2019

MEASURING GLUTAMATE AND OXYGEN IN BRAIN REWARD CIRCUITS IN ANIMAL MODELS OF COCAINE ABUSE AND DECISION-MAKING

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Digital Object Identifier: https://doi.org/10.13023/etd.2019.335

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DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Arts & Sciences at the
University of Kentucky

By

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2019

ABSTRACT OF DISSERTATION

MEASURING GLUTAMATE AND OXYGEN IN BRAIN REWARD CIRCUITS IN ANIMAL MODELS OF COCAINE ABUSE AND DECISION-MAKING

Drug-specific reward and associated effects on neural signaling are often studied between subjects, where one group self-administers drug and a separate group self-administers a natural reinforcer. However, exposure to drugs of abuse can cause long-term neural adaptations that can affect how an organism responds to drug reward, natural reward, and their reward-associated stimuli. Thus, to isolate drug-specific effects it is important to use models that expose the same organism to all of the aforementioned. Multiple schedules provide a means of dissociating the rewarding effects of a drug from the rewarding effects of food within a single animal. Further, drug users do not take drugs in isolation; rather, they are often faced with several concurrently available commodities (e.g. monetary goods, social relationships). Thus, using choice measures to assess the relative subjective value of drug reinforcers in both humans and animals promotes a translational understanding of mechanisms that govern drug-associated decision-making. Thus, in order to gain a more translational view of the neurobehavioral mechanisms that underlie drug-associated behavior, in the first study, glutamate was measured in the nucleus accumbens core (NAcC) and prefrontal cortex (PrL) in freely-moving rats as they behaved in a cocaine-food multiple schedule procedure. In the second study, oxygen dynamics were measured in the orbitofrontal cortex (OFC) of freely-moving rats as they behaved in a cocaine/food choice procedure. The results from the first study showed that, in the NAc and PrL, there was an increase in glutamate release when animals earned cocaine. Further, the number of glutamate peaks that occurred per cocaine lever press and per cocaine reinforcer was increased compared to food. In the second study, OFC oxygen dynamics were positively correlated with cocaine/food choice and generally tracked preference. Further, OFC oxygen dynamics were greater to cocaine related events. Taken together, these results showed the feasibility of combining electrochemical measurements with complex drug-related behavioral procedures. These results also highlight the importance of the PrL, NAcC, and OFC in the valuation of drug and non-drug commodities. Overall, these results add to our understanding of the neurobehavioral mechanisms that guide drug-associated behavior and create more precise experimental avenues to research potential treatments.

KEYWORDS: Cocaine, Glutamate, Orbitofrontal Cortex, Nucleus Accumbens, Oxygen, Prelimbic Cortex

Seth R. Batten

July 16, 2019

MEASURING GLUTAMATE AND OXYGEN IN BRAIN REWARD CIRCUITS IN ANIMAL MODELS OF COCAINE ABUSE AND DECISION-MAKING

By

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July 16, 2019

Dedicated to my Mother & Father

You two have given me every opportunity possible to succeed.
I could not have done this without your unwavering support.
Hopefully, one day I can repay you.

It starts now-ish.

(Mom: beach house, maybe?)

ACKNOWLEDGEMENTS

In 1990 Carl Sagan instructed NASA to turn *Voyager 1* around and take a picture of earth from 3.7 billon miles away. What you see above is the resulting picture known as the "Pale Blue Dot". That arrow points to earth– here nothing more than a pixel. I find this to be one of the most beautiful pictures I have ever seen. I am not alone in that feeling– Carl Sagan thought this was a beautiful picture as well. Here are a few words he had to say when he saw it:

> "Consider again that dot. That's here. That's home. That's us. On it, everyone you love, everyone you know, everyone you've ever heard of, every human being who ever was lived out their lives. The aggregate of all our joys and sufferings; thousands of confident religions, ideologies and economic doctrines; every hunter and forager; every hero and coward; every creator and destroyer of civilizations; every king and peasant, every young couple in love; every mother and father; hopeful child; inventor and explorer; every teacher of morals; every corrupt politician; every supreme leader; every superstar; every saint and sinner in the history of our species, lived there— on a mote of dust suspended in a sunbeam."

Science is for humanity. However, being a scientist can be a dehumanizing experience. This is no fault of any one individual. That is just the way it is. I have personally been so caught up in the minutia of my 'own science' that I have often forgotten why I am doing it in the first place. I forgot about the purpose. Our

purpose as scientist (as I see it) is to do the best we can to figure out how the "Pale Blue Dot" works. Specifically, our job as behavioral scientists and neuroscientists is to figure out how the people that live on the "Pale Blue Dot" work. I have looked at the above picture to remind me of this purpose when I feel I have lost my way. In hard times, the above picture has kept me going, as have all of you.

To my family: through fostering my education and dreams you allowed me to be a "hopeful child". Without your support I would have never had the opportunity to write the first word on this page.

To my committee: thank you for taking the time out of your busy schedules to shape my educational experience– your guidance helped greatly with these experiments.

Greg Gerhardt: if you had not given me my first lab job so many years ago I may have never taken this path (so, maybe I should be cursing your name?). Thank you for putting faith in a kid from Eastern Kentucky and for sticking with me.

Josh Lavy, Jonathan Chow, and Aaron Smith: you all made this bearable and you made it fun (most of the time). I will miss our time together but know that it has changed me forever.

Josh Beckmann: one could not have asked for a more dedicated friend and mentor. You have given me the one thing no one can ever take from me: you taught me how to think.

To all of you: thank you for affording me the opportunity to be an "inventor and explore".

To my fiancé, Quinn Adams: it takes a special person to put up with someone as eccentric and neurotic as me. Thank you for sticking it out, for supporting me, and for being patient through this process. You have made my life on the "Pale Blue Dot" richer and more incredible than I could have ever imagined. Carl Sagan would be happy to see another "young couple in love".

Lastly,

Beckmann: you have always told me that, no matter what, to own what you do.

I hope I owned it.

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CHAPTER 1

INTRODUCTION TO GLUTAMATE DYNAMICS IN COCAINE SUBSTANCE-USE DISORDER

Cocaine Substance-use Disorder: General Overview

Substance-use disorders are defined as chronically relapsing disorders, characterized by compulsion to seek and take the drug, a loss of control in limiting intake, and the presentation of a negative emotional state when access to the drug is prevented (American Psychiatric Association, 2013). Substance-use disorders involving cocaine are a major issue in the United States with approximately 913,000 Americans affected (NSDUH, 2015). Cocaine misuse or abuse also accounts for 40% of drug-related emergency room visits with a 52.4% increase in cocaine overdoses between 2015-2016 (CBHSQ, 2013; MMWR, 2018). Thus, understanding the neurobehavioral mechanisms that lead to cocaine use and abuse are of paramount importance considering current trends.

The reinforcing properties of cocaine are mostly due to its mechanism of action on dopamine neurons in the nucleus accumbens (NAc; for review see Kuhar et al., 1991). Specifically, cocaine functions as an indirect agonist of dopamine receptors by inhibiting the dopamine transporter (DAT) and causing an increase in extracellular dopamine levels in the NAc (Kuzcenski, 1983; Kalivas & Duffy, 1990). This fact initially spurred the production of a large body of research studying cocaine abuse through the lens of dopamine signaling from the ventral tagmental area (VTA) to the NAc (see Nestler, 2004 for review). However, in the last 10-20 years research has shown that many different brain regions and neurotransmitter systems are important in the acquisition, maintenance, and relapse seen in those with substance-use disorder (for review see Koob & Volkow, 2016).

A dysfunction in a number of brain regions are suggested to be involved in the etiology of cocaine-use disorder beyond those found in the VTA and NAc, which are primarily involved in the rewarding properties of the drug and the

conditioning of drug cues (Shultz et al., 1997; Volkow et al., 2003). Some of these other brain regions include areas of the prefrontal cortex (PFC) such as the prelimbic (PrL), infralimbic (IL), and orbitofrontal (OFC) cortices (Jentsch & Taylor, 1999; Hutcheson & Everitt, 2003) as well as areas such as the dorsal striatum and amygdala (Whitelaw et al., 1996; Belin et al., 2009). For example, research suggests that cocaine related dysfunctions in areas of the prefrontal cortex might promote compulsive drug seeking and drug use (Hester & Graven, 2004). Further, the dorsal striatum is primarily involved in 'habit-like' formations that occur with repeated drug exposure (Faure et al., 2005) while the amygdala is thought to be primarily involved in the negative states experienced during drug withdrawal (Koob et al., 2014). Further, within these brain regions, dysregulated neurotransmitter systems are suggested to be driving the maladaptive behavioral patterns observed in those with addiction issues (Koob & Volkow, 2016).

As mentioned above, dysregulated dopamine signaling is observed in those with cocaine-use disorder. Specifically, data suggest that drugs of abuse cause an increase in dopamine levels in the ventral striatum (Kuhar et al., 1991) and that the steep increase in dopamine levels, along with the binding of dopamine to D_1 receptors, is responsible for the rewarding properties of drugs and the subjective feeling of the 'high' (Volkow et al., 2003; Caine et al., 2007). However, other neurotransmitters such as opioid peptides, glutamate, γ-aminobutyric acid (GABA), serotonin, acetylcholine, and endocannabinoids also seem to be dysregulated in substance-use disorder (Dani & Heinemann, 1996; Kalivas, 2009; Sidhpura & Parsons, 2011; Mitchell et al., 2012; Cunningham & Anastasio, 2014; Vashchinkina et al., 2014). How dysregulations in these neurotransmitter systems contribute to substance abuse is not yet completely clear; however, there is evidence that they may all have a role in drug seeking, drug taking, and relapse (Koob & Volkow, 2016). Of these aforementioned neurotransmitters, much research has focused on glutamate in the past 10-20 years, especially with regard to the specific role of glutamate in the reinstatement of drug seeking (Kalivas & Volkow, 2011).

Research suggests that the infusion of an AMPA glutamate receptor antagonist into the nucleus accumbens core (NAcC) prevents cocaine-primed reinstatement whereas the infusion of a D_1/D_2 dopamine receptor antagonist fails to do so (Cornish et al., 1999; McFarland & Kalivas, 2001). Further, cocaine-primed reinstatement causes an increase in dopamine release in animals that previously self-administered cocaine as well as in yoked-cocaine and yoked-saline controls; however, an increase in glutamate release and drug-seeking behavior is only observed in animals that previously self-administered cocaine (McFarland et al., 2003). These data suggest that, while dopamine signaling is important in abuse behavior (as are other neurotransmitters), glutamate signaling may be more involved in the long-term plastic changes that promote drug seeking and drug taking.

In the first part of this dissertation the role of glutamate in cocaine abuse is explored. The glutamate system in general will be discussed first, including glutamate-dopamine interactions, followed by the relationship between glutamate and cocaine-use disorder. The important brain regions involved will be considered, as will the animal models that contributed to the discussed results.

Glutamate Dynamics Under Baseline Physiological Conditions

Glutamate is the major excitatory neurotransmitter in the mammalian brain and is necessary for proper cognitive functioning and memory formation (Ozawa et al., 1988; Platt, 2007). It is thought that glutamate promotes memory formation and synaptic plasticity through long-term potentiation (LTP) and long-term depression (LTD) (Lüscher & Malenka, 2012). Here the glutamate system is discussed with regard to how it functions under 'normal', non-diseased conditions.

Synthesis and Release

Glutamate is synthesized from either glutamine via the enzyme glutaminase or from α-ketoglutarate (made from the Krebs Cycle) via a transamination reaction (Anderson & Swanson, 2000; Daikhin & Yudkoff, 2000).

Glutamate is then taken up by vesicular glutamate transporters (VGLUTs), in an energy dependent fashion, and packaged into vesicles (Fonnum et al., 1998). Once packaged into vesicles, glutamate is released into the synaptic cleft in a Ca^{2+}-dependent fashion (Turner, 1998; Meldrum, 2000). When glutamate is released it can either bind to pre and postsynaptic glutamate receptors, be actively taken up by glia and synthesized back into glutamine, be actively transported by presynaptic neurons and repackaged, or diffuse away from the synapse (Anderson & Swanson, 2000; Attwell, 2000; Daikhin & Yudkoff, 2000).

Transporters

Research suggests that there are five transporters present in the mammalian central nervous system (CNS) (Meldrum, 2000). Two of the five transporters (excitatory amino acid transporter 1 [EAAT1/GLAST] and EAAT2/GLT-1]) are found on glial cells and are responsible for 90% of glutamate uptake (Danbolt et al., 1998; Iverson et al., 2009). The remaining three transporters, EAAT3, EAAT4, and EAAT5, are found postsynaptically in cortical, cerebellar Purkinje, and retinal neurons, respectively (Danbolt et al., 1998; Iverson et al., 2009). All transporters are Na^+-dependent (Kataoka et al., 1997). As well as being Na^+-dependent, EAAT3-5 are also linked to Cl^- channels; thus, when glutamate binds there is a decrease in synaptic activity via hyperpolarization, which is thought to be a negative feedback system for glutamate release (Levy et al., 1998). Once glutamate is taken back up by glia or neurons it is metabolized and recycled as mentioned above. However, before glutamate leaves the synaptic space it is free to bind to a number of different receptor types.

Inotropic Receptors

The three classes of ionotropic glutamate receptors are *N*-methyl-*D*-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainic acid (Meldrum, 2000; Iverson et al., 2009). Each receptor was identified first by their pharmacology then later by their molecular biology (Meldrum, 2000; Tzschentke, 2002). Here most attention is given to NMDA and

AMPA receptors due to their role in cocaine induced synaptic plasticity (for review see Kalivas, 2009).

NMDA Receptor

The NMDA receptors (NMDARs) are postsynaptic, ligand and voltage-gated ion channels that are permeable to Na^+, K^+, and Ca^{2+} (Madden, 2002; Kew & Kemp, 2005). NMDARs are found in their highest concentration in the CA1 of the hippocampus, thalamus, and cerebral cortex (Riedel et al., 2003). The NMDAR has several modulatory binding sites: (a) glutamate binding site that binds the transmitter as well as related agonists, (b) glycine binding site (glycine is a necessary co-factor for glutamate activation of the NMDAR), (c) a site inside the receptor that binds phencyclidine (PCP) as well as other non-competitive antagonists, (d) a voltage-dependent Mg^{2+} binding site inside the channel, (e) an inhibitory site located near the mouth of the receptor that causes a voltage-independent block when Zn^{2+} is bound, (f) a polyamine site that enhances NMDAR activity when spermine and spermidine are bound at low concentrations but inhibit the receptor when they are bound at high concentrations (Anson et al., 1998; Kew et al., 2000; Madden, 2002; Mayer, 2005; Iverson et al., 2009).

NMDARs are composed of different subunits that house the different regulatory sites mentioned above (Anson et al., 1998; Kew et al., 2000). The two primary subunits that compose NMDARs are the NR1 subunit and the NR2 (A-D) subunit (Mori & Mishina, 1995; Meldrum, 2000; Kew & Kemp, 2005). There is also evidence that NR3A and NR3B subunits exists and that they function to decrease Ca^{2+} permeability; however, the exact physiology of these subunits is not well understood (Nishi et al., 2001; Matsuda et al., 2002; Sasaki et al., 2002). The glutamate-binding site is located on the NR2 subunit; thus, most functional NMDARs are heteromeric complexes mostly comprised of the NR1 subunit with NR2 subunits acting as the functional unit that affects channel kinetics and sensitivity (Mori & Mishina, 1995; Kew & Kimp, 2005). Note that the glycine-binding site is found on the NR1 subunit and thus it is also necessary for NMDAR activation (Lynch & Guttmann, 2001).

