical model of the Dorsal Striatum. Highlighted in green are the two regions of the dorsal striatum known as the caudate and putamen (CPu). The section shown above is a coronal section of the brain. Permission granted by Elsevier Publishing to use image from The Mouse Brain in Stereotaxic Coordinates (Franklin & Paxinos, 2007). This image has been modified from the original image.

Figure 2: Differential innervation of direct and indirect pathway striatal neurons. Figure adapted from Wall et al., 2013.
Anatomically, the brain has been found to have fine-scale specificity, meaning neighboring neurons of the same anatomical type have been found to receive information from unique precisely interconnected networks of neurons ((Yoshimura, Dantzker, & Callaway, 2005), (Song, Sjöström, Reigl, Nelson, & Chklovskii, 2005)). Importantly, it was demonstrated that when neighboring cells were connected, the probability that reciprocally connected cells shared common inputs was increased compared to randomly connecting neighboring cells (Yoshimura et al., 2005) (Figure 3). Specifically, it was shown that on a fine scale, inhibitory neurons have precise connectivity to inhibitory and excitatory neurons. These findings follow the Hebbian Learning Theory stating that coactivation of cells strengthens synaptic connections between them, and is a plausible and important explanation for memory formation. These findings suggest a rule of non-random neuronal connectivity; an architecture of unique information transmission and processing.
Another 2015 study by Cao et al. used a rotarod training task reiterated over multiple days to study neuronal recruitment and consolidation during motor learning, ultimately coming to the conclusion of cellular involvement in the formation and strengthening of task-specific neuronal ensembles as a result of motor learning (rotarod task) (Cao et al., 2015). An essential conclusion of this 2015 study was that repeated experience (rotarod learning) locked Arc, an immediate early gene (IEG) indicating neuronal activity initiation, expression patterns in place, and these Arc-containing cells were found to consistently reactivate Arc transcription during future rotarod sessions. This helped to identify Arc as a critically important molecule in the consolidation of task-specific neuronal ensembles as a result of motor learning. Arc-GFP heterozygous mice,
Arc being an immediate early gene (IEG), were gathered to help identify neuronal activation patterns in the motor cortex as a result of the rotarod task (Cao et al., 2015).

The present study used a combination of advanced genetic and viral techniques to map out corticostriatal neural ensembles, and used gathered results to attempt to decipher information transmission pathways for complex experiences. Using a Cre$^+$ transgenic mouse system, partnered with a two-virus injection protocol, this study captured, at a single-synapse level, the monosynaptic retrograde projections onto striatal medium spiny neurons (MSNs) activated by a chronic cocaine experience. To target specific striatal cells and trace retrograde monosynaptic projections onto these cells, a first round of AAV virus was injected into the striatum. With the expression of both avian tumor virus receptor A (TVA) and rabies glycoprotein (RG) as a result of the expression of Cre, under control of the Arc promoter, targeted striatal cells acquired the necessary characteristics for rabies infection and monosynaptic retrograde mapping of projecting cells. With the final viral injection of (EnvA)-SAD-ΔG-GFP rabies virus into the striatum, cells with expression of RG and TVA membrane proteins were able to both uptake rabies virus and monosynaptically spread the rabies virus onto projecting cells. In this way, the mapping of neuronal ensembles representing the brain-wide projections of cocaine-activated cells onto cocaine-activated striatal cells was developed. The question of interest for the present study was to describe experience-specific activation of a unique corticostriatal subcircuit. A three pronged experimental plan was implemented: (1) genetically isolate cells activated by a strong cocaine experience; (2) assay anatomical
and physiological connectivity between activated neurons; (3) perturb network
connectivity by providing mice with repeated prior experience. All of this was done to
identify unique corticostriatal subcircuits activated by a specific experience.

Arc expression in response to acute cocaine administration was accomplished via
cocaine exposure. Previous research showed that rapid increases in \([\text{Ca}^{2+}]_i\) influx in D1
(D1 receptor) expressing striatal neurons (direct-pathway) and progressive decreases in
\([\text{Ca}^{2+}]_i\) influx in D2 (D2 receptor) expressing striatal neurons results from cocaine
administration (Z. Luo, Volkow, Heintz, Pan, & Du, 2011). Cocaine’s rewarding effects
result from both fast activation of D1 expressing direct-pathway neurons and slow
stimulation of D2 expressing indirect-pathway neurons (inhibition of indirect-pathway
neurons).

In the present study, transgenic mice with CreER\(^{T2}\) knocked into the \(Arc\) locus of
striatal MSNs were used to target striatal MSNs activated by a strong cocaine experience.
With the administration of a high dose (30 mg/kg) of cocaine in conjunction with 4-
Hydroxytamoxifen (4-OHT), Cre expression was activated, and translocation of Cre
recombinase back into the nucleus occurred. In this way, Cre recombination was
temporally restricted due to its 4-OHT dependence. Specifically, only when AAV
infection of the striatum was followed by an intra-peritoneal injection of 30 mg/kg
cocaine to activate expression of Cre-recombinase, under the control of the Arc promoter,
and a 50 mg/kg intra-peritoneal injection of 4-OHT, leading to nuclear localization of Cre
recombinase, can TVA and RG be expressed on the cell membrane of cocaine-activated
striatal cells. The present study also used an Ai14 Cre-dependent tdTomato reporter line to assay global Cre expression patterns, making a brain-wide map representing cocaine-activated neurons (Figure 4).