The various regulatory sites found on the NMDAR, as well as its voltage dependence, has prompted the appellation of the NMDARs as 'coincident receptors' (Nestler et al., 2009). The NMDAR opens after the voltage-dependent removal of the Mg^{2+} ion, binding of glycine to the NR1 subunit, and the binding of glutamate to the NR2 subunit (Nowak et al., 1994; Ozawa et al., 1998; Lynch & Guttmann, 2001). Once the channel opens, Na^{+} and Ca^{2+} can enter the neuron and there is an efflux of K^{+} (Riedel et al., 2003). Note that the NMDARs role as a 'coincident receptor' as well as its permeability to Ca^{2+} is thought to be the driving force in this receptors role in synaptic plasticity including the aberrant plasticity seen in those with cocaine-use disorder (Kalivas, 2009; Lüscher & Malenka, 2012).

AMPA Receptor

AMPA receptors (AMPARs) are found ubiquitously in the brain with the highest concentrations found in the CA1 and CA3 subregions of the hippocampus, the cerebral cortex, basal ganglia, thalamus, hypothalamus, cerebellum, and spinal cord (Blackstone et al., 1992). AMPARs are ligand-gated, postsynaptic ion channels with two glutamate binding sites (Dingledine et al., 1999). When glutamate binds to AMPARs there is an influx of Na^{+} and Ca^{2+} and an efflux of K^{+} (Forman et al., 2008). AMPARs have faster kinetics than NMDARs and are responsible for the initial component of excitatory postsynaptic potentials (EPSPs); however, they have a lower affinity for glutamate compared to NMDA receptors (Dingledine et al., 1999).

AMPARs are tetramers composed of four subunits (GluR1-4; Rosenmund et al., 1998). The majority of AMPARs contain the GluR2 subunit and can only pass Na^{+} and K^{+} ions; however, the AMPARs that lack the GluR2 subunit are able to pass Ca^{2+} (Bowie & Mayer, 1995). AMPARs can also exist in different 'Flip or Flop' splice variants that influence the rate of desensitization and the efficacy of certain allosteric modulators (Kew & Kemp, 2005). Further, evidence suggests that Ca^{2+} entering through GluR2-lacking AMPARs may promote the migration of GluR2-containing subunits to the neuronal membrane (Liu & Cull-Candy, 2002). These results suggest not only another level of synaptic plasticity

but also a glutamate/AMPAR self-regulatory mechanism. Interestingly, there is an increase in GluR2-lacking AMPARs in the NAc after cocaine withdrawal that is associated with an increase in cocaine craving and relapse (Conrad et al., 2008). Thus, changes in AMPAR subunit composition may also contribute to cocaine-use disorder.

Kainate Receptor

AMPA receptors and kainate receptors are difficult to dissociate leading to them often being discussed as one entity (Riedel et al., 2003). Due to this aforementioned fact, kainate receptors will only be briefly discussed.

Kainate receptors are ligand-gated ion channels located both pre and postsynaptically (Lerma, 2003). When activated by glutamate, presynaptic kainate receptors can facilitate or inhibit neurotransmission whereas postsynaptic receptor stimulation causes slow EPSPs (Kidd & Isaac, 1999; Lauri et al., 2001; Cossart et al., 2002). Kainate receptors contain two glutamate binding sites that must be bound to allow Na^+ and Ca^{2+} influx and K^+ efflux (Sommer et al., 1991; Pinaheiro & Mulle, 2006). These receptors are tetrameric complexes of GluR5-7 or KA1-2 subunits (Bleakman et al., 2002). However, only homotetrameric complexes of GluR5-7 subunits or heterotetrameric complexes of GluR5-7 and KA1-2 subunits make functional receptors (Bleakman et al., 2002; Alt et al., 2004). Similar to AMPARs, the GluR5-7 subunits of kainate receptors can be edited to increase their permeability to Ca^{2+} (Dingledine et al., 1999). Kainate receptors are also found in high concentrations in the temporal lobe and show differential expression between subregions of the hippocampus (Contractor et al., 2000; Rogawski et al., 2003). Thus, with differential brain expression and varying permeability to Ca^{2+}, these receptors allow for enhanced complexity when it comes to synaptic plasticity. Ionotropic receptors are not the only receptors found within the glutamate system nor are they the only ones potentially affected by cocaine use. Metabotropic glutamate receptors are also found in the glutamate system and play an important role in glutamate homeostasis.

Metabotropic Receptors

Metabotropic glutamate receptors (mGluRs) are seven *trans*-membrane spanning G-coupled protein receptors that signal to various second messenger systems and generally have a slower more modulatory role than ionotropic receptors (Kunishima et al., 2000; Pin & Acher, 2002). There are currently eight subtypes of mGluRs that are separated into three groups based on sequence homology, second messenger system interactions, and pharmacology (Dingledine et al., 1999; Kunishima et al., 2000). These receptors are critical to maintaining glutamate homeostasis and research suggests that they are affected by chronic cocaine abuse (Kalivas, 2009).

Group I mGluRs

Group I mGluRs consist of mGluR1 and mGluR5 metabotropic receptors (Niswender & Conn, 2010). These receptors are found postsynaptically and have an excitatory effect on neurons (Coutinho & Knopfel, 2002; Niswender & Conn, 2010). When mGluRs1/5s are bound by glutamate they activate second messenger systems via G_q proteins, which stimulate phospholipase C (PLC) to release 1,4,5-triphosphate (IP3) and diacylglycerol (DAG; Hermans & Challiss, 2001). IP3 and DAG function to release Ca^{2+} from intracellular stores to several effector proteins (Hermans & Challiss, 2001). DAG also stimulates protein kinase C (PKC), which can also stimulate several downstream effectors (Hermans & Challiss, 2001). These receptors can undergo alternate splicing thus increasing receptor variation and their effect on cellular function (Joly et al., 1995; Pin & Duvoisin, 1995). When activated, these receptors can also promote synaptic plasticity via LTP and LTD (Bellone et al., 2008; Kullman & Lamsa, 2008).

Group II mGluRs

Group II mGluRs consist of mGluR2 and mGluR3 metabotropic receptors (Niswender & Conn, 2010). These receptors are found pre and postsynaptically on excitatory, inhibitory, and modulatory neurons and when bound by glutamate have an inhibitory effect (Tamaru et al., 2001; Niswender & Conn, 2010). The mGluR2/3s are located primarily in the pre-terminal area on presynaptic neurons and mGluR3s may also be present on glial cells (Tamaru et al., 2001; Ferraguti &

Shigemoto, 2006). Presynaptic mGluR2/3s can be activated by excess synaptic glutamate or glutamate release from glial cells via the cystine-glutamate transporter (xCT; Kalivas, 2009).

The mGluR2/3s work through $G_{i/o}$ proteins that inhibit adenylyl cyclase (AC) and cyclic AMP (cAMP) formation (Tanabe et al., 1992; Pin & Duvoisin, 1995). AC and cAMP inhibition cause the release of $G_{\beta\gamma}$, which then affects downstream signaling proteins and directly activates K^+ channels and inhibits voltage-sensitive Ca^{2+} channels (Tanabe et al., 1992; Pin & Duvoisin, 1995). Note that alternate splicing can increase mGluR2/3s diversity and their effect on neurons (Sartorius et al., 2006).

Group III mGluRs

Group III mGluRs include mGluR4, mGluR7, and mGluR8 and are primarily found presynaptically in the active zone of neurons (Niswender & Conn, 2010). When activated by glutamate these neurons inhibit neurotransmitter release and, due to their location in the active zone, they often regulate neurons via negative-feedback mechanisms (Niswender & Conn, 2010). These receptors have different affinities for glutamate with mGluR7 needing a greater concentration of glutamate to be activated compared to the others (Schoepp et al., 1999). These receptors are coupled to $G_{i/o}$ proteins that inhibit AC and cAMP and promote $G_{\beta\gamma}$ release (Pin & Duvoisin, 1995). Note that the mGluR7/8 receptors show more diversity than mGluR4 receptors because they can be alternately spliced (Corti et al., 1998; Malherbe et al., 1999).

On a molecular level it should now be clear that the glutamate system is quite complex and can promote synaptic plasticity in a multitude of ways. However, the receptors discussed can be found on a myriad of different cell types and due to this fact glutamate has the ability to regulate many different types of neurons. Thus, discussed next is a more network-wide view of how the glutamate system can interact with other neurotransmitter systems. Considering the scope of this dissertation, how the glutamate system interacts with the dopamine system will be the primary focus.

Glutamate System Interactions: A Focus on Dopamine

Research suggests that the glutamate system interacts with several different neurotransmitter systems including the serotonin, acetylcholine, norepinephrine, GABA, and dopamine systems (Martin et al., 1998; Egli et al., 2004; Tseng & O'Donnell, 2004; Li et al., 2006; Parikh et al., 2008). However, most work has focused on glutamate-dopamine interactions especially in the addiction field (Sesack et al., 2003; Kalivas & Volkow, 2011). Thus, here the basics of what is known about non-pathological, homeostatic glutamate-dopamine interactions are highlighted.

Anatomical Associations

Midbrain DA neurons project from the VTA to the NAc (termed the mesolimbic pathway or 'reward pathway'), from the substantia nigra (SN) to the dorsal striatum (termed nigrostriatal pathway), and from the VTA to the prefrontal cortex (termed the mesocortical pathway; Miller et al., 2013). Considering the mesolimbic and mesocortical pathways have a large role in reward processing and addiction (Schultz, 2001; Volkow et al., 2004; Berridge, 2007), connections centering on these regions are highlighted.

Evidence suggests that dopamine neurons from the VTA synapse on to glutamatergic pyramidal cells in the PFC (Goldman-Rakic et al., 1989). Dopamine projections from the VTA specifically seem to innervate pyramidal cells that have projections to the NAc and to the contralateral PFC (Carr et al., 1999; Carr & Sesack, 2000). There is evidence that dopamine projections from the VTA may also regulate PFC pyramidal cells indirectly by synapsing on GABA interneurons (Williams et al., 1992; Condé et al., 1994). Further, evidence suggests that VTA dopamine may also have extrasynaptic actions on PFC pyramidal neurons (Sesack, 2002). Reciprocally, research shows that glutamate neurons from the PFC have projections to the NAc and the VTA (Sesack & Pickel, 1992; Geisler et al., 2007). Specifically, projections from the PrL innervate the NAcC and projections from the lL innervate the NAc shell (NAcSh; Geisler et al., 2007). Further, both the PrL and lL have connections to the VTA (Geisler et al., 2007). Thus, taken together, there is strong evidence that glutamate and

dopamine are strongly linked in the anatomical sense. Note that evidence also shows a strong molecular and physiological association between these two systems.

Molecular and Physiological Associations

Beyond their anatomical associations, glutamate and dopamine also interact on a physiological level. For example, in the PFC, D_1 agonists can act synergistically with glutamate agonists to increase pyramidal cell excitability whereas D_2 agonists have the opposite effect (Tseng & O'Donnell, 2004). Specifically, evidence suggests that D_1 stimulation potentiates NMDA-mediated responses (Tseng & O'Donnell, 2004). Conversely, D_2 receptor stimulation has the downstream effect of inhibiting NMDA receptors and thus weakening the excitatory response in neurons (Kotecha et al., 2002). Similarly, the stimulation of D_4 receptors in pyramidal neurons in the PFC depressed AMPA receptor-mediated excitatory synaptic transmission by decreasing AMPA receptors in the synapse (Yuen et al., 2010).

At the level of the midbrain, research suggests that glutamate projections from the PFC can regulate phasic dopamine signaling as well as burst firing and pauses (Grace & Bunney, 1984a; Grace & Bunney, 1984b; Sesack et al., 2003). Further, evidence shows that NMDA receptors are involved in activating dopamine neurons in the VTA (Martinez-Fong et al., 1992). Glutamate may also regulate dopamine release in the NAc indirectly through exciting GABA neurons (Montaron et al., 1996). Notably, research from the drug addiction field provides strong support that glutamate release in the NAc from PFC projections is important in drug-seeking behavior (Kalivas, 2009; Russo et al., 2010)

Glutamate Dynamics in Cocaine-use Disorder

A large amount of research has accumulated over the past 20 years suggesting that glutamate signaling in brain reward centers may play a key role in substance abuse (for review see Kalivas, 2004; Kalivas, 2009; Kalivas et al., 2009). Much of this work has focused primarily on the reciprocal connections

between the PFC (specifically, the PrL and lL cortices) and the NAc (Cornish & Kalivas, 2000; McFarland et al., 2003; Kalivas et al., 2005; LaLumiere et al., 2012). Further, much of this work has focused on cocaine seeking and relapse and was mostly conducted using animal models (e.g. McFarland & Kalivas, 2001; Park et al., 2002; Kalivas & McFarland, 2003). Here the evidence for dysregulated glutamate signaling in cocaine abuse is reviewed, focusing on the brain regions involved and the models used. This section culminates in discussing what has been termed "The Glutamate Homeostasis Hypothesis of Addiction" and with the limitations of current research.

Glutamate in Cocaine-use Disorder: A Brief Look at The Evidence

Historically, drug abuse research has focused on the role of dopamine signaling in areas of the reward system, such as the NAcC, NAcSh, PrL, and lL, in promoting and maintaining abuse-like behavior (Berridge & Robinson, 1998). However, as mentioned above, evidence suggests that glutamatergic signaling also plays a role in drug-abuse behavior (Kalivas & Volkow, 2011). For example, the infusion of an AMPA glutamate receptor antagonist into the NAcC prevents cocaine-primed reinstatement whereas the infusion of a D_1/D_2 dopamine receptor antagonist fails to do so (Cornish et al., 1999; McFarland & Kalivas, 2001). Evidence also suggests that cocaine reinstatement causes an increase in dopamine release in animals that previously self-administered cocaine as well as in yoked controls. However, an increase in glutamate release and drug-seeking behavior is only observed in animals previously exposed to cocaine (McFarland et al., 2003). These data suggest that, while dopamine signaling is important in abuse behavior, glutamate signaling may be more involved in the long-term plastic changes that promote drug abuse.

Along with an increase in glutamate release, chronic cocaine exposure may also causes a decrease in basal glutamate levels (via a decrease in the cystine-glutamate exchanger [xCT]) and a decrease in glutamate uptake (via a decrease in GLT-1) in the NAcC; an effect that is not seen in animals that only self-administer food (McFarland et al., 2003; Madayag et al., 2007; Miguens et al., 2008; Kalivas, 2009; Knackstedt et al., 2010). Further, pharmaceuticals that

increase extrasynaptic tone on mGluR2/3 receptors or that increase GLT-1 expression and glutamate uptake, normalize glutamate signaling in the NAcC and reduce cue-induced drug seeking (Tzschentke & Schmidt, 2003; Peters & Kalivas, 2006; Zhou & Kalivas, 2007).

Evidence also suggests that the inhibition of the PrL prevents the aforementioned glutamatergic changes in the NAcC and inhibits drug-seeking behavior (Park et al., 2002; McFarland et al., 2003) suggesting that glutamate release in the NAcC is primarily from PrL neurons. Conversely, inhibition of glutamate signaling from the IL to the NAcSh increases drug-seeking behavior suggesting that different glutamate tracts have different regulatory roles (Peters et al., 2008). Thus, cocaine-induced changes in glutamate-mediated plasticity appear to play a key a role in the development of addictive behavior and have lead to a general hypothesis regarding the role of glutamate in cocaine-use disorder (for review Kalivas, 2009).

The Glutamate Homeostasis Hypothesis of Addiction

Critical to a homeostatic glutamate system is a balance between glutamate release and elimination. Again, like all neurotransmitters, glutamate is released from presynaptic neurons, binds to postsynaptic receptors, and is eliminated by high-affinity, Na^+-dependent transporters (Diamond & Jahr, 1997; Herman & Jahr, 2007). However, some evidence suggests that the majority of extracellular glutamate is derived from non-synaptic, glial sources (Warr et al., 1999; Barbour, 2001). This non-synaptic glutamate is primarily responsible for regulating perisynaptic mGluRs (Warr et al., 1999; Barbour, 2001) and the xCT is responsible for approximately 60% of basal extracellular glutamate in the NAcC (Baker et al., 2002). Generally, ionotropic receptors in the synaptic cleft are not affected by non-synaptic glutamate due to uptake mechanisms (and, for the same reason, synaptic glutamate often does not affect perisynaptic mGluRs; Warr et al., 1999; Barbour, 2001). However, research suggests that all of these aspects of glutamate signaling are dysregulated by chronic exposure to cocaine.

Cocaine-Induced Changes in Glutamate Signaling

Chronic cocaine reduces membrane levels of functional xCT exchangers thus reducing basal glutamate levels by roughly 50% (Xi et al., 2002; Baker et al., 2003). This decrease in basal glutamate results in decreased tone on mGluR2/3s (Moran et al., 2005). Without the inhibitory regulation of these mGluR2/3s, synaptic glutamate release is increased (McFarland et al., 2003; McFarland et al., 2004; Miguens et al., 2008). Cocaine also decreases the number of GLT-1 transporters in glial membranes causing decreased glutamate uptake in the NAcC (Knackstedt et al., 2010). Thus, an increase in release of synaptic glutamate (due to decreased tone on mGluR2/3s) coupled with a decrease in glutamate uptake (due to a decrease in GLT-1) is likely the reason an overflow of synaptic glutamate is seen during cocaine reinstatement (McFarland et al., 2003). Evidence supports these claims in that activation of the xCT by *N*-acetylcysteine restores basal glutamate levels and prevents the reinstatement of cocaine seeking (Baker et al., 2003; Zhou and Kalivas, 2007; Moussawi et al., 2009). The administration of mGluR2/3 agonists also prevents cocaine reinstatement (Baptista et al., 2004; Peters & Kalivas, 2006). This mechanistic link is further strengthened by the fact that *N*-acetylcysteine activation of xCT does not prevent cocaine reinstatement if mGluR2/3 antagonists are also present (Moran et al., 2005). Ceftriaxone was also shown to prevent cue and cocaine-induced reinstatement by increasing GLT-1 levels (Knackstedt et al., 2010).

Cocaine-Induced Morphological Changes

Research shows that dendritic spine head diameter increases in animals that are in withdrawal from non-contingent cocaine (Shen et al., 2009). Further, when animals in withdrawal are given acute cocaine injections spine head diameter changes over a 120-minute time course (Kalivas, 2009). Specifically, an increase in spine head diameter is seen 45-minutes after injection and a decrease in diameter is seen 120-minutes after injection (Kalivas, 2009). Of importance here is the fact that this increase in spine head diameter is likely caused by an increase in AMPA receptors and the decrease caused by AMPA receptor internalization (Kalivas et al., 2009). Interestingly, after contingent or

non-contingent cocaine administration an increase in GluR1 containing AMPA receptors is seen in the NAc (Boudreau & Wolf, 2005; Conrad et al., 2008). Further, after extensive periods of withdrawal, AMPA receptors lacking GluR2 subunits are observed (Conrad et al., 2008). If acute cocaine is given after the withdrawal period there is rapid surface expression of GluR1 containing AMPA receptors (Anderson et al., 2008). Together, this suggests AMPA receptor composition and dynamics may play an important role in the progression of cocaine abuse.

Cocaine-Induced Metabotropic Receptor Changes

Evidence shows that mGluR2/3 receptors are downregulated after non-contingent administration of cocaine (Xi et al., 2002). This decrease in mGluR2/3 function could be due to decreased protein expression, increased receptor phosphorylation, and/or an upregulation of activator of G protein signaling 3 (AGS3; a negative regulator of mGLuR2/3 function via negative regulation of G_i coupled signaling; Takesono et al., 1999; Xi et al., 2002; Bowers et al., 2004; Ghasemzadeh et al., 2009). The upregulation of AGS3 is further supported by the fact that inhibiting AGS3 restores mGluR2/3 signaling and reduces cocaine-seeking behavior (Bowers et al., 2004, Yao et al., 2005; Bowers et al., 2008). Withdrawal from cocaine has also been shown to decrease the expression of mGluR1/5 and its binding protein, Homer1b/c, in the NAc (Swanson et al., 2001; Ary & Szumlinski, 2007; Ghasemzadeh et al., 2009). The importance of the downregulation of mGluR2/3 and mGluR1/5 is seen in the fact that stimulating mGluR2/3 signaling attenuates cocaine seeking and inhibiting mGluR1/5 or downregulating Homer 1 attenuates reinstatement (Chiamulera et al., 2001; Ghasemzadeh et al., 2003; Tessari et al., 2004; Olive et al., 2005; Peters & Kalivas, 2006; Palmatier et al., 2008). These data suggest that the downregulation of mGluR1/5 may be a compensatory mechanism whereas the downregulation of mGluR2/3 may promote drug-seeking behavior.

Cocaine-Induced Changes in LTP and LTD

Animals in extended withdrawal from cocaine show an increase in the AMPA/NMDA ratio in the NAcC indicating increased synaptic strength (Kourrich

et al., 2007; Conrad et al., 2008). Further, cocaine withdrawal also attenuates LTD in the NAcC (Martin et al., 2006). These results are at odds with convention because neurons in a potentiated state tend to have a greater dynamic range towards LTD (Kauer & Malenka, 2007). Evidence suggests that mGluR2/3s and mGluR1/5 regulate LTP and LTD, respectively; thus, this bidirectional loss in synaptic plasticity may be due to loss of glutamatergic tone on mGluRs (Grover & Yan, 1999; Wu et al., 2004; Kauer & Malenka, 2007). Evidence is given to this claim by the fact that increased xCT activity caused by *N*-acetylcysteine increases glutamate tone on mGluRs and restores the ability of neurons to induce LTP and LTD (Grover & Yan, 1999; Malenka & Bear, 2004; Wu et al., 2004; Moussawi et al., 2009).

Summary: Mechanisms of Cocaine-Induced Glutamatergic Changes

Following withdrawal from cocaine (after chronic exposure) the key presynaptic changes that occur in the NAcC are reduced mGluR2/3 signaling due partially from an increase in AGS3 and partially due to reduced glutamatergic tone from the xCT (Kalivas, 2009). Under basal conditions there is also a decrease in metabolic activity in the PFC of those with substance-use disorder and the firing rates of neurons from the PFC to the NAcC are reduced (Sun & Rebec, 2006). This fact accounts for decreased basal synaptic release in the presence of reduced mGluR2/3-mediated inhibition of release (Kalivas, 2009). This decrease in basal synaptic and non-synaptic release may account for the observed decrease in GLT-1 and the increase in postsynaptic AMPA receptors (Boudreau & Wolf, 2005; Kourrich et al., 2007; Conrad et al., 2008; Pendyam et al., 2009). In fact, the cocaine-induced upregulation of GluR2-lacking AMPA receptors has been interpreted as homeostatic synaptic scaling in response to decrease glutamatergic activity (Conrad et al., 2008).

When drug-seeking behavior is reinstated, there is an increase in neuronal activity in the PFC and an increase in glutamate release in the NAcC (McFarland et al., 2003; Sun & Rebec, 2006; Madayag et al., 2007; LaLumiere & Kalivas, 2008). The glutamate released from PFC afferents is synaptic and results partially from decreased mGluR2/3 signaling due to reduced xCT function (Baker

et al., 2002; Xi et al., 2002). The overflow of glutamate outside of the synapse is partially due to a down regulation of GLT-1 (Knackstedt et al., 2010). Forty-five minutes after cocaine administration there is an increase in spine head diameter due to an increase postsynaptic AMPA receptor expression (Toda et al., 2006; Anderson et al., 2008; Shen et al., 2009). However, by 120 minutes, glutamate levels are no longer elevated and the spine head diameter is reduced (Toda et al., 2006; Kalivas, 2009; Shen et al., 2009). By 120 minutes there is also a reduction in membrane bound AMPA receptors and a decrease in the AMPA/NMDA ratio (Thomas et al., 2001; Boudreau et al., 2007; Kourrich et al., 2007; Shen et al., 2009).

The bidirectional loss of LTP and LTD after cocaine self-administration suggests that mechanisms indicated in synaptic plasticity are impaired (Martin et al., 2006; Kourrich et al., 2007; Moussawi et al., 2009). This is likely due to the cocaine-induced increase in actin cycling, making it difficult to sustain the morphological changes that accompany the induction of LTP or LTD (Toda et al., 2006), as well as the decreased glutamatergic tone on mGluRs (Xi et al., 2002; Baker et al., 2003). The latter reasoning is supported by the fact that *N*-acetylcysteine restores the induction of LTP and LTD by increasing glutamatergic tone via increased functioning of the xCT (Moussawi et al., 2009). Note that further credence is given to the aforementioned fact in that the administration of *N*-acetylcysteine also prevents cocaine-seeking behavior (Baker et al., 2003; Zhou & Kalivas, 2007).

Limitations of Current Research

A limitation of the current research studying glutamate dynamics in models of substance-use disorder is that studies primarily focus on measuring glutamate in the NAcC; thus, very little is known about glutamate signaling in the PrL (for review Kalivas, 2009). Understanding glutamatergic signaling in the PrL could further aid in elucidating cocaine-specific glutamatergic changes especially considering the close relationship in glutamate signaling between the NAcC and the PrL in abuse behavior (McFarland et al., 2003). Also, most data assessing glutamate release in drug abuse models has primarily focused on reinstatement.

Thus, cocaine-specific glutamatergic changes that occur during drug taking are unknown. Also, most studies use microdialysis to collect glutamate measures. Considering that microdialysis can only collect data on the order of minutes (see Nandi & Lunte, 2009 for review) specific release events and their kinetics cannot be measured. Further, microdialysis probes usually sample from a large area on the order of millimeters (see Nandi & Lunte, 2009 for review) making it hard to isolate signaling from specific circuits. Thus, methods that collect data on a more physiologically relevant time scale and that sample from a smaller population of neurons may elucidate cocaine-specific changes in the glutamate system that have yet to be detected. Use of different behavioral designs may also aid in isolating cocaine-specific changes in glutamate signaling.

To date, most preclinical research attempts to study drug-specific neural changes in animals that only self-administer drug, while controls only self-administer food, water, or receive yoked saline (Cunningham et al., 2015; Huff & LaLumiere, 2015; Saddoris et al., 2016). However, controls likely do not have the same neuronal adaptations as rats with a history of drug taking, limiting the ability to study drug-specific glutamatergic adaptations that specifically contribute to drug-taking behavior. This is particularly important when considering that no persons with substance-use disorder are drug naïve, and they exhibit a wide array of behavior beyond drug taking that is maintained by non-drug reinforcement. Thus, to adequately study drug-taking behavior, including drug-specific glutamatergic adaptations, a design must be used that exposes the same individual to drugs, natural rewards, and their associated cues.

Multiple schedules of reinforcement allow for the study of drug and food reinforcement, as well as their associated stimulus effects, within a single individual (Weissenborn et al., 1995; Weissenborn et al., 1996; Carelli et al., 2000; Stairs et al., 2010). Previous studies have used multiple schedules to study primary reinforcers as well as the effects of associated conditioned and discriminative stimuli (Weiss et al., 2000; Weiss et al., 2001; Weiss et al., 2003; Kearns & Weiss, 2005; Kearns & Weiss, 2007; Weiss et al., 2007). However, no one to date has utilized multiple schedules to conduct a systematic within-subject

investigation into how cocaine specifically changes glutamatergic signaling in the NAcC and the PrL, compared to a natural reinforcer, such as food.

Overview of Experiment 1

Experiment 1 was conducted to increase understanding about the complex interactions that occur in glutamatergic signaling in the NAcC and PrL during cocaine self-administration. Specifically, biosensor technology was used to measure sub-second glutamate release in freely-moving animals behaving in a cocaine/food multiple schedule. Considering a behavioral procedure was used that exposed animals to both cocaine and food, it allowed for the isolation of cocaine-specific glutamatergic adaptations. The overall hypothesis for the experiment was that cocaine related behavioral events would cause an increase in glutamate release compared to food related behavioral events. Considering the connection between the PrL and NAcC glutamate release to cocaine behavioral events was expected to increase in both brain areas; however, it was expected that the dynamics of the release events would be markedly different.

CHAPTER 2

EXPERIMENT 1:
COMBINING MULTIPLE SCHEDULES OF REINFORCEMENT WITH GLUTAMATE BIOSENSORS TO EXAMINE THE EFFECTS OF COCAINE AND FOOD ON PRELIMBIC AND ACCUMBAL GLUTAMATERGIC SIGNALING IN FREELY-MOVING RATS

Introduction

Although there is evidence that glutamate signaling in the PrL and NAcC are dysregulated in cocaine-use disorder (Park et al., 2002; McFarland et al., 2003; Kalivas, 2009; Knackstedt et al., 2010) the exact nature of these changes and their implication for behavior are not fully known. This lack of knowledge partially comes from the use of methodologies that cannot measure glutamate at the high temporal and spatial resolution needed to detect fast changes in glutamate signaling (e.g. McFarland et al., 2003; Nandi & Lunte, 2009). Further, the use of behavioral paradigms that only expose animals to drug or food (e.g. Cunningham et al., 2015; Huff & LaLumiere, 2015; Saddoris et al., 2016) make it difficult to elucidate drug-specific glutamatergic changes. This study was conducted to address these issues by measuring glutamate with biosensors capable of detecting physiological relevant changes in glutamate signaling in the PrL and NAcC while animals behaved in a cocaine-food multiple schedule.

Materials and Methods

Animals

Twenty-two adult male Sprague-Dawley rats (Harlan, Inc.; Indianapolis, IN, USA) weighing approximately 250-300 g were used for experimentation. Rats were individually housed in a temperature-controlled environment on a 12:12 h light:dark cycle with lights on at 0600 h. All rats were acclimated to the colony

room and handled one week before any experimentation began. All rats had *ad libitum* access to food and water during the experiment proper. The Institutional Animal Care and Use Committee at the University of Kentucky approved all experimental protocols.

Drugs

Cocaine hydrochloride (COC; NIDA, Rockville, MD) was prepared in 0.9% sterile saline for self-administration. COC was self-administered at 1.0 mg/kg/infusion (Tella, 1995) based on weight.