Figure 4: Global Cre expression pattern assay via Ai14 Cre-dependent tdTomato reporter line. (A) Chronic cocaine activation of Cre recombinase without 4-OHT. (B) Acute cocaine and TM driven Cre Recombinase expression of striatal and corticostriatal-projecting cells resulting in tdTomato expression. TM binding to Cre Recombinase allows for Cre/TM complex to translocate back into the nucleus. Figure modified from Luo et al., 2013.
**Basal ganglia anatomy and functionality**

The striatum is an important part of the circuits in the brain that control motivated behaviors. Classically, cognitive control of executive function has been the realm of the prefrontal cortex ((PS, 1995), (Fuster, 2000)). Literature has built on this classical belief suggesting that executive function relies on reward-related circuitry that links prefrontal, premotor, and sensorimotor cortices with the dorsal striatum ((Dobrossy, Svendsen, & Dunnett, 1996),(Kimura, 1995), (White, 1997)). Interestingly, studies have shown the existence of reciprocal feedback-loop between the frontal cortex and basal ganglia, suggesting possible anatomical explanation for the involvement of the basal ganglia in executive functions ((Berendse, Graaf, & Groenewegen, 1992), (Middleton & Strick, 2002),(Sesack & Pickel, 1992)). Two important afferent projecting structures of the striatum are the cortex and substantia nigra. The cerebral cortex projects to the striatum, and both ipsilateral and contralateral portions of the cortex contact dendritic spines of MSNs via glutamatergic projections (Lanciego, Luquin, & Obeso, 2012). The nigrostriatal pathway is a major source of dopamine to the striatum. The major projections of the directly pathway are the internal globus pallidus (GPi), and the substantia nigra pars reticulata (SNr), while the indirect pathway major projections run to the external globus pallidus (GPe) (Figure 5).
Figure 5: Diagram of the closed loop motor circuit. A simplified schematic of the basal ganglia model. Dashed lines represent the direct pathway and solid lines represent the indirect pathway. See key for neurotransmitter of connections. Substantia Nigra (SNr), Globus pallidus internal (GPi), Globus pallidus external (GPe), Subthalamic Nucleus (Subthalamic N.).
The dorsal striatum is a structure, specifically a nucleus, of the basal ganglia containing two unique classes of inhibitory GABAergic medium spiny neurons (MSNs). Information flows through the basal ganglia back to the cortex through two pathways that have opposing consequences on executive function, specifically voluntary, motivated movement. These two pathways, while both inhibitory MSNs, differ in projection target. The direct pathway contains MSNs that express D1 dopamine (DA) receptors and project directly to the substantia nigra pars reticulata (SNr), a major output nuclei of the basal ganglia to the motor thalamus. Classically, the direct pathway was viewed to play the “go” role, facilitating movement; a second class of MSNs, the indirect projection neurons expressing D2 receptors, form a circuit that indirectly projects to the SNr. Studies on hyperkinetic and hypokinetic movement disorders helped to elucidate the important role of striatal projection neurons in the initiation of movement (Albin, Young, & Penney, 1989). Conversely, the indirect pathway was seen as part of a “no go” pathway suppressing undesired movement. Importantly, SNc neurons project to both direct and indirect MSNs. These dopaminergic inputs from the SNc to the striatum are facilitory towards direct pathway MSNs, and act in an inhibitory fashion on D2 indirect MSNs. These two seemingly dichotomous impacts of SNc neuron dopamine release on direct and indirect MSNs are theorized to be a model for the involvement of indirect and direction pathways in movement.
The Arc Promoter

Since its 1995 discovery, numerous studies have explored and spelled out the role of Arc as an indicator of neuronal activity and the mechanisms by which Arc expression couples changes in neuronal activation to synaptic plasticity. Ultimately, because of the link found between the expression of Arc and its role in synaptic plasticity as a result of changing patterns of neuronal activation, it is a molecule of unique interest for its potential role in the consolidation of “cellular memory”. This “cellular memory” results from stimuli induced neuronal activity, leading to Arc transcription and ultimately protein synthesis-dependent local gene expression.

The molecular signaling pathways associated with activation of Arc transcription and the molecular machinery necessary for Arc’s translocation to synaptic spines has been elucidated (Figure 6). Of particular interest for the present study was the use of the Arc promoter to couple neuronal activity to the expression of the recombinase gene Cre. To achieve this, Knock-in Arc-Trap mice in a C57 background mice were bred. In this way, genetic/neuronal activity dependent Cre expression was achieved.

The Arc promoter contains several key response elements that contribute to its activity dependent transcriptional regulation. A 2009 study identified two key enhancer elements (Pintchovski, Peebles, Joo Kim, Verdin, & Finkbeiner, 2009). The first of these enhancer elements, approximately 6.5 kb upstream of the Arc transcription start site, included a highly conserved serum response element (SRE) to which a protein called a serum response factor (SRF) binds to, a result of synaptic activity. A second important enhancer
element was found at approximately 1.4 kb upstream of the Arc transcription start site, containing what is termed a “zest-like” enhancer element. Another important component of the Arc promoter was the synaptic activity-responsive element (SARE), a 100bp sequence located 7 kb upstream of the Arc transcription start site (Kawashima et al., 2008). This SARE was found to be both sufficient and necessary for the initiation of activity-dependent rapid Arc expression in hippocampal neurons (Kawashima et al., 2008). Figure 6 illustrates the multiple receptors, downstream transcription factors, enhancer elements, and ultimately protein complexes associated with activity-dependent Arc transcription and translocation to synaptic spines. An important function of Arc is its role in long-term synaptic plasticity. Arc knockout mice were found to have an increased early-phase long term potentiation LTP but a blocked long-phase LTP (Plath et al., 2006). A later study deciphered a novel molecular pathway regulated by Arc that likely contributes to late-phase synaptic plasticity (Chowdhury et al., 2006). Put together, the Arc system is a highly adaptive and specialized system where the intersection of memory consolidation and synaptic plasticity at a genetic level becomes reality, and Arc expression is a significant indicator for neural activity.
Figure 6: Regulation of Arc transcription, transport and translation. Synaptic activity leads to the induction of a signal cascade resulting in transcription factor activation, and ultimately Arc expression. The genomic composition of upstream regulatory elements of the Arc open reading frame such as the synaptic activity-response elements (SARE) are depicted. Post transcriptionally, with the aid of mRNA cis-regulatory elements, Arc mRNA is packaged for transport, and is transported to developing synaptic spines down microtubules. Docking of mRNA at synaptic spines is dependent on f-actin (not depicted), and following docking, synaptic translation occurs with rapid mRNA degradation within the spine. Figure adapted from Bramham et al. (Bramham et al., 2009).
**Viral Gene Therapy Systems**

Prior to the development of recombinase activated intracranial DNA-based recombinant adeno-associated viruses (rAAVs), retrograde anatomical tracers such as biotinylated dextran amine allowed for mapping of projections between brain regions; however, such tracing lacked cell specificity (Reiner et al., 2000). A relatively new technique that combines the use of Cre driven transgenic mice designed to influence specific cell types in unique brain regions in combination with an intracranial injection of rAAV transcriptionally activated by Cre recombinase allows for cell-type specific targeting of AAV expression. The present study took advantage of Cre-dependent AAVs to facilitate the targeted infection and retrograde spread of modified rabies virus.

**Adeno-associated viruses**

AAVs are single-stranded (ss) DNA viruses surrounded by an icosahedral capsid, and are a member of the *Parvoviridae* taxonomic family. Characteristic among the Parvovirus family are self-priming hairpin telomeres and ssDNA that replicate via a “rolling hairpin” mechanism (Cotmore & Tattersall, 2013). Through a myriad of cell surface glycan-mediated capsid interactions, AAV viruses infect host cells ((Murlidharan, Samulski, & Asokan, 2014), (Asokan, Schaffer, & Jude Samulski, 2012), (Van Vliet, Blouin, Brument, Agbandje-McKenna, & Snyder, 2008)). Differences in capsid-receptor interactions drive regional and cellular specificity of infection. The rAAV ssDNA can self-prime in the nucleus becoming dsDNA, or alternatively can integrate into the host...
genome in unstable and/or repair regions of DNA sequences ((Yan, Zak, Zhang, & Engelhardt, 2005), (Deyle & Russell, 2010), (Miller, Petek, & Russell, 2004), (Inagaki et al., 2007)). Intracranial injections of AAV vectors using stereotaxic coordinates allows for minimal damage to surrounding tissue and is relatively benign leading to stable and long-lasting expression of TVA and RG on targeted cells.

**Rabies Virus**

Wall et al., 2010 described a powerful system for the mapping of direct monosynaptic inputs to specific cell types in Cre-expressing transgenic mice (Wall, Wickersham, Cetin, La Parra, & Callaway, 2010). This study went on to use a Cre-dependent helper virus in conjunction with the injection of a modified rabies virus. The two-virus system described by Wall et al., 2010 defined a powerful system for revealing monosynaptic inputs of targeted cells in Cre-expressing mice. Only striatal cells expressing both TVA and RG membrane proteins were able to be infected by RV and monosynaptically retrogradely transport RV. Unlike standard anterograde and retrograde neuronal tracers that posses the ability to elucidate the region from which neurons projecting to or from, in vivo monosynaptic circuit tracing resulting from modified RV and Cre-dependent targeting gives researchers the ability to deliver genes of interest in a spatially controlled manner, and to temporally regulate protein synthesis of the gene of interest.
The specific RV injected was an (EnvA)SAD-ΔG-GFP modified virus. Importantly, this modified RV lacked the membrane glycoprotein necessary for retrograde monosynaptic transport. In this way, only cells expressing TVA and RG post AAV Cre dependent helper virus injection possessed the ability to uptake (EnvA)SAD-ΔG-GFP RV and monosynaptically retrogradely spread RV. In Arc-Ai14 mice, only cocaine-activated cells express Cre recombinase. Cre recombinase expression results in recombination of Cre-dependent AAVs resulting in the expression of TVA and RG in activated cells. Upon modified RV injection, only those cells infected with AAV that express TVA are infected with the modified RV. From here, only activated cells expressing RG possess the capability to spread RV retrogradely to cells that project onto them. In this way, monosynaptic inputs to activated neurons can be mapped. In other terms, in order for the modified RV to be taken up by a cell and undergo only monosynaptic, retrograde transport, two membrane proteins are required, TVA and RG. Due to TRAPing of Cre$^+$ neurons, TVA and RG (AAV-DIO-TVA viral vector & AAV-DIO-RG viral vector) were only present on the membrane of cocaine-activated striatal cells, thus spatially limiting rabies virus infection to Cre$^+$ cocaine-activated striatal MSNs (Wickersham et al., 2007). This allowed for direct labeling of presynaptic neurons (Wall et al., 2010).

**Cre-Recombinase & Targeted Recombination in Active Populations (TRAP)**

The birth of recombinant DNA technology, molecular cloning, and the advent of transgenic mice ushered in a new era of genetic engineering. The field of neuroscience
takes advantage of these new technologies to characterize neural circuits, mapping neural pathways. The development of Cre-recombinase-based conditional expression methods allows for both single-neuron resolution and precise spatiotemporal regulation of gene expression (Van Deursen, Fornerod, Van Rees, & Grosveld, 1995). Subsequently, the development of numerous Cre (Causes Recombination) driver lines has greatly increased the number of neuronal populations susceptible to targeted gene regulation (Taniguchi et al., 2011).

Despite significant structural differences between prokaryotic and eukaryotic DNA, specific prokaryotic enzymes known as recombinases (such as Cre recombinase) act in vivo at specific eukaryotic DNA sequences ((Sauer, 1987), (Sauer & Henderson, 1988)). Of particular interest for the present study was the development of the Cre-Lox recombinase system. This powerful genetic tool results in site-specific DNA recombination that, under the control of specific promoters, allows for precise spatial regulation of Cre expression and ultimately the expression of genes of interest. This system allows for precise manipulation of transgenic mice and regulation of gene expression, leading to the activation or deactivation of the expression of specific DNA sequence.