Apparatus

Experiments were conducted in an operant conditioning chamber (ENV-008, Med Associates) housed within a sound-attenuating compartment (ENV-018M, Med Associates). Each chamber was connected to a computer (SG-502, Med Associates) and ran using MED-PC. Each operant chamber contained a 5.1 cm x 5.1 cm recessed food receptacle (ENV-200R2MA) on the front response panel with two retractable levers on either side (ENV-122CM; 6 cm above metal rod floor). Above each lever was one white cue light (ENV-221M; mounted 4.1 cm above each lever). A Sonalert tone (ENV-223 AM) was located above the top left cue light and another Sonalert tone (ENV-223 HAM) was located above the top right cue light. A house light (ENV-227M) was placed 17 cm above the metal floor in the middle of the back wall. Food pellets (45 mg, Dustless Precision Pellets; Bio Serv) were delivered via a dispenser (ENV-203M-45) placed behind the food receptacle. COC was delivered through a watertight swivel attached via tygon tubing to a back-mounted cannula via a syringe pump (PHM-100) located outside of the sound-attenuating chamber.

Glutamate Biosensor

Microelectrode Array Preparation

Microelectrode arrays (MEAs, S2 configuration; CenMeT, University of Kentucky) consisting of four platinum recording sites (15 µm x 333 µm) arranged in dual pairs were first built into an implantable headcap (Figure 2.1A) as previously described (Rutherford et al., 2007). Briefly, both ends of an ~2.5 cm long varnished 30 AWG copper wire (RadioShack, Fort Worth, TX) were scraped

to expose ~ 0.25 cm of copper wire and fluxed (#186 Rosin flux type RMA, Kester). One end of the wire was soldered (~200 °C) to a gold-plated socket (Ginder Scientific, Nepean, ON). The other end of the wire was soldered into the paddle portion of the MEA. This was done four times in order to wire up all four measuring sites. The four wires containing gold-plated sockets were then inserted into four holes in a nine-hole ABS plug and the wires were wrapped around the plug. A Teflon coated, 5 cm long Ag wire (A-M Systems, Carlberg, WA), which was electroplated to form an Ag/AgCl wire to serve as a reference electrode once in contact with CSF containing Cl^-, was then scraped (~0.25 cm), fluxed, soldered into a gold-plated socket, and placed in the ABS plug. The assembly was then covered with a heavy layer of marine quality epoxy and allowed to cure for at least 48 hours to ensure a waterproof seal (Rutherford et al., 2007).

MEAs were then configured for selective measures of glutamate (Figure 2.1B). Specifically, a solution of 1% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO), 0.125% glutaraldehyde (Sigma-Aldrich), and 1% glutamate oxidase (GluOX; US Biological, Salem, MA) was coated on the bottom two recording sites of the MEA by syringe application (3 coats) to allow for conversion of glutamate into α-ketoglutarate and the reporter molecule, peroxide (Burmeister and Gerhardt, 2001; Day et al., 2006). The top two recording sites were coated with the BSA/glutaraldehyde matrix (without GluOX) to allow for background current subtraction thus resulting in a self-referenced glutamate signal (Burmeister and Gerhardt, 2001). After the MEAs were configured with enzymes to measure glutamate, they were allowed to cure for at least 72 hours and then all four recording sites were electroplated with m-phenylenediamine (mPD; Acros, Fisher Scientific, Waltham, MA). mPD is a size exclusion layer that eliminates signals from interferent molecules such as ascorbic acid and dopamine thus allowing for more selective glutamate measurements (Miller et al., 2015). Electrodes were allowed to sit for at least 24 hours before implantation.

In Vitro Calibration

Amperometric recordings were collected at 4 Hz using the FAST16 mkIII electrochemical recording system (Fast Analytical Sensing Technology, Quanteon, LLC, Nicholasville, KY). Immediately before *in vivo* implantation, all electrodes underwent an *in vitro* calibration to determine sensitivity (slope, nA/μM), selectivity (glutamate vs. ascorbic acid sensitivity), limit of detection (in μM, signal-to-noise = 3), and linearity ($R^2 \geq 0.9$) (Burmeister and Gerhardt, 2001).

In Vivo Implantation

Immediately before surgery all rats were given subcutaneous injections of carprofen (Rimadyl©, Pfizer, NYC) at a dose of 10mg/kg and 1 mL of 0.9% sterile saline. All rats were anesthetized using 4% isoflurane (Isothesia, Henry Schein, Melville, NY). Once anesthetized, animals were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and maintained at an isoflurane level of 1-3%. Body temperature was maintained at 37°C using a circulating water bath attached to a water pad (Gaymar Industries, Orchard Park, NY). Artificial tears (Henry Schein, Melville, NY) were then applied to rats' eyes, the rats' heads were shaved, and Hibiclens scrub (Mölnlycke Health Care, Norcross, GA) and 70% ethanol were used to disinfect the surgery site. The skin overlying the skull was then reflected. A craniotomy was performed exposing the right hemisphere of either the PrL (AP: +3.2 mm; ML: ±0.8 mm; DV: -3.5 mm [from brain surface]) or NAcC (AP: +2.0 mm; ML: ±1.5 mm; DV: -7.0 mm [from brain surface]) (Paxinos and Watson, 2009). A small burr hole was made contralateral to the site of the craniotomy for implantation of the small Ag/AgCl reference electrode. Three screws (Amazon supply, part No. B00FN0K02) were then screwed into the skull. The dura was then reflected where the craniotomy was performed and the MEA was implanted in the respective brain region and the Ag/AgCl reference was placed into the contralateral burr hole epidurally. Anterior-posterior and medial-lateral coordinates for MEA implantation were calculated relative to bregma and dorsal-ventral coordinates from the brain surface. The MEA headcap was then set in place on rats' skulls using dental acrylic (Ortho Jet Powder and Jet Acrylic Liquid, Lang Dental Manufacturing Co., Wheeling, IL). The animals were allowed

to recover for 3 days before the recordings began; they were given carprofen (10 mg/kg) and 1 mL 0.9% sterile saline subcutaneously during this period.

Electrochemical Recordings

Glutamate measurements were performed using the FAST-16 mkIII recording system using a low noise 4-channel Rat Hat amplifier (Quanteon, LLC, Nicholasville, KY) system connected through a low-noise commutator (Plastics One, Inc., Roanoke, VA). For every session, each animal behaved in the procedure while neurochemical measurements were made at +0.7 V potential vs. Ag/AgCl reference (able to oxidize the recorder molecule, peroxide [i.e. able to detect glutamate]).

Establishing Procedures

Rats were first trained to retrieve food pellets from the food receptacle for two consecutive days. Following magazine shaping, rats were trained to lever press (left and right, randomly presented) on a fixed-ratio 1 (FR1) schedule of reinforcement; completion of the FR1 resulted in lever retraction and delivery of a food pellet. Each session consisted of 15 left and 15 right-lever trials. Rats were incrementally moved from an FR1 to a terminal FR3 over six days.

Catheter Surgeries

After lever training, rats underwent chronic indwelling jugular catheter implantation surgery. Rats were anesthetized with a ketamine/xylazine/acepromazine (75/7.5/0.75 mg/kg) mixture at 0.15 ml/100 g body weight (i.p.). Next a catheter was inserted into the right jugular vein, extended under the skin, and exited through the body via an incision on the back. A cannula attached to the catheter was then implanted in the back. Animals were given 7 days to recover after surgery.

Drug Self-Administration Training

After recovery, animals self-administered COC on an FR1 in the presence of a drug discriminative stimulus (cue light over lever). All COC infusions were paired with a drug conditioned stimulus (solid house light + tone). After 3 days the FR requirement was increased to an FR3. Animals were then allowed to self-administer COC until stable responding on the FR3 was reached (> 10 infusion

earned three consecutive days in a row). Animals were moved on to multiple schedule training (see below) after stability was reached.

Multiple Schedule Procedure

After stably responding for COC, rats were trained on a multiple schedule procedure (see Batten & Beckmann, 2018) where each session consisted of 12 alternating 5-minute components (with the first component being randomly selected). Either drug (6 components) or food (6 components) could be earned in a component. Each component was separated by 2-minute inter-component intervals (ICI); a period between components where all stimuli and manipulanda were off and no reinforcers could be earned. For drug components, the cue light above the drug lever was used as the discriminative stimulus signaling that lever presses (FR3) on the COC lever would result in a COC infusion. The COC infusion was paired with the compound conditioned stimulus of a solid house light and tone for the duration of the infusion (5.9 seconds). For food components, the cue light above the food lever served as the discriminative stimulus signaling that lever presses (FR3) on the food lever would result in food reinforcement. The food pellet was paired with the conditioned stimulus of a blinking house light (5.9 seconds). During each component, both levers were present with reinforcement being set up for either COC or food on one lever with the other lever being inactive (counterbalanced). After stable behavior was reached (overall discrimination ratio ≥ 75%; see below), the glutamate biosensor was implanted (see above) into the PrL (n = 11) or NAcC (n = 11) and cemented in place with dental acrylic (Figure 2.1C). After three days of recovery, glutamate measurements were collected while animals performed on the multiple schedule procedure. After all data were collected, animals were euthanatized and the brains were extracted, flash frozen, and 40 μm slices were prepared using a cryostat. The slices were stained using Cresyl Violet (Sigma-Aldrich, St. Louis, MO) and were visualized to confirm biosensor placements into the PrL or NAcC (Figure 2.2).

Data Analysis

Behavioral data were analyzed by calculating discrimination ratios for the

entire session (expressed as a percentage) as follows:

$$D.Ratio_{overall} = \frac{L_c + L_f}{L_c + L_f + I_c + I_f} \times 100 \qquad (1)$$

where L_c represents lever presses for COC during COC components, L_f represents lever presses for food during food components, I_c represents inactive lever presses during COC components, and I_f represents inactive lever presses during food components. Discrimination ratios were also calculated separately for COC and food component as follows:

$$D.Ratio = \frac{L_R}{L_R + I_R} \times 100 \qquad (2)$$

where L_R represents lever presses for COC or food during the respective components and I_R represents inactive lever presses during COC or food components. For the overall discrimination ratios, a one-sampled t-test was conducted comparing the average overall discrimination ratio to 75%. For the COC and food discrimination ratios, a one-sampled t-test was conducted comparing the average COC or food discrimination ratios to 50%. Individual behavioral events were analyzed with linear mixed-effects models (Gelman & Hill, 2007) using JMP Pro 12.0.0. statistical software (SAS Institute, Inc., Cary, NC) with subject as a random factor, brain region as a fixed between-subjects factor, and behavioral events associated with each component as a fixed, within-subjects factor.

Neurotransmitter data were analyzed using custom MATLAB®-based software (Quanteon LLC, Nicholasville, KY) and a custom-written MATLAB® (MathWorks, Inc., Natick, MA) program. Subtracted glutamate data and the location of respective behavioral events were extracted using the custom MATLAB®-based software. The glutamate signals related to the behavioral events were analyzed using the custom-written MATLAB® program. Specifically,

a glutamate peak was defined as an event that was 5 standard deviations above the mean of the baseline (Gunaydin et al., 2014). The baseline was defined as the last 1-minute average of all COC ICI's for COC components and the last 1-minute average of all food ICI's for food components. Peaks were considered related to a given behavioral event if they occurred within a 10-second window of the behavioral event (Malvaez et al., 2015) and were not interrupted by another type of behavioral event. Figure 2.3 and Figure 2.4 show example glutamate traces highlighting glutamate events considered to be peaks based on the previously mentioned criteria. The measures assessed were the absolute maximum of the glutamate peak (μM), the maximum amplitude of the glutamate peak above baseline (μM), the percent increase of the glutamate peak above baseline, the peak width (s), the peak prominence (μM), and the number of glutamate events that occurred to a given behavioral event. Statistical analyses were conducted with JMP Pro 12.0.0. statistical software using linear mixed-effects (LME) models (Gelman & Hill, 2007) with subject as a random factor, brain region as a fixed between-subjects factor, and component as a fixed, within-subjects factor.

Akaike information criterion (AIC) values were used to compare models; only statistics from the models that were most likely to describe the data are presented. Further, differences in AIC values (ΔAICs) were also calculated in order to assess the relative difference of information loss of all the other models compared to the best model. Evidence ratios for the best model relative to the second-best model were calculated from the ΔAICs (Burnham & Anderson, 2002; Burnham et al., 2011). The evidence ratios indicate the relative strength of the preferred model to the second-best model. Thus, by using evidence ratios one can say that the evidence for the preferred model is 'x' times stronger than that of the second-best model.

Where necessary linear regressions were performed and correlation coefficients, as well as if the slopes of the lines were statistically different than zero, were assessed. Any interactions were probed using the Tukey HSD, and statistical significance was defined as $p < 0.05$.

Results

Behavioral Responses During Baseline and Recording Conditions

Figure 2.5 shows the overall discrimination ratio for baseline behavior (the day before MEA implantation) and behavior during glutamate recordings. Overall discrimination ratios for both baseline [$t(21) = 14.22$, $p < 0.0001$] and recording [$t(21) = 3.50$, $p = 0.002$] behavior were statistically greater than 75%. Figure 2.6 shows the discrimination ratio for the COC and food components during baseline (Figure 2.6A) and recording behavior (Figure 2.6B). Discrimination ratios for COC [$t(21) = 13.33$, $p < 0.0001$] and food [$t(21) = 19.56$, $p < 0.0001$] during baseline were statistically greater than 50%. Discrimination ratios for COC [$t(21) = 3.03$, $p = 0.006$] and food [$t(21) = 7.09$, $p < 0.0001$] during recording were statistically greater than 50%.

Total Number Glutamate Peaks Found During COC and Food Components

Figure 2.7 shows the number of glutamate peaks found to behavioral events per component type and the total number of glutamate peaks found overall per component type (regardless of behavioral events). Specifically, a main effect of event type was found with more glutamate peaks occurring to events during food components compared to COC components (Figure 2.7A) [$F(1,20) = 13.02$, $p = 0.0018$]. This statistic came from the full model (brain x event type as factors), which had the lowest AIC and was 441.42 times more likely to describe the data than the second best model that used only event type as a factor.

Figure 2.7B shows the total number of glutamate peaks found during components (not just the peaks related to behavioral events). The full model (brain region x event type as factors) had the lowest AIC and was 158.38 times more likely to describe the data compared to the model with only event type as a factor. Specifically, a main effect of event type was found with significantly more glutamate peaks occurring during COC components compared to food components [$F(1,20) = 4.81$, $p = 0.040$].

Percentage of Peaks Related to Behavioral Events

Figure 2.8 shows the percentage of glutamate peaks that were related to behavioral events out of the total number of glutamate peaks found in a session. Specifically, Figure 2.8A shows that roughly an equal number of glutamate peaks were found between the PrL (11.51%) and the NAcC (12.19%). When looking specifically at each brain region, more glutamate peaks were found to food related events (PrL = 8.32% vs. NAcC = 8.27%) compared to COC related events (PrL = 3.19% vs. NAcC = 3.92%) in both the PrL (Figure 2.8B) and the NAcC (Figure 2.8C).

Total Number of Behavioral Events and Total Number of Glutamate Peaks Related to Behavioral Events

Figure 2.9A shows the total number of behavioral events that occurred during the COC and food components for rats with electrodes implanted in the PrL and NAcC. The full model had the lowest AIC and was 376.15 times more likely to describe the data compared to the model with only event type as a factor. The full model shows that there was a main effect of event type where a significantly greater number of behavioral events occurred during food components compared to COC components [$F(1,20) = 17.35$, $p = 0.0005$].