To achieve cell specific targeting, a combination of genetic and viral strategies have been implemented. Binary expression systems such as the P1 bacteriophage Cre recombinase system along with its associated LoxP recognition sites allow for highly efficient and flexible gene expression. When under the control of the Arc promoter, the
Cre recombinase system allows for regulated expression of *LoxP* flanked genes in active neurons. By combining the arsenal of tools now at the disposal of neuroscientists including but not limited to transgenesis, recombination-based gene targeting, and the Cre-recombinase system, it is possible to generate nearly any mouse genome modification, achieving both spatial and temporal regulation of desired genes.

*Lox P* flox sites are known to be 34 bp consensus sequences of DNA consisting of one 8 bp non-palindromic core “spacer sequence” sandwiched between two 13 bp palindromic “flanking/recognition sequences”. The orientation of the *LoxP* site is determined by the orientation of the spacer sequence, and the orientation of Lox sites ultimately determines how Cre-recombinase impacts floxed DNA sequence. Importantly, regardless of cellular environment, no cofactors or additional sequence elements are required for efficient recombination. A past limitation was in the ability to develop Cre transgenic lines; however, this became an active area of research and has already made huge strides, making controlled expression of nearly any gene possible (Taniguchi et al., 2011). Cre recombinase, a 38kDa enzyme derived from the P1 bacteriophage, is used to catalyze the recombination between mutually exclusive *Lox* recognition sites on DNA. The result of such Cre dependent recombination depends on the orientation of *LoxP* sites.

*LoxP* recombination includes targeting of specific DNA sequences and the use of the enzyme Cre recombinase to splice a DNA sequence in a site-specific manner. It is widely accepted that Cre recombinase catalyzes the recombination between two 34 base-pair consensus base pair DNA recognition sites known as *loxP* sites. In the present study,
Cre-dependence resulted from a FLEX double-floxed system where Cre recombinase acted specifically on mutually exclusive sets of lox sites (loxP and Lox2272) (Figure 7). This recombination event of the floxed genes oriented them properly for transcription, and the insertion of a kozak sequence at the beginning of the gene cassette and the removal of all start codons from floxed genes ensured proper transcription initiation. By acting on mutually exclusive sets of lox sites and not across sites, Cre recombination lead to stably locked gene cassettes in the proper orientation for transcription. Cre recombinase allows for DNA recombination at lox sites, making possible the use of mice expressing Cre recombinase under the control the Arc promoter to drive selective gene expression.
Figure 7: LoxP flanked Cre dependent helper virus allows for rabies virus infection and monosynaptic retrograde tracing. (A) Cre dependent AAV helper virus vectors (Lerner et al., 2015). (B). Helper virus (AAV) infection of Cre+ striatal cells, expression of TVA and RG membrane proteins, and monosynaptic retrograde infection of rabies virus. Red cells represent monosynaptic inputs onto active Cre+ cells labeled as a result of rabies virus infection. B19G indicates membrane RG resulting from Cre+ TRAPed cells. Figure modified from Luo et al, 2013.
The genetic technique known as Targeted Recombination in Active Populations (TRAP) developed in the laboratory of Dr. Liqun Luo made possible the study of information segregation and behavior generation \textit{in vivo} (L. Luo, Guenthner, Miyamichi, Yang, & Heller, 2013). The TRAP approach allows for dissection of neural circuits by genetically hijacking access to neurons activated by defined stimuli. To achieve spatiotemporally precise activity dependent expression of tamoxifen-dependent recombinase CreER$^{T2}$, CreER$^{T2}$ was knocked into the endogenous \textit{Arc} loci. To visualize activity-dependent expression of CreER$^{T2}$, utilization of Ai14, Cre-dependent tdTomat knock-in allele of the \textit{Rosa26 (R26)} locus, allows for pervasive expression of the red fluorescent protein tdTomato due to the excision of a \textit{LoxP}-flanked stop sequence (Madisen et al., 2009). Without tamoxifen (TM) present, CreER$^{T2}$ is unable to translocate from the cytoplasm back into the nucleus, and no recombination of \textit{LoxP}-flanked sites occurs. This results in no excision of the early transcriptional stop sequence. The transient activation of IEG regulated CreER$^{T2}$ during neuronal activity and the limited lifetime of TM due to metabolism results ultimately in TRAPed cells. Only neurons activated within a very narrow time frame post TM administration and that contain the CreER$^{T2}$ transgene lead to Ai14 expression and ultimately expression of tdTomato, resulting in a red signal during examination under fluorescence microscopy.

4-OHT, the active metabolite of Tamoxifen, is an estrogen receptor antagonist called a Selective Estrogen Receptor Modulator (SERM). A critical parameter for inducible Cre-LoxP recombination is the verification that the 4-OHT dose used is
appropriate for the experiment to maximize the special range for recombination, but limit temporal duration of recombination. Importantly, exposure to stimulus needs to be time-locked to injection of 4-OHT (L. Luo et al., 2013). In this way, visualization of specific cells activated by a strong experience during a specific time frame was made possible.

In the 2013 paper by Wall et al., it was determined that D1 and D2 MSNs have unique input networks (Wall et al., 2013). In the present study, the question asked was whether it was possible to identify functional subcircuits that transmit unique types of information embedded within the overall corticostriatal circuit. We wanted to investigate at a brain wide scale whether cells that are co-activated by a single experience preferentially wire onto one another. The first model hypothesized that chronic cocaine administration would result in unique groups of corticostriatal cells projecting to unique target MSNs. Model two hypothesized that asymmetric information transmission is not due to parallel information pathways, but possible differences in synaptic strength, both chemically and anatomically, or changes in neural excitability. To assess the two above hypotheses, the described two-virus system was implemented (AAV + RV). This experimental design allowed for the determination of whether cocaine-activated cells in the cortex specifically projected onto co-activated striatal neurons, or if there was random connectivity of corticostriatal neurons onto activated MSNs in the striatum. Even more specifically, my study aimed to determine whether prior experience refined neural circuits involved in transmitting cocaine-relevant information. The question at hand was
whether repeated low dosages of cocaine, followed by a withdrawal period, lead to the refinement of wiring between coactive striatal neurons and their projections.
OBJECTIVES & LIMITATIONS

Recent decades have been witness to a revolution in the identification and mapping of neural circuits. New viral technologies and the Cre-recombinase system have made precise genetic regulation a reality. It has been suggested that information pathways into the striatum can be segregated, hinting at a mechanism of differential information transmission to two unique cell types (Wall et al., 2013). The goal of the present study was to decipher brain wide monosynaptic retrograde tracing of inputs onto cocaine-activated striatal MSNs.

A limitation of the present study was that Arc is not a perfect reporter for neuronal activity, but rather is a marker for IEG expression. In addition, because the present study was purely anatomical, no conclusions regarding changes in the physiology of the active corticostriatal circuit due to chronic cocaine exposure can be drawn. Lastly, from collected data, it is difficult to determine whether chronic cocaine administration reveals pre-existing circuitry or leads to the production of new circuitry.

The primary focus for the present study is one of information processing, and how changes in synaptic connectivity and function lead to circuit remodeling following powerful experiences. The specific task was to decipher how relevant information for a complex experience (e.g. cocaine exposure) is transmitted throughout the brain, received by appropriate cells in the striatum, ultimately leading to behavior modification and overall change in phenotype. More precisely, after repeated prior experience with cocaine, does the brain rewire to form a refined circuit representing chronic cocaine
exposure, resulting in a change in proportion of active cells communicating with active cells. This study aimed to find parallel information pathways using the same overall circuitry, but that convey experience-specific information.

Experimental Design

(1) Previously developed virus injection protocols designed for precise spatiotemporal regulation of Cre-recombinase activity were used.

(2) Took advantage of the unique properties of Cre-dependent AAV and modified RV ((EnvA)SAD-ΔG-GFP) viruses to trace monosynaptic retrograde projections onto cocaine-activated MSNs.

(3) Provided mice with repeated prior cocaine experience, resulting in a refined circuit representation of cocaine that is then TRAPed to map out the refined circuit.

(4) Compare the refined circuit map of cocaine-activated corticostriatal pathway resulting from chronic cocaine administration to that of acute administration. This potentially induces brain wide plasticity that is detectable using the present experiment timeline of events.
METHODS

Figure 8: Experimental timeline.

Chronic Cocaine Habitation

Arc-Trap mice (tamoxifen-dependent recombinase CreER\textsuperscript{T2} knocked into the endogenous IEG Arc locus) were crossed with Ai14 Cre-dependent tdTomato reporter mice (hereafter referred to as Arc/Ai14 mice). At 5-6 weeks of age, Arc/Ai14 mice were
exposed to 10 mg/kg cocaine for one week. Control mice were injected with saline. Intra-peritoneal injections of 2 mg/ml cocaine or saline (control) were performed at 0.1 ml of cocaine per gram of mouse (10 mg/kg). Mice were age-and sex-matched Arc/Ai14 mice. Following one week of chronic cocaine/saline administration, injection was withdrawn.

**Arc-Ai14 TRAPed Mice**

One week post chronic cocaine administration, AAV virus injections were conducted (Figure 8). Three days post AAV injection, intra-peritoneal injections of 0.1 mL of saline for four days were performed on Arc/Ai14 mice. One-week post AAV injection, an acute intra-peritoneal injection of 50 mg/kg 4-OHT partnered with a strong intra-peritoneal injection of 30 mg/kg cocaine were performed, TRAPing Arc/Ai14 mice, resulting in nuclear localization of Cre-Recombinase and expression of TVA, RG, and tdTomato in cells active during the strong 30 mg/kg cocaine experience.

**Virus Injection Protocol & Mouse Surgeries**

All live animal experiments were performed in compliance with the protocols of both the Administrative Panel on Laboratory Animal Care (APLAC) and the Institutional Animal Care and Use Committee (IACUC) at Stanford Medical Center. All statistical analyses were performed using GraphPad Prism 6.0 software, with statistical significance assumed at a $P$ value less than 0.05.
**AAV Production**

AAV helper viruses containing Cre-dependent plasmids using a FLEX TVA and RG double-floxed gene cassette were generated at Stanford Medical Center following a previously determined protocol (Grieger, Choi, & Samulski, 2006). Both the pAAV-CAG-FLEX-TVA vector and pAAV-eF1α-DIO-B19G vectors were contained within Cre-dependent AAVs. Genomic titers were approximately $3.3 \times 10^{12}$ colony forming units (cfu)/mL for AAV serotype 2 (pAAV-CAG-FLEX-TVA).

**Rabies Production**

EnvA pseudotyped glycoprotein deficient rabies virus was generated as previously described ((Wickersham, Sullivan, & Seung, 2010)) by Dr. Beier. (EnvA)SAD-ΔG-GFP has a functional titer of $5.0 \times 10^8$ colony forming units (cfu)/mL (Beier et al., 2015).