Figure 2.9B shows the number of glutamate peaks that occurred to behavioral events during COC and food components for rats with electrodes implanted in the PrL and NAcC. The full model had the lowest AIC and was 441.42 times more likely to describe the data compared to the model with only event type as a factor. The best model suggests that there was a main effect of event type where a significantly greater number of glutamate peaks occurred during food components compared to COC components [$F(1,20) = 13.02$, $p = 0.0018$]. Note that this graph is the same graph that was presented in Figure 2.7A with the y-axis changed for the ease of comparison.

Total Number of Glutamate Peaks are Correlated with Behavioral Events

Figure 2.10 shows correlations between the number of glutamate peaks found to COC or food events during a session and the number of behavioral events that occurred to COC or food during a session for rats with MEAs

implanted in both brain regions. Figure 2.10 also shows correlations between the number of glutamate peaks found per COC or food event in a session (created by taking the number of glutamate peaks that occurred to a behavioral event and dividing it by the number of behavioral events) and the number of behavioral events that occurred in the session. Figure 2.10A (left) shows a significant positive correlation between the number of glutamate peaks that occurred to COC events and the number of COC behavioral events that occurred ($r = 0.82$, $p < 0.001$). Figure 2.10A (right) shows a significant negative correlation between the number of glutamate peaks that occurred per COC event and the number of COC responses that occurred during a session ($r = 0.47$, $p = 0.03$). Figure 2.10B (left) shows a significant positive correlation between the number of glutamate peaks that occurred to food events and the number of food behavioral events that occurred in a session ($r = 0.95$, $p < 0.001$). Figure 2.10B (right) shows a significant negative correlation between the number of glutamate peaks that occurred per food event and the number of food behavioral events that occurred during a session ($r = 0.47$, $p = 0.03$).

Distribution of Glutamate Peaks Related to Behavioral Events

In order to get an idea of how the glutamate peaks that were related to behavioral events were distributed, the number of glutamate peaks found to a given behavioral event was divided by the total number of glutamate peaks found to all behavioral events and was expressed as a percentage. Figure 2.11 represents the percentage of glutamate peaks that occurred to all the different behavioral events measured. Specifically, Figure 2.11A shows that there was a main effect of event type where a statistically greater percentage of glutamate peaks occurred to the start of food components compared to the start of COC components [$F(1,21) = 9.20$, $p = 0.006$]. This statistic came from a model that included only event type as a factor and was 2.9 times more likely to describe the data compared to the full model.

Figure 2.11B shows a main effect of event type where a significantly greater percentage of glutamate peaks were seen to responses on the food lever compared to responses on the COC lever [$F(1,21) = 13.02$, $p = 0.002$]. This

statistic came from a model that included only event type as a factor and was 1.97 times more likely to describe the data compared to the full model.

Figure 2.11C shows that there were no statistical differences in the percentage of glutamate peaks that occurred to COC or food reinforcers [$F(1,20) = 0.44$, $p = 0.52$]. The model for this statistic included only brain region as a factor and was 1.32 times more likely to describe the data compared to the full model.

Figure 2.11D shows all head entries into the food receptacle during COC and food components in rats with electrodes implanted in the PrL and NAcC. The model with the lowest AIC was the full model; however, the ΔAIC between the best model and second best model was lower than 4. Thus, the simpler of the two models (event type only as a factor) was selected. Specifically, a main effect of event type was found with a significantly larger percentage of glutamate peaks observed with head entries during food components compared to head entries during COC components [$F(1,21) = 11.46$, $p = 0.003$].

Figure 2.11E shows all head entries into the food receptacle directly after a COC infusion was earned and all head entries directly after a food pellet was delivered. Presumably, head entries after a pellet was delivered are 'eating' responses. The model with the lowest AIC included only event type as a factor and was 1.58 times more likely to describe the data compared to the full model. Significantly more glutamate peaks were observed to head entries for eating during food components compared to the number of glutamate peaks that occurred to head entries after COC infusions [$F(1,21) = 27.70$, $p = 0.0001$].

Figure 2.11F shows inactive lever responses during COC and food components. There were no statistical differences in the number of glutamate peaks observed [$F(1,21) = 0.44$, $p = 0.51$]. The model with the lowest AIC included only event type as a factor and was 1.24 times more likely to describe the data compared to the full model.

Number of Glutamate Peaks Related to The Beginning of Components

Figure 2.12 shows the number of glutamate peaks that were related to the beginning of the COC and food components as well as the number of glutamate

peaks that occurred per component beginning (calculated by dividing the number of glutamate peaks by the number of COC or food components) in animals that had electrodes implanted in the PrL and NAcC. Specifically, Figure 2.12A shows that significantly more glutamate peaks occurred to the beginning of food components compared to the beginning of COC components [$F(1,21) = 9.20$, $p = 0.006$]. The model with the lowest AIC was the full model; however, the ΔAIC between the best model and second best model was lower than 4. Thus, the simpler of the two models (event type only as a factor) was selected. Figure 2.12B shows that significantly more glutamate peaks per food component beginning occurred compared to the beginning of COC components [$F(1,21) = 10.08$, $p = 0.0046$]. This statistic came from the model that included only event type as a factor, which had the lowest AIC and was 13 times more likely to describe the data compared to the full model.

Number of Glutamate Peaks Related to Lever Presses, Reinforcers, and Inactive Lever Presses

Figure 2.13 shows the behavior exhibited to lever presses, reinforcers earned, and inactive lever presses as well as the number of glutamate peaks that happened to each of those events. Figure 2.13A (left) shows that there was a significant main effect of event type with a greater number of responses occurring on the food lever compared to the COC lever [$F(1,20) = 18.35$, $p = 0.0004$]. This statistic came from the full model, which was 156.02 times more likely to describe the data than the model with just event type as a factor. Figure 2.13A (right) shows a main effect of event type with a greater number of glutamate peaks being observed to responses on the food lever compared to responses on the COC lever [$F(1,20) = 11.96$, $p = 0.0025$]. This statistic came from the full model, which had the lowest AIC and was 45.83 times more likely to describe the date than the model that used only event type as a factor.

Figure 2.13B (left) shows a significant main effect of event type with a greater number of food reinforcers being earned compared to COC reinforcers [$F(1,20) = 16.82$, $p = 0.0006$]. This statistic came from the full model, which had the lowest AIC and was 6.33 time more likely to describe the data than the model

with only event type included as a factor. Figure 2.13B (right) shows that there were no differences observed in the number of glutamate peaks that occurred to COC or food reinforcers [$F(1,20) = 0.04$, $p = 0.84$]. This statistic came from the full model, which had the lowest AIC and was 29.22 time more likely to describe the data compared the to the model where only event type was a factor.

Figure 2.13C (left) shows that there were not any statistical differences between COC and food inactive lever presses [$F(1,20) = 0.01$, $p = 0.92$]. This statistic came from the full model, which had the lowest AIC and was 10.86 times more likely to describe the data than the model with only event type as a factor. Figure 2.13C (right) shows that there were no differences observed in the number of glutamate peaks associated with inactive lever presses [$F(1,20) = 0.0013$, $p = 0.97$]. This statistic came from the full model, which had the lowest AIC and was 18.08 times more likely to describe the data than the model that used brain region only as a factor.

Number of Glutamate Peaks Per Lever Presses, Reinforcers, and Inactive Lever Presses

Figure 2.14 shows the behavior exhibited to lever responses, reinforcers earned, and inactive lever presses as well as the number of glutamate peaks per each of those events (calculated by taking the number of glutamate peaks that occurred to an event and dividing it by the amount of behavior that occurred). Note that the behavior is the same as seen in Figure 2.13 and is only shown in Figure 2.14 for purposes of comparison.

Figure 2.14A (right) shows a main effect of event type with a greater number of glutamate peaks occurring per COC lever presses compared to food lever presses [$F(1,21) = 6.26$, $p = 0.02$]. This statistic came from the model with only event type included as a factor, which had the lowest AIC and was 11.65 times more likely to describe the data compared to the model where only brain region was a factor.

Figure 2.14B (right) shows a main effect of event type where significantly more glutamate peaks occurred per COC reinforcer compared to food per reinforcer [$F(1, 21.27) = 67.32$, $p < 0.001$]. This statistic came from the model

where only event type was included as factor, which had the lowest AIC and was 44.70 times more likely to describe the data compared to the full model.

Figure 2.14C (right) shows that the number of glutamate peaks that occurred per inactive lever press were not different between COC and food [$F(1,20.16) = 3.35$, $p = 0.08$]. This statistic came from the model where only event type was included as a factor and was 4.39 times more likely to describe the data compared to the model where only brain region was a factor.

The Number of Glutamate Peaks Are Correlated with Lever Presses, Reinforcers, and Inactive Lever Presses

Figure 2.15 shows correlations between lever presses for COC and food and the number of glutamate peaks that occurred to those events or the number that occurred to those events per response. Specifically, Figure 2.15A (left) shows a significant positive correlation between the number of glutamate peaks that occurred to COC responses and the number of COC responses that occurred ($r = 0.80$, $p < 0.001$). Figure 2.15A (right) shows that there is no correlation between the number of glutamate peaks that occurred per COC response and the number of COC responses that occurred ($r = 0.076$, $p = 0.74$). Figure 2.15B (left) shows that the number of glutamate peaks that occurred to food responses and the number of food responses that occurred are positively correlated ($r = 0.94$, $p < 0.001$). Figure 2.15B (right) shows that the number of glutamate peaks that occurred per food lever press and the number of food lever presses that occurred were negatively correlated ($r = -0.51$, $p = 0.01$).

Figure 2.16 shows correlations between the number of COC and food reinforcers earned and the number of glutamate peaks that occurred to each reinforcer or the number of glutamate peaks that occurred per each reinforcer. Specifically, Figure 2.16A (left) shows a significant positive correlation between the number of glutamate peaks that occurred to COC reinforcers and the number of COC reinforces that occurred ($r = 0.84$, $p < 0.0001$). Figure 2.16A (right) shows that the number of glutamate events that occurred per COC reinforcer and the number of COC reinforcers that occurred are significantly negatively correlated ($r = -0.44$, $p = 0.04$). Figure 2.16B (left) shows a significant positive

correlation between the number of glutamate peaks that occurred to the food reinforcer and the number of food reinforcers that occurred (r = 0.85, $p < 0.0001$). Figure 2.16B (right) shows that there was no correlation between the number of glutamate peaks that occurred per food reinforcer and the number of reinforcers that were delivered (r = -0.28, $p = 0.21$).

Figure 2.17 shows correlations between the number of COC and food inactive lever presses and the number of glutamate peaks that occurred to inactive lever presses or the number of glutamate peaks that occurred per inactive lever press. Specifically, Figure 2.17A (left) shows a significant positive correlation between the number of glutamate peaks that occurred to inactive lever presses on the COC lever and the number of inactive COC lever presses that occurred (r = 0.91, $p < 0.0001$). Figure 2.17A (right) shows that the number of glutamate peaks that occurred per inactive lever press was not correlated with inactive COC responding (r = -0.44, $p = 0.1$). Figure 2.17B (left) shows a significant positive correlation between the number of glutamate peaks that occurred to inactive lever presses on the food lever and the number of food inactive lever presses that occurred (r = 0.91, p < 0.0001). Figure 2.17B (right) shows that there was no correlation between the number of glutamate peaks that occurred per food inactive lever press and inactive food responding (r = -0.35, p = 0.19).

Number of Glutamate Peaks Related to Head Entries, Head Entries After Reinforcer Delivery, and COC Earned/Eat Head Entries

Figure 2.18 shows the behavior exhibited to head entries, head entries after reinforcer delivery, and to COC earned and head entries associated with eating as well as the number of glutamate peaks to each of those events.

Figure 2.18A (left) shows that there were no difference in head entries during COC or food components [$F(1,20) = 0.26$, $p = 0.62$]. This statistic came from the full model, which was 32.95 times more likely to describe the data than the model with just event type as a factor. Figure 2.18A (right) shows a main effect of event type with a greater number of glutamate peaks occurring to food head entries compared to COC head entries [$F(1,20) = 9.02$, $p = 0.007$]. This

statistic came from the full model, which had the lowest AIC and was 90.02 times more likely to describe the data compared to the model where only event type was a factor.

Figure 2.18B (left) shows a significant main effect of event type with a greater number eat head entries occurring compared to COC infusion head entries [$F(1,20) = 26.09$, $p = 0.0001$]. This statistic came from the full model, which had the lowest AIC and was 3.56 times more likely to describe the data than the model with only event type as a factor. Figure 2.18B (right) shows a main effect of event type where significantly more glutamate peaks occurred to head entries after a pellet was delivered compared to head entries during/after a COC infusion [$F(1, 20) = 20.58$, $p = 0.002$]. This statistic came from the full model, which had the lowest AIC and was 39.25 times more likely to describe the data compared to the model that included only event type as a factor.

Figure 2.18C (left) shows that there was a main effect of event type with more eat head entry responses occurring than the number of COC reinforcers earned [$F(1,20) = 16.04$, $p = 0.0007$]. This statistic came from the full model which had the lowest AIC and was 3.67 times more likely to describe the data than the model with only event type as a factor. Figure 2.18C (right) shows that the number of glutamate peaks that occurred to head entries after a pellet was delivered was significantly greater than the number of peaks that occurred when COC was earned [$F(1,20) = 6.85$, $p = 0.02$]. This statistic came from the full model and was 44.82 times more likely to describe the data compared to the model where only event type was a factor.

Number of Glutamate Peaks Per Head Entries, Head Entries After Reinforcer Delivery, and COC Earned/Eat Head Entries

Figure 2.19 shows the behavior exhibited to head entries, head entries after reinforcer delivery, and to COC earned and head entries associated with eating as well as the number of glutamate peaks that occurred per each of those events. Note that the behavior is the same as seen in Figure 2.18 and is only shown in Figure 2.19 for purposes of comparison.

Figure 2.19A (right) shows that there are no statistical differences between

the number of glutamate peaks that occurred per COC and food head entries [$F(1,19.34) = 4.10$, $p = 0.06$]. This statistic came from the model with only event type included as a factor, which had the lowest AIC and was 4.01 times more likely to describe the data compared to the model where only brain region was a factor.

Figure 2.19B (right) shows that there were no statistical difference in the number of glutamate peaks that occurred per head entries during/after COC infusions or after a food pellet was delivered [$F(1,14.91) = 0.26$, $p = 0.62$]. This statistic came from the model where only event type was included as a factor, which had the lowest AIC and was 1.03 times more likely to describe the data compared to the model that included only brain region as a factor.

Figure 2.19C (right) shows that the number of glutamate peaks that occurred per COC reinforcer earned was significantly greater than the number of glutamate peaks that occurred per eating response [$F(1,19.91) = 85.71$, $p < 0.0001$]. This statistic came from the model where only event type was included as a factor and was 57.69 times more likely to describe the data compared to the full model.

The Number of Glutamate Peaks Are Correlated With Head Entries, Head Entries After Reinforcer Delivery, and COC Earned/Eat Head Entries

Figure 2.20 shows correlations between the number of COC and food head entries and the number of glutamate peaks that occurred to each type of head entry as well as the number or glutamate peaks that occurred per each head entry. Specifically, Figure 2.20A (left) shows a significant positive correlation between the number of glutamate peaks that occurred to head entries during COC components and the number of COC head entries that occurred ($r = 0.95$, $p < 0.0001$). Figure 2.20A (right) shows that the number of glutamate peaks that occurred per COC head entry and the number of COC head entries that occurred were not correlated ($r = -0.32$, $p = 0.24$). Figure 2.20B (left) shows a significant positive correlation between the number of glutamate peaks that occurred to head entries during food components and the number of food head entries that occurred ($r = 0.93$, $p < 0.0001$). Figure 2.20B (right) shows that there

was no correlation between the number of glutamate peaks that occurred per food head entry and the number of food head entries that occurred (r = -0.21, p = 0.87).