**AAV & RV Surgery and Viral Injection Parameters**

Arc/Ai14 mice were maintained in a C57 background. These mice were selected for experiments at five to six weeks of age. Mice received 0.5µl monohemispheric injections of AAVs expressing Cre-dependent TVA and RG at the following coordinates: 2.0 mm lateral, 0.6 mm rostral, and 3.5 mm ventral. Three days post AAV injection, a four day habituation of 0.1ml saline was followed by an intra-peritoneal injection of 4-Hydroxytamoxifen (4-OHT) and 30 mg/kg of cocaine to TRAP striatal cells activated by
acute cocaine administration. Three weeks post TRAPing, mice received 0.5µl monohemispheric injections of the modified rabies virus (EnvA)SAD-ΔG-GFP at the following coordinates at a 15° angle medial to lateral: 1.02 mm lateral, 0.6 mm rostral, and 3.89 mm ventral. Please see Figure 8 for timeline of events.

**Tissue Processing**

To preserve brain tissue for later imaging and analysis, intracardial perfusions at 50 mL of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2), or formalin, were performed. Post perfusion, mouse brains were isolated and kept in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2), or formalin and incubated overnight at 4°C. One day post fixation, tissue was transferred to PBS and kept at 4°C until further processing was needed. 50 micron sagittal sections were prepared using a microtome and separated into three separate groups to allow for multiple tissue manipulations. Two groups that did not undergo analysis shortly after sections were prepared and placed into a PBS solution, and stored at 4°C until further manipulations were performed. One group of sections were mounted and stained with a DAPI-containing mounting medium. Three days post DAPI staining, mounted sections were imaged using an Olympus Fluorescence Whole-slide scanning microscope.
RESULTS

To determine the impact of chronic cocaine exposure on corticostriatal circuit refinement, Arc/Ai14 mice had both AAV and RV injected within the striatum, eventually resulting in the ability to identify monosynaptic inputs from cortical projecting neurons onto cocaine-activated striatal neurons. One of five potential outcomes of the above experiment were expected: (1) Formation of a refined circuit that contains a higher probability of co-activated cells wiring together as compared to previous studies due to a pruning away of non-activate corticostriatal inputs; (2) Non-activated cortical neurons wire onto a subpopulation of striatal neurons that are no longer activated following chronic cocaine exposure resulting in a high proportion of co-connectivity of activated cells; (3) In refined corticostriatal circuits, cocaine habituation leads to an overall reduction in the proportion of active inputs onto cocaine-activated striatal cells. While the total number of cortical inputs onto active-striatal cells remains constant, due to chronic cocaine administration, previously active cortical inputs become habituated and are no longer “active”, and therefore will not be tagged as a co-active inputs onto activate striatal cells. Thus, there is a decrease in the proportion of active inputs onto cocaine-activated striatal cells; (4) A completely different corticostriatal circuit occurs as a result of both an increase in inputs relaying pertinent signals and reduction of inputs carrying unwanted information; (5) Chronic cocaine exposure does not lead to any significant
brain wide changes in connections between active neurons as compared to acute cocaine exposure.

To parse out which of the five outcomes to the corticostriatal circuit occurred as a result of chronic cocaine administration, a two-virus system in combination with a Cre-expressing mouse line was utilized to target specific subtypes of projection neurons in the striatum and label their unique monosynaptic inputs (Wall et al., 2010). Arc/Ai14 mice were injected with 500 nl of helper virus. One week later, 50 mg/kg of 4-OHT + a single challenge of 30 mg/kg of cocaine was injected into the mice, resulting in nuclear localization of Cre recombinase, and membrane expression of TVA and RG in striatal cells activated by cocaine (Appendix 1A, Figure 1A). Three weeks following 4-OHT and single challenge of cocaine administration, mice were injected with 500 nl of modified RV at the same location as the helper virus (Appendix 1B, Figure 1B). One week between RV injections and tissue processing was needed to allow for replication and monosynaptic spread of RV (Appendix 1C, Figure 1C). Brain sections were mounted and scanned using a semiautomatic fluorescence slide scanner. RV GFP labeled somata were counted to determine retrogradely labeled cells in different brain regions.

It is necessary here to parse out the significant differences between the acute vs. chronic cocaine schemes, and how data interpretation was accomplished. In the acute state, only a single strong dose of 30 mg/kg of cocaine along with 50 mg/kg 4-OHT were injected post AAV infection and saline habituation, effectively TRAPing activity dependent striatal MSNs. Note–all experiments, data collection, and data analysis for the
acute study were performed by Dr. Nicholas Wall, and this data has yet to be published. Post TRAP, monosynaptic or retrograde rabies was injected to map corticostriatal circuit of acute cocaine exposure. Retrograde RV infection traces not only monosynaptic inputs on cocaine-activated striatal cells, but in addition is able to label many upstream neurons. This results because the rabies glycoprotein gene is not deleted from the retrograde RV genome. In comparison, in the chronic condition, cocaine was administered to Arc/Ai14 mice for one week. One week post chronic cocaine exposure, AAV intracranial injections were performed, after which saline habituation, TRAPing with 30 mg/kg of cocaine with 50 mg/kg 4-OHT, and lastly RV intracranial injections were performed.

Previous work determined that acute cocaine administration lead to preferential wiring of active cortical cells onto active striatal cells (Figure 9A, Table 2). Chronic cocaine habituation resulted in the preferential wiring of cocaine-activated corticostriatal neurons onto co-activated striatal cells (Figure 9B, Table 1). While retrograde tracers such as (RG)SAD-DG-GFP non-selectively label inputs onto the striatum, the use of the (EnvA)SAD-DG-GFP viral tracer made it possible to selectively label projections onto cocaine-activated striatal cells. By using a selective tracer paired with a one-week cocaine administration protocol, preliminary results of chronic cocaine exposure while statistically insignificant were found to have a trend towards preferential wiring of co-activated corticalstriatal cells. Significantly, it was determined that chronic cocaine exposure resulted in an increase in proportion of activated prefrontal and medial cortical neurons wiring onto co-activated Arc/Ai14 striatal cells as compared to control samples
(Figure 9B). Note—in the chronic cocaine trials, control samples (referred to as C\textsubscript{c} controls) followed exactly the same protocol for monosynaptic input tracing of cortical cells onto active striatal cells, with the exception that during the initial week of habituation, saline was injected into mice and not cocaine. In the acute cocaine trials, controls (referred to as C\textsubscript{A}) refer to mice that received saline in place the single 30 mg/kg cocaine injection post AAV infection. Therefore, data suggests that chronic cocaine pre-exposure lead to corticostriatal circuit refinement. Specifically, neurons within the pre-frontal cortex (PFC) and medial cortices had a higher probability of connecting to co-active striatal cells (Figure 9C, D).