Figure 2.21 shows correlations between the number of COC and food head entries that occurred after reinforcer delivery and the number of glutamate peaks that occurred to each type of head entry as well as the number or glutamate peaks that occurred per each head entry. Specifically, Figure 2.21A (left) shows a significant positive correlation between the number of glutamate peaks that occurred to head entries during/after a COC infusion and the number of COC infusion head entries that occurred (r = 0.82, p < 0.0001). Figure 2.21A (right) shows that the number of glutamate peaks that occurred per COC infusion head entry and the number of COC infusion head entries that occurred are not correlated (r = -0.13, p = 0.63). Figure 2.21B (left) shows a significant positive correlation between the number of glutamate peaks that occurred to food head entries after a pellet was delivered and the number of food pellet head entries that occurred (r = 0.91, p < 0.0001). Figure 2.21B (right) shows that there was no correlation between the number of glutamate peaks that occurred per food pellet head entry and the number of food pellet head entries that occurred (r = -0.21, p = 0.87).

Glutamate Peaks Percent Increase From Baseline for Lever Presses, Reinforcers Earned, and Inactive Lever Presses

Figure 2.22 shows the behavioral events that occurred to lever presses, reinforcers, and inactive lever presses and the percent increase from baseline of the glutamate peaks to the different behavioral events. Note that the behavior is the same as seen in Figure 2.13 and thus was already statistically analyzed and is only shown in Figure 2.22 for purposes of comparison. Specifically, Figure 2.22A (right) shows the percent increase from baseline of the glutamate peaks to responses on the COC and food lever. No statistical differences were observed [F(1,20) = 1.71, p = 0.21]. This statistic came from the full model, which had the lowest AIC and was 11.36 times more likely to describe the data than the model with only brain region as a factor.

Figure 2.22B (right) shows a significant main effect of event type where there was a greater percent increase above baseline for glutamate peaks when COC was earned compared to when food was earned [$F(1,19.56) = 5.03$, $p = 0.037$]. This statistic came from the full model, which had the lowest AIC and was 10.33 times more likely to describe the data than the model with only event type as a factor.

Figure 2.22C (right) shows that there were no statistical differences in the percent increase for glutamate peaks above baseline for inactive lever presses [$F(1,17.12) = 0.40$, $p = 0.54$]. This statistic came from the full model, which had the lowest AIC and was 18.08 times more likely to describe the data than the model with only brain region as a factor.

Glutamate Peaks Percent Increase From Baseline for Head Entries, Head Entries After Reinforcer Delivery, and COC Earned/Eat Head Entries

Figure 2.23 shows the behavioral events that occurred to food receptacle head entries, head entries associated with COC infusions and eating, and COC earned and eating and the percent increase from baseline of the glutamate peaks to the different behavioral events. Note that the behavior is the same as seen in Figure 2.18 and is only shown in Figure 2.23 for purposes of comparison. Specifically, Figure 2.23A (right) shows the percent increase from baseline of the glutamate peaks for COC and food head entries. There was an event type x brain region interaction with a greater percent increase seen to COC head entries compared to food head entries in the PrL [$F(1,14.64) = 9.39$, $p = 0.008$]. This statistic came from the full model, which had the lowest AIC and was 363.22 times more likely to describe the data than the model with only brain region as a factor.

Figure 2.23B (right) shows that there were no percent baseline differences in glutamate to eat head entries and COC infusion head entries [$F(1,14.2) = 2.47$, $p = 0.14$]. The model with the lowest AIC was the full model; however, the ΔAIC between the best model and second best model was lower than 4. Thus, the simpler of the two models (event type only as a factor) was selected.

Figure 2.23C (right) shows that there were no statistical differences in the

percent increase from baseline for glutamate peaks between eating head entries and COC earned [$F(1,17.64) = 2.25$, $p = 0.15$]. The model with the lowest AIC was the full model; however, the ΔAIC between the best model and second best model was lower than 4. Thus, the simpler of the two models (event type only as a factor) was selected.

Discussion

To the knowledge of the author this is the first study to examine how COC affects glutamatergic signaling in the PrL and NAcC compared to a natural reinforcer (food) within subject using a multiple schedule procedure. The results from this experiment showed differential glutamate signaling to COC and food related behavioral responses with the directionality of these differences being similar between brain regions. Specifically, it was found that the percentage of glutamate peaks related to COC or food behavioral events was roughly equal between the PrL and NAcC. Within each brain region, it was also observed that the percentage of glutamate peaks related to food behavioral events was approximately 2.5 times greater than the percentage of glutamate peaks related to COC behavioral events. Overall, the number of COC behavioral events was positively correlated with the number of glutamate peaks that occurred to COC behavioral events, and the number of food behavioral events was positively correlated with the number of glutamate peaks found to food behavioral events. Interestingly, this trend tended to reverse when correlating behavioral responses with the number of glutamate peaks that occurred per response. Thus, the number of glutamate peaks that occurred per behavioral response generally increased as the number of behavioral responses decreased; this relationship was most pronounced with COC lever presses and when the COC reinforcer was earned. Lastly, it was also found that there was a greater percent increase from baseline in glutamate when COC was earned compared to when food was earned.

Previous work has shown that glutamate neurotransmission in the NAcC and the PrL are involved in drug seeking and relapse (McFarland et al., 2003;

Kalivas et al., 2005). Specifically, evidence suggests that the glutamate projections from the PrL to the NAcC and ventral tagmental area (VTA) are especially important in promoting drug seeking and cue-induced relapse (Kalivas, 2004). For example, pharmacological inhibition of the prefrontal cortex prevents cue-induced reinstatement and associated glutamate release in the NAcC (McFarland et al., 2003; McLaughlin & See, 2003). Further, evidence suggests that COC induced reinstatement increases glutamate release in the NAcC (Kalivas, 2009; Pendyam et al., 2009). In this study, after every session, the rats went approximately 24 hours without drug, which could have caused a withdrawal state. Thus, when the rats began to self-administer COC, it could be thought of as similar to COC primed reinstatement. This could account for the increased glutamate release observed to COC delivery compared to food delivery (Figure 2.22B). Evidence also suggests that COC self-administration increases glutamate levels above baseline after approximately 60 minutes in rats chronically exposed to COC (Miguens et al., 2007). The sessions here were approximately 82 minutes; thus, the observed increase in glutamate is also in line with previous self-administration studies. Further, considering the link between the PrL and NAcC in drug-seeking behavior (e.g. McFarland et al., 2003; McLaughlin & See, 2003) it is not surprising that glutamate release was in the same direction in both brain regions. It is worth noting that an increase in glutamate release above baseline was observed to COC delivery even though more food was earned than COC on average (Figure 2.22B). This disparity between glutamate signaling and behavior suggests that the increase in glutamate release observed is specific to COC and is not just due to the number of reinforcers earned. Note that differences in the number of glutamate peaks that occurred to behavioral events were also found.

A significant increase in the number of glutamate peaks was observed to food events compared to COC events (Figure 2.9B, Figure 2.12A, Figure 2.13, and Figure 2.18). This result is somewhat contradictory to the studies that show an increase in the probability of glutamate release during COC reinstatement and self-administration compared to food controls (Moran et al., 2005; Madayag et

al., 2007; Miguens et al., 2007; Kalivas 2009). However, as mentioned, most studies looking at the effects of COC on glutamate use separate animals for experimental (COC) and control (food/saline) conditions (e.g. McFarland et al., 2003; Miguens et al., 2007). Thus, the increase in the number of glutamate events to food related behavioral events compared to COC related behavioral events observed here could be due to the fact that all animals in this study were exposed to both reinforcers. Further, using multiple schedules, Carelli et al. (2000, 2002) found that neural populations in the NAc showed differential firing patterns to COC vs. natural reinforcers. However, in the Carelli study, electrophysiology was used; thus, what type of cells (dopaminergic, GABAergic, glutamatergic, etc.) were more active to COC vs. the natural reinforcer is unknown. Considering this fact, it is possible (albeit unlikely) that glutamatergic cells fire more frequently to food compared to COC, which may explain the results presented here. In fact, there is evidence that food-related events cause glutamate release in the PrL and NAcC (e.g. Batten et al., 2018), which gives some support to this idea. However, it is more likely that more glutamate peaks were observed to food events compared to COC events due to the fact that animals on average had more history with food than COC (Figure 2.9A; Figure 2.13A/B). This is especially so considering that differential reinforcer experience has been shown to affect drug/food preference and corresponding brain activity compared to when reinforcer history is held constant (Chow 2018; Beckmann et al., 2019). However, more research will need to be conducted to adequately explain the discrepancies between these results and the current literature.

Another important finding in this study was that the number of glutamate peaks that occurred to a given behavioral event was positively correlated to that respective event (Figure 2.10). This finding is similar to glutamate measures taken from the basolateral amygdala and OFC in other behavioral paradigms (Malvaez et al., 2015, Malvaez et al., 2019). However, while these results are reassuring, they should be interpreted with caution because it is possible that the number of glutamate peaks increased simply because the amount of behavior increased. Thus, it could be that more glutamate peaks were more likely to be

found simply because more behavior occurred. That being said, there is evidence that the NAcC does not participate in the processing of movements (Schultz et al., 1992; Carelli & Deadwyler, 1997). Also, the relationship between the number of glutamate peaks and the number of behavioral responses was not 1:1 suggesting that the number of glutamate peaks may be encoding something other than just movement. Further, the correlation for inactive lever presses (an action that could be considered to have less value in the current paradigm; Figure 2.17) was generally stronger (albeit not by much) than those associated with a reinforcer (lever presses, delivery; Figure 2.15 and Figure 2.16). Thus, this could indicate that the number of glutamate peaks for inactive lever presses has more to do with motor responses whereas reinforcer-related responses have to do with motor activity as well as the value of the action. The fact that there were statistical differences to reinforcer related lever presses but not inactive lever presses lends some support to this idea (Figure 2.13A/C). Further, the number of peaks that occurred to the start of the food component was greater than the number of peaks that occurred to the start of the COC component (Figure 2.12A). Considering that there were an equal number of COC and food components it would suggest that this difference is not simply due to the number of components that occurred. While not completely comparable to other behavior (when the component starts the animal is not engaging in a programmed response) it suggests that the number of glutamate peaks is encoding something more than just responses. Nevertheless, these results have the potential to be confounded.

In an attempt to control for the problematic nature of the number of peaks analysis discussed above we divided the number of glutamate peaks that occurred to a specific event by the number of responses that occurred. Standardizing the number of peaks to the number of behavioral responses in theory should control for the disproportionate number of responses between event types thus creating a measure that is less confounded by the amount of behavior. The number of glutamate peaks that occurred per response type is informative in two ways: (1) the measure creates quick metric for assessing how

the number of peaks and behavior are directly related (i.e. less than 1 means there were a greater number of behavioral responses than peaks, equal to 1 means that the number of behavioral responses and the number of peaks were equal, and greater than 1 means that more peaks were observed than behavioral responses) and (2) shows the number of glutamate peaks that occurred per a behavioral event (on average). Interestingly, when the data were expressed as the number of glutamate peaks that occurred per event type (Figure 2.14 and Figure 2.19) more glutamate peaks were observed per COC lever press and per COC reinforcer earned. Thus, even though more glutamate peaks are associated with food lever presses overall, there are more glutamate peaks generated per a single COC lever press and per a COC reinforcer. Thus, when looking at this measure, there is a dissociation between behavior and glutamatergic activity. This result is similar to what has previously been found in the literature (e.g. McFarland et al., 2003; Miguens et al., 2007; Kalivas, 2009 for review) in that chronic exposure to COC causes an increase in the probability of synaptic glutamate release.

As mentioned, the number of glutamate peaks to a behavioral event is positively correlated with the number of responses that occurred (Figures 2.10 [left]). However, the number of glutamate peaks that occurred per behavioral event was either not correlated or was negatively correlated with the number of behavioral responses that occurred (Figures 2.10 [right]). For example, there seems to be no relationship between the number of glutamate peaks that happened per COC lever press and the number of COC lever presses that occurred (Figure 2.15A [right]). However, there is a significant negative correlation between the number of glutamate peaks that occurred per food lever press and the number of food lever presses that were exhibited (Figure 2.15B [right]). This suggests that as the number of food responses decreased the number of glutamate peaks that occurred per food lever press increased. Conversely, the relationship between reinforcers earned was switched; thus, as the number of COC reinforcers decreased the number of glutamate peaks observed per COC reinforcer increased (Figure 2.16A [right]). This relationship is

counterintuitive based on previous literature showing positive correlations between glutamate and behavior using a similar 'rate' measure (Malvaez et al., 2015). However, based on previously mentioned research, it is likely more peaks are seen per COC event due to the pharmacology of the drug. Nevertheless, more research needs to be conducted in order to better understand this relationship.

As highlighted above a potential weakness of this study is in interpreting the meaning of glutamate frequency data in relation to behavior. While effort was taken separate frequency of release and frequency of behavior, it is likely that these measures are still partially confounded. Thus, it is difficult to say for certain that there were reinforcer specific effects. Further, even though animals were exposed to both reinforcers they could still only respond for one reinforcer at a time. Also, the reinforcer histories were not controlled. These latter two factors make it difficult to say anything about brain representations of value in relation to these two reinforcers or the actions taken to earn them. It is also worth noting that all glutamate measurements in these experiments were taken from the right hemisphere of both brain regions. However, others (e.g. McFarland et al., 2003; LaLumiere et al., 2008) have conducted similar experiments and have collected their data by either counterbalancing hemispheres or collapsing data from both hemispheres into a single data point. Thus, these previous studies suggest that robust hemispheric differences in glutamate signaling do not exist in the PrL and NAcC at least in relation to appetitive behavior. Therefore, only having right hemisphere data in this study likely did not skew these results.

In conclusion, the results presented here show that the percentage of glutamate peaks found between the PrL and NAcC are approximately equal. Within a given brain region a larger percentage of glutamate peaks were found to food related events compared to COC related events. Likewise out of all the glutamate peaks found to behavioral events, the majority were related to food-associated behavior. Overall, there were no differences in glutamate signaling between brain regions; however, there was evidence of glutamate specific signaling to different behavioral events. Namely, more glutamate peaks were

observed to food lever presses than COC lever presses. However, the number of glutamate peaks that occurred per COC lever presses was greater than the number that occurred per food lever press. Similarly, the number of glutamate peaks that occurred per COC reinforcer earned was greater than the number that occurred per food reinforcer earned. There was also a significant percent increase above baseline in glutamate when the COC reinforcer was earned compared to when the food reinforcer was earned. Taken together, these data suggest that differential glutamate signaling does exist between COC and food related events in the PrL and NAcC.