A specific brain region of interest for comparison between corticostriatal circuits resulting from either acute or chronic cocaine exposure was the orbitofrontal cortex (Figure 10). Specifically, the proportion of cocaine-activated orbitofrontal inputs onto cocaine-activated striatal MSNs for chronic cocaine exposure in three mice were 37.9\%, 52.2\%, and 50.0\% with a mean of 46.7\%. These same numbers in three acute cocaine mice were found to be 29.2\%, 32.9\%, and 28.6\% with a mean of 30.2\%. After performing a one-way ANOVA comparing the means of acute cocaine (TRAP), chronic cocaine and acute control for orbitofrontal projections onto active striatal cells, a $P$ value of 0.0012 was calculated, indicating a statistically significant difference between means (Table 2). These collections of data suggest differential corticostriatal circuitry as a result of acute vs. chronic cocaine administration, and show an increased proportion of active
critical inputs from three active brain regions, the PFC, OFC, and medial cortex onto active striatal cells (Figure 9, Figure 10, Table 1, Table 2).

Figure 9: Proportion of active cortical inputs onto active striatal cells in Arc-Ai14 mice. (A) Proportion of active cortical inputs onto active striatal inputs in acute cocaine exposed mice vs. control (C_A). (B) Proportion of active cortical inputs onto active striatal inputs in chronic cocaine exposed mice vs. C_C controls. (C) Proportion of active cortical inputs onto active striatal inputs in chronic cocaine and chronic control (C_C) (saline) exposed mice. (D) Comparison of the proportion of active cortical inputs onto active striatal inputs in acute and chronic cocaine exposed mice by brain region. All experiments conducted and data collection for (A) were performed by Dr. Nicholas Wall, and this data is yet to be published.
Figure 10: Proportion of active orbitofrontal inputs onto active striatal cells. Represented by red dots, TRAP indicates the condition where a single acute dose of cocaine was administered post AAV injection. Represented by teal dots, chronic indicates the condition where chronic cocaine was followed by an acute dose of cocaine to Trap active striatal cells. Lastly, the control condition (CA) (dark blue dots) is the same condition as TRAP, however saline is injected in place of cocaine.

| Table 1. | Results from two-way ANOVA studying chronic cocaine vs. control CA. The impact of exposure type (chronic vs. CA) on the proportion of active cortical inputs onto active striatal cells. |
|---|---|---|---|
| Statistical Quantity | Exposure Type | Brain Region | Interaction |
| Sum of Squares | 0.00096 | 0.051 | 0.046 |
| Degrees of Freedom (df) | 1.0 | 4.0 | 4.0 |
| Mean Squares | 0.00096 | 0.13 | 0.012 |
| F | 0.23 | 3.1 | 2.8 |
| p-value | 0.63 | 0.038 | 0.053 |

| Table 2. | Results from two-way ANOVAs studying chronic and acute cocaine exposure impact on corticostriatal circuitry. One-way ANOVA on TRAP, Chronic, and Control (CA) P value for proportion of active inputs from orbitofrontal cortex to cocaine-activated striatal cells. |
|---|---|---|---|
| Statistical Quantity | Chronic vs. Control | Chronic vs. Acute | TRAP, Chronic, and Control (CA) |
| $P$ value | Factor 1 $P$ value =0.63, Factor 2 $P$ value =0.038, Interaction $P$ value=0.053 | Factor 1 $P$ value=0.0687, Factor 2 $P$ value=0.0038, Interaction $P$ value 0.0008 | 0.0012 |
Table 2 reports results of two-way ANOVAs comparing continuous variables respectively across cocaine-exposure types (chronic vs. control (C\textsubscript{C}), and chronic vs acute) and brain regions (somatosensory, motor cortex, prefrontal cortex (PFC), retrosplenial/cingulate, other). Table 2 also reports results of a one-way ANOVA comparing continuous variables (proportion of projections from the active orbitofrontal regions) across exposure type (TRAP=acute, chronic, and acute control=saline). Factor 1, the column factor, for both chronic vs. C\textsubscript{C} and chronic vs. acute exposure types were not statistically insignificant (P values 0.63 & 0.687 respectively). This means that in each case, exposure type was not found to significantly impact the proportion of active cortical inputs. Factor 2 for both groups, the row factor, analyzed how brain region impacted the proportion of active cortical inputs onto co-active striatal cells. Both groups were found to be statistically significant (P value 0.038 & 0.0038 respectively), suggesting that different brain regions have statistically different proportions of active cortical inputs onto co-active striatal cells. Chronic vs. C\textsubscript{C} (saline) interaction P value (0.053) indicated that the difference between exposure types were consistent across brain regions. Lastly, chronic vs. acute interaction P value (0.0008) rejects the null hypothesis, indicating that the difference between exposure types (chronic vs. acute) were not consistent across brain regions; there was a difference in proportion of active cortical inputs onto co-active striatal cells between exposure types and brain region.
DISCUSSION

The present study demonstrated that chronic cocaine exposure in Arc/Ai14 mice followed by AAV helper virus intracranial injection, TRAPing of cocaine-activated striatal cells, and RV intracranial injection, resulted in changes of the proportion of cocaine-activated prefrontal cortex, medial cortex, and orbitofrontal inputs onto cocaine activated striatal cells (Figure 9B & C, Figure 10, Table 2). In a side-by-side comparison to previous data on active cortical inputs projecting onto active-striatal cells resulting from acute-cocaine exposure (data not yet published), chronic cocaine exposure showed significantly different proportions of active cortical (prefrontal and medial) inputs onto active striatal cells (Figure 9D, Table 2). These data put together suggest that pre-exposure to chronic cocaine in Arc-Ai14 mice lead to a detectable corticostriatal circuit refinement.