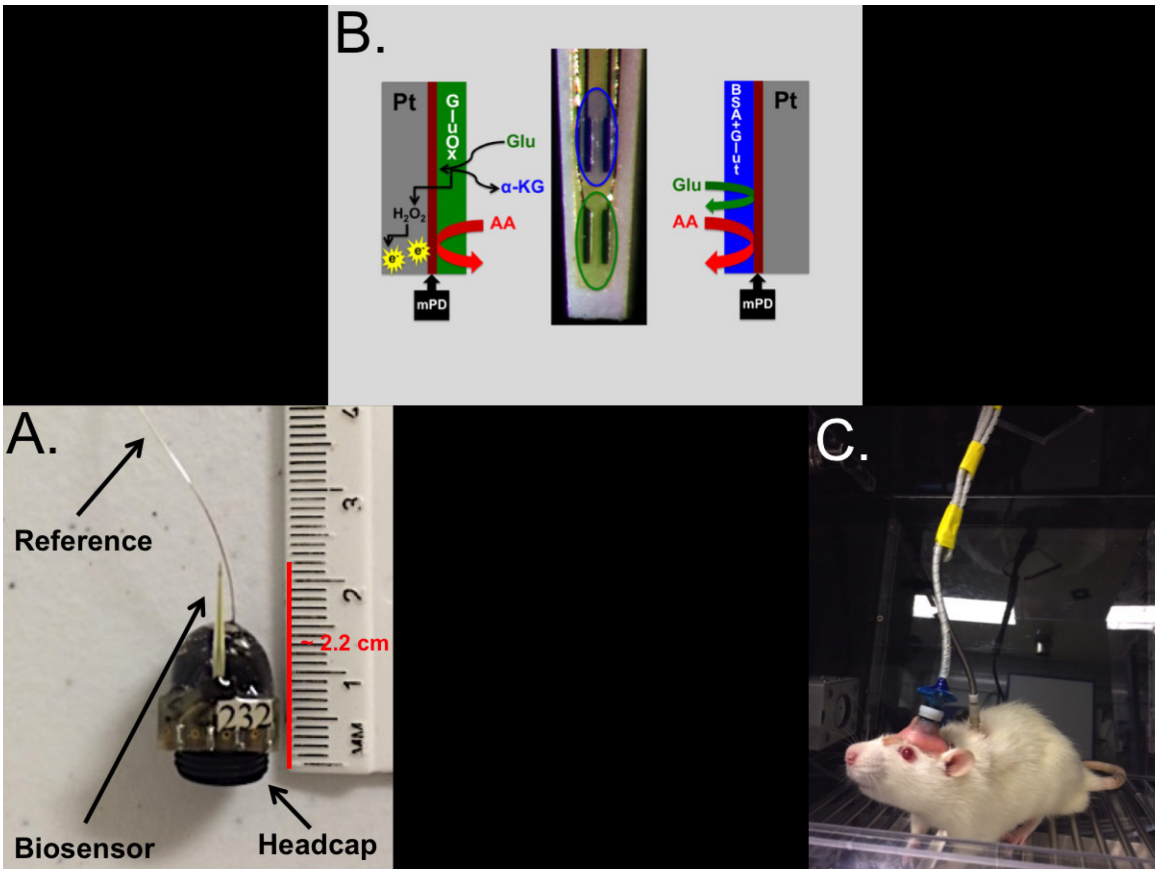


Figure 2.1 Biosensor Setup & Implantation

(A) Example of an implantable biosensor highlighting the different components of the headcap and the overall size (~ 2.2 cm). **(B)** S2 Biosensor Image. Green shaded sites: contain GluOx and thus can generate hydrogen peroxide (H_2O_2) from glutamate, which can be oxidized by the biosensor. Blue shaded sites: sentinel sites that contain an inert protein matrix and thus can only measure background current but not glutamate; the sentinel sites are subtracted from the glutamate recording sites to acquire basal glutamate levels in the brain (termed self-referencing). mPD excludes ascorbic acid (AA) and other large molecules (DA; DOPAC) by size thus stopping them from reaching the platinum recording surfaces. **(C)** An example of a rat with a glutamate biosensor implant and an indwelling backmount catheter. Note that the animal is hooked up to the FAST recording system and drug syringe pump.

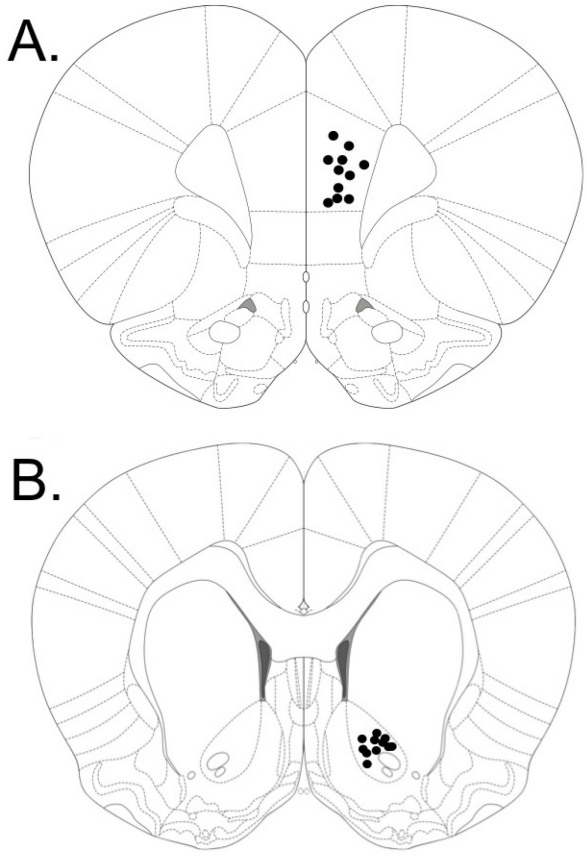


Figure 2.2 Glutamate Biosensor Placements

The black circles represent the approximate placement of the tip of the glutamate biosensor for each subject (n = 11/brain region) in **(A)** The prelimbic cortex (PrL) and **(B)** The nucleus accumbens core (NAcC).

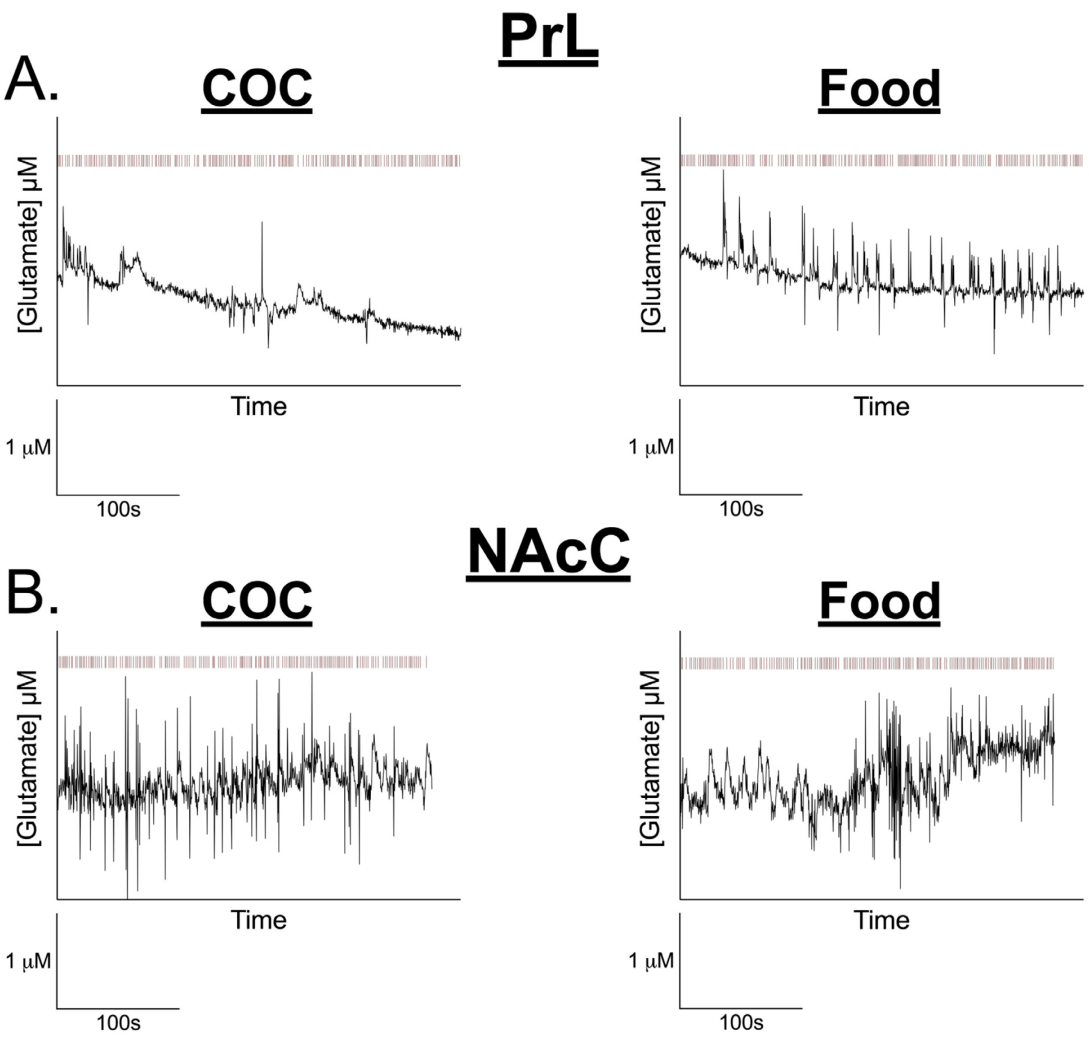


Figure 2.3 Glutamate Traces to COC and Food Components in The PrL and NAcC

(A) Example of a glutamate trace during a COC component (left) and a food component (right) from the PrL. **(B)** Example of a glutamate trace during a COC component (left) and a food component (right) from the NAcC. Note that the red lines above the traces indicate all locations where a glutamate peak was detected.

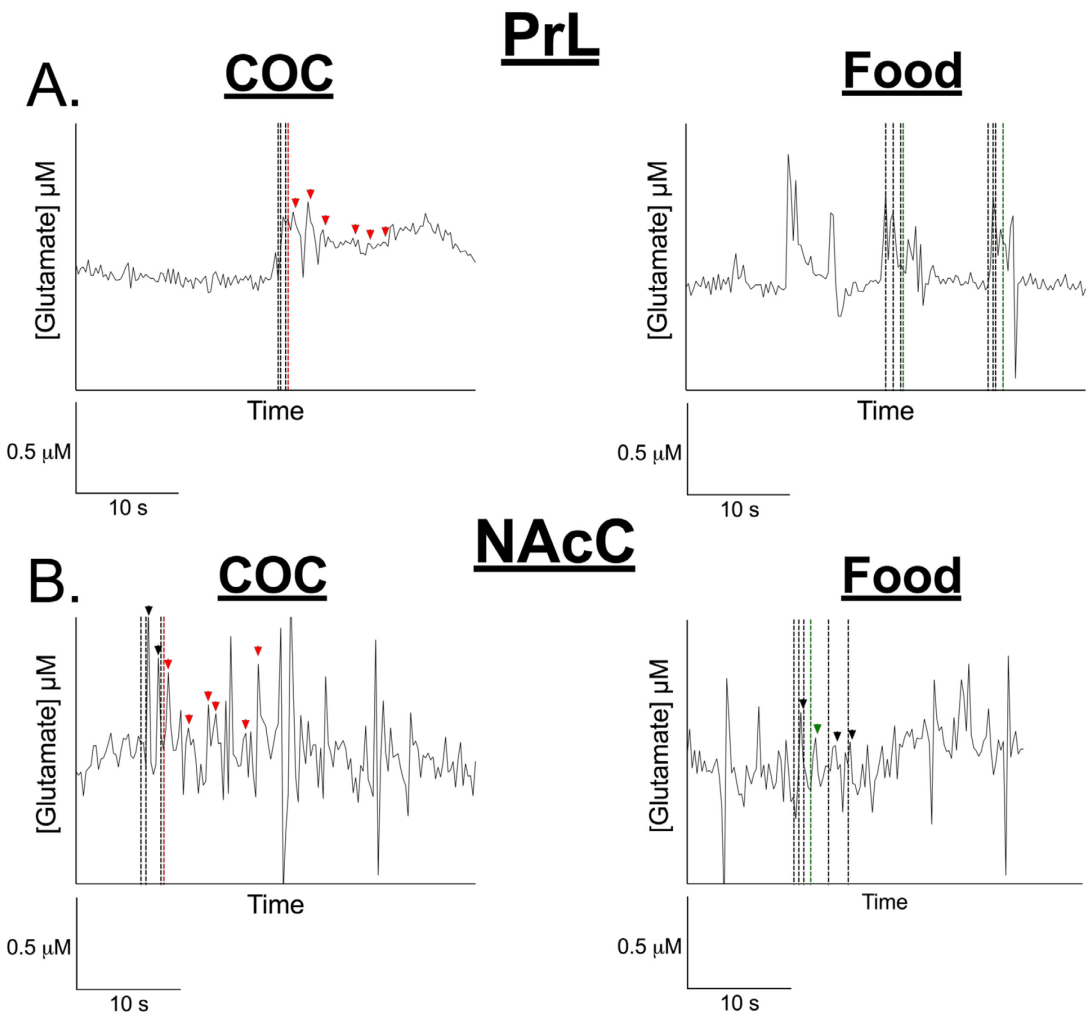


Figure 2.4 Glutamate Traces to COC and Food Events in The PrL and NAcC

(A) Example of a glutamate trace during a COC event (left) and a food event (right) from the PrL. **(B)** Example of a glutamate trace during a COC event (left) and a food event (right) from the NAcC. Note that the **black-hatched** lines represent lever presses and the red- and green-hatched lines represent COC infusions and food deliveries, respectively. The **black triangles** represent glutamate peaks associated with lever presses. The red triangles represent glutamate peaks associated with a COC infusion. The green triangles represent glutamate peaks associated with earned food pellets. Note that there are no black or green triangles on the PrL food trace because, due to the timing of events, the only peaks found were to the responses/reinforcer.

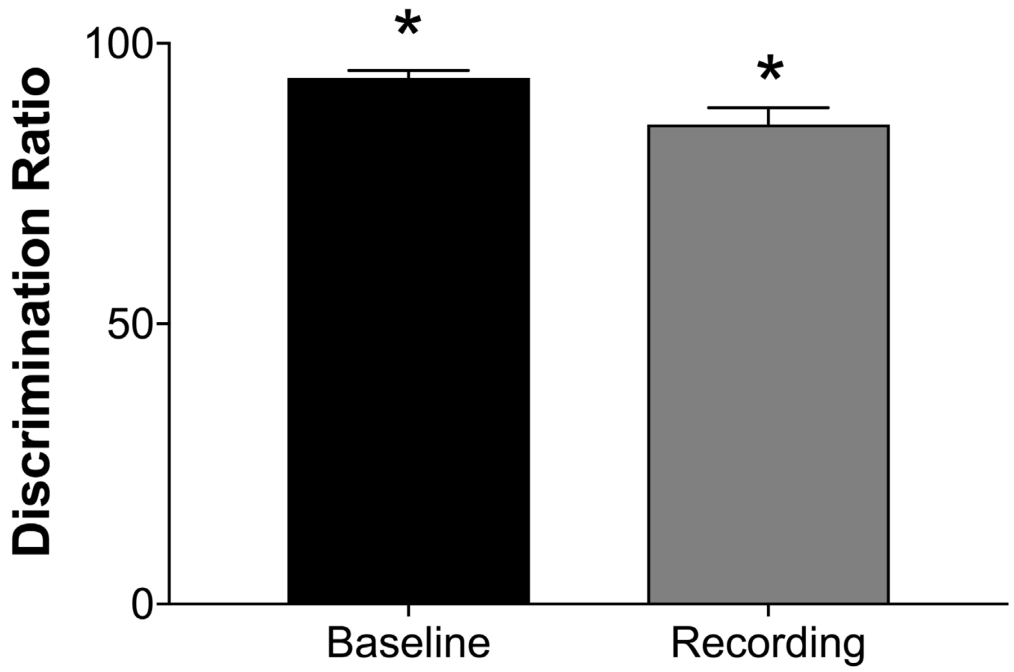


Figure 2.5 Overall Discrimination Ratios For Baseline & Recording Behavior

The overall discrimination ratios for baseline behavior (session before biosensor implantation) and for behavior during glutamate recordings were significantly greater than 75%. One-sample t-test, $*p < 0.05$. Data represented as mean ± SEM.