All statistical analysis for the present study was performed using GraphPad Prism 6.0 software, and statistical significance was assumed at a $P$ value less than 0.05. Two-way ANOVAs were used to compare continuous variables respectively across cocaine-exposure types and brain regions. Data indicated the presence of an interaction between cocaine-exposure time (acute vs. chronic) and brain region, with a calculated $P$ value of 0.0008 (Table 2). This result suggests that there was a statistically significant difference in the proportion of wiring between co-activated cortical and striatal cells in acute vs. chronic cocaine exposed mice. However, in the case where the exposure type of chronic cocaine vs. $C_C$ were analyzed using a two-way ANOVA, no interaction was found.
between brain region and exposure type, with a calculated $P$ value of 0.053 (Table 1, Table 2). A plausible explanation for a lack of statistical significance for the previous analysis may be attributable to the low power of the present study.

A potential extrapolation of the present study may be that by mapping out with single neuron resolution how information is transferred from throughout the brain to targeted brain regions of interest in mice as a result of a chronic strong experience, we may elucidate biological underpinnings of the activation patterns of people studied using functional MRI. It is important to realize that complex neural networks are distributed throughout the human brain, and do not work in isolated brain regions, but rather allow for information transfer between brain regions. Functional connectivity networks are defined as the co-activation of scattered brain regions to perform specific complex brain functions. By preliminarily concluding that pre-exposure to chronic cocaine resulted in detectable circuit refinement of the corticostriatal circuit, the present study may validate previous studies using fMRI testing “functional connectivity” within the human brain, elucidating possible biological mechanisms by which “functional connectivity” in the human brain occurs.

While the present study provides a model to begin to understand how complex experiences result in circuit refinement in the brain, details regarding the specificity of changes still remain a puzzle. Data showed a statistically significant difference in the proportion of active cortical inputs from the frontal and medial cortices onto active striatal cells as a result of chronic cocaine exposure (Figure 9C). However, these data do
not allow for interpreting the exact mechanism by which this difference in proportion of active cortical inputs from the frontal and medial cortices occurs. To determine if it is the result of inactive cortical inputs from frontal and medial cortices being specifically pruned away, leaving a refined population of striatal neurons with different connectivity, we can in the future, first inject AAV helper virus, then TRAP CreER<sup>T2</sup> mice with an acute dose of 30 mg/kg of cocaine and 50 mg/kg of 4-OHT, followed by a one week chronic cocaine exposure, and lastly inject RV. This would allow us to specifically tag neurons that were naively activated by cocaine, and trace the cortical circuit resulting from chronic cocaine administration, probing corticostriatal connectivity following circuit refinement.

Another question left unanswered is whether chronic cocaine pre-exposure modifies in any way the gross connectivity (active and non-active projections) patterns between the cortex and the striatum. A future study tackling this question could take advantage of a retrograde rabies virus injected in the same location as the modified rabies virus. Following the same protocol as the present study, with the only modification being the injection of retrograde rabies virus, gross connectivity patterns between the cortex and striatum could be elucidated.

Lastly, investigation into the mechanism by which information is asymmetrically transferred by cocaine-activated cortical neurons onto cocaine-activated striatal cells. Physiological and behavioral experiments would be of particular interest to parse out asymmetrical information transfer between co-activated cortical and striatal cells.
Figure 11: Experimental sequence from AAV infection through to monosynaptic rabies spread. Blue shaded striatal neurons in A3 represent AAV infected striatal cells. Pink labeled striatal and cortical neurons in B1 represent activated neurons as a result of 50 mg/kg 4-OHT + acute 30 mg/kg cocaine. Dark red cells in B2 represent TRAPed striatal cells expressing TVA and RG on cell membrane. Monosynaptic rabies specifically infects TRAPed striatal neurons in C1. Retrograde monosynaptic spread of rabies onto cocaine-activated cortical neurons in C2,3. Figure adapted from Wall et al., 2013, and a 2015 power point presentation by Dr. Nicholas Wall.
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Research Experiences

1. Graduate research assistant | Laboratory of Prof. Robert Malenka (July 2015-May 2016)
   The Stem Cell Biology and Regenerative Medicine Institute | Stanford University; Stanford, CA
   - Using a combination of advanced genetic, viral and physiological tools to examine the shared wiring between groups of neurons displaying coordinated hyperactivity
2. Undergraduate research assistant | Laboratory of Prof. Robert Langer (January–August, 2012)
*The Koch Institute for Integrative Cancer Research | Massachusetts Institute of Technology; Cambridge, MA*
  - Developed electrically conductive polymers to increase peripheral nerve growth

3. Ruhlman Conference Presenter (Spring 2013)
*Wellesley College: Wellesley, MA*
  - Presented MIT-based research titled “Investigating electrical stimulation, using conducting polymeric materials, for Peripheral Nerve Regeneration”

4. Research Assistant | Laboratory of Professor Catherine Olsen
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  - Genetic profiles to aid in the diagnosis of Multiple Sclerosis via qPCR and Gel Electrophoresis

Activities

Barton Road Tutoring Program (Spring 2014)

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NCAA Student-Athlete Advisory Committee (SAAC) Member (2011–2012)

Honors and Awards

Academic All-Conference, Women’s Soccer, NEWMAC Conference (2011, 2012, 2013)

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