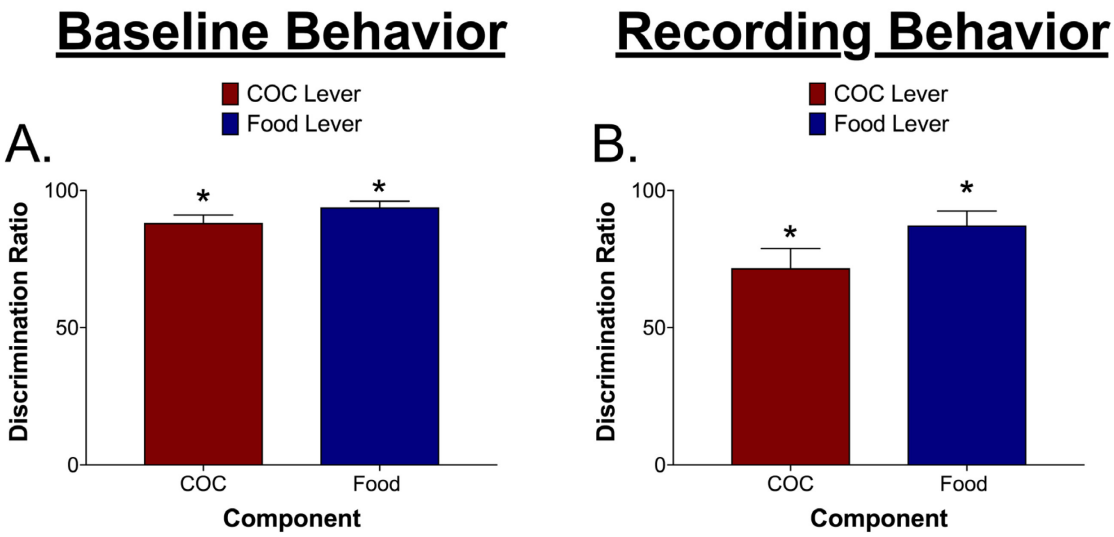


Figure 2.6 Discrimination Ratios for COC and Food Components for Baseline & Recording Behavior

(A) Baseline behavior discrimination ratios during the COC component and the food component were significantly greater than 50%. **(B)** Recording behavior discrimination ratios during the COC component and the food component were significantly greater than 50%. One-sample t-test, $*p < 0.05$. Data represented as mean ± SEM.

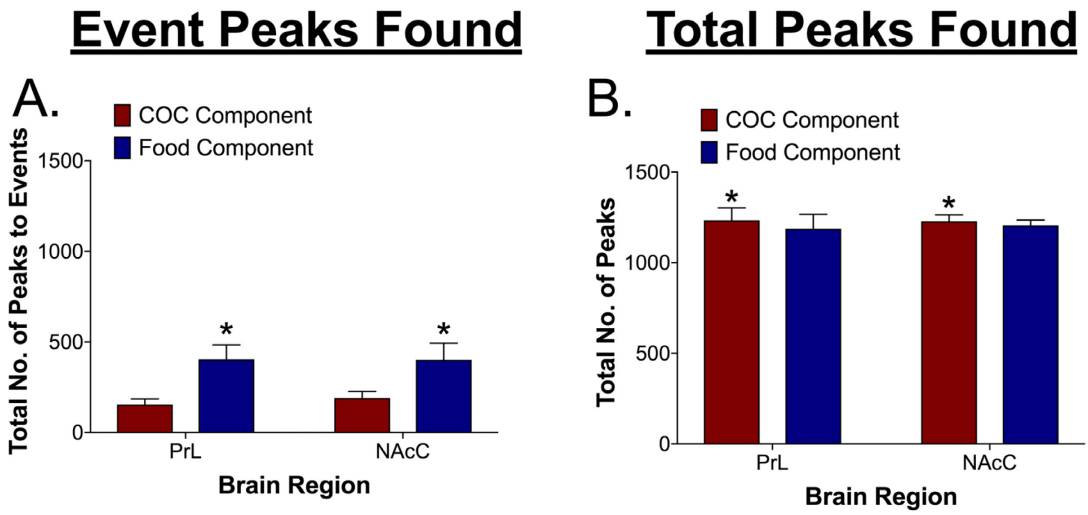


Figure 2.7 Total Number of Glutamate Peaks Found To Behavioral Events in Each Component vs. The Total Number of Peaks Found in Each Component

(A) Significantly more glutamate peaks were found to behavioral events during food components compared to COC components. **(B)** Significantly more glutamate peaks were found overall (not just those related to behavioral events) during COC components compared to food components. LME, $*p < 0.05$. Data represented as mean ± SEM.

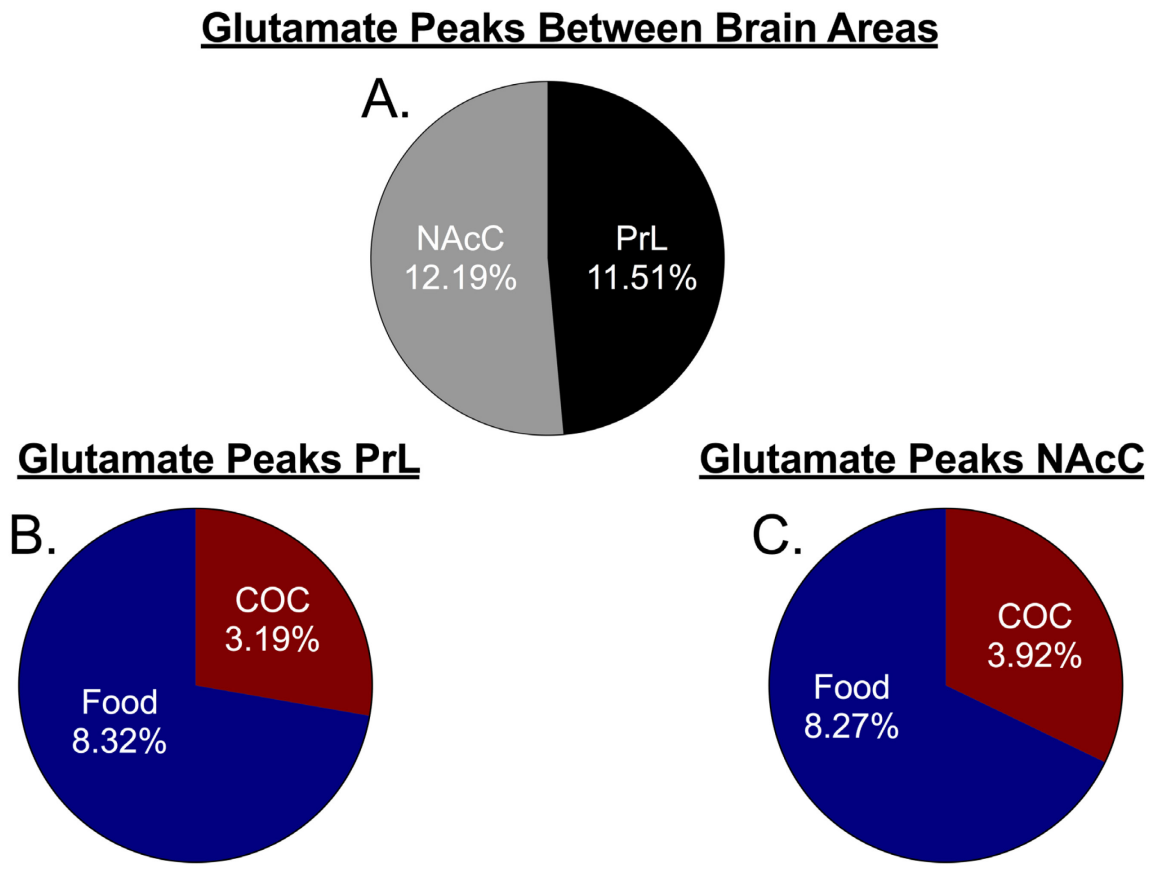


Figure 2.8 The Percentage of Glutamate Peaks Related to Behavioral Events Relative to The Total Number of Glutamate Peaks Found in Both Brain Regions

(A) Roughly an equal number of glutamate peaks were found to behavioral events between the PrL and the NAcC. **(B)** More glutamate peaks were found to food related events compared to COC related events in the PrL. **(C)** More glutamate peaks were found to food related events compared to COC related events in the NAcC. Note that the distribution of peaks to COC and food related events are roughly equal between brain regions.

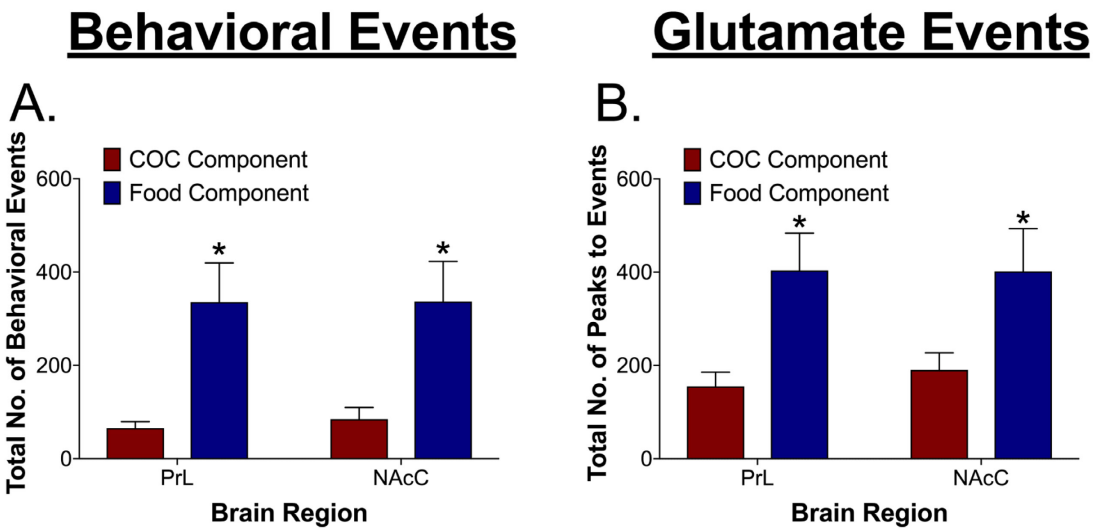


Figure 2.9 Total Number of Behavioral Events & Total Number of Glutamate Peaks to Behavioral Events During Components

(A) Significantly more behavior was exhibited during food components compared to COC components in rats with biosensors implanted in both brain regions. **(B)** Significantly more glutamate peaks were observed to food related events compared to COC related events in rats with biosensors implanted in both brain regions. LME, $*p < 0.05$. Data represented as mean ± SEM.

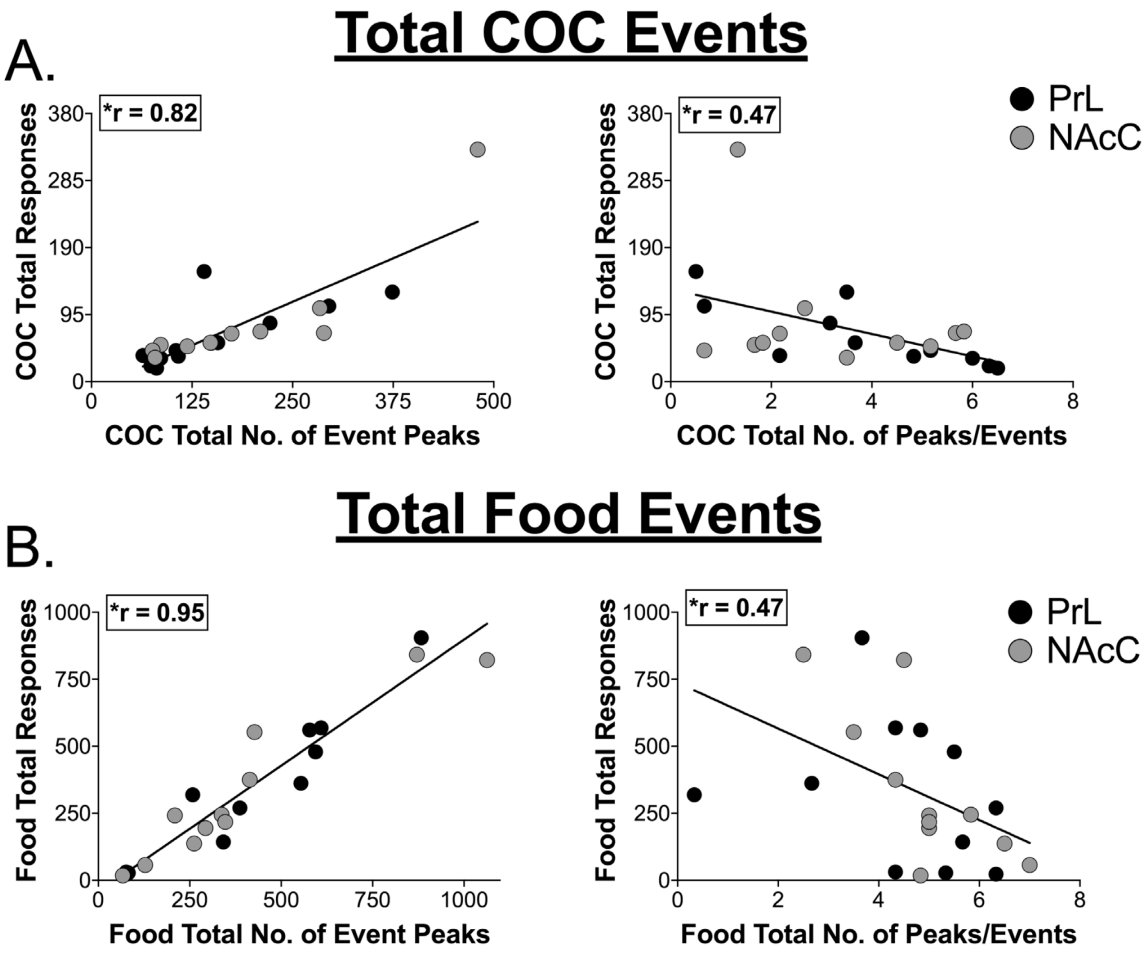


Figure 2.10 Correlation Between Total Number of Glutamate Peaks to Behavioral Events & Total Number of Behavioral Events

(A) The total number of glutamate peaks found to behavioral events for COC during a session and the total number of COC behavioral events that occurred in a session was significantly positively correlated (left); the number of glutamate peaks that occur per COC event was significantly negatively correlated with the number of COC responses (right). **(B)** The total number of glutamate peaks found to food behavioral events during a session and the total number of food behavioral events that occurred during a session were significantly positively correlated (left); the number of glutamate peaks that occurred per food event was significantly negatively correlated with the number of food responses (right). Note the difference in scale between COC and food. Linear regression, $*p < 0.05$.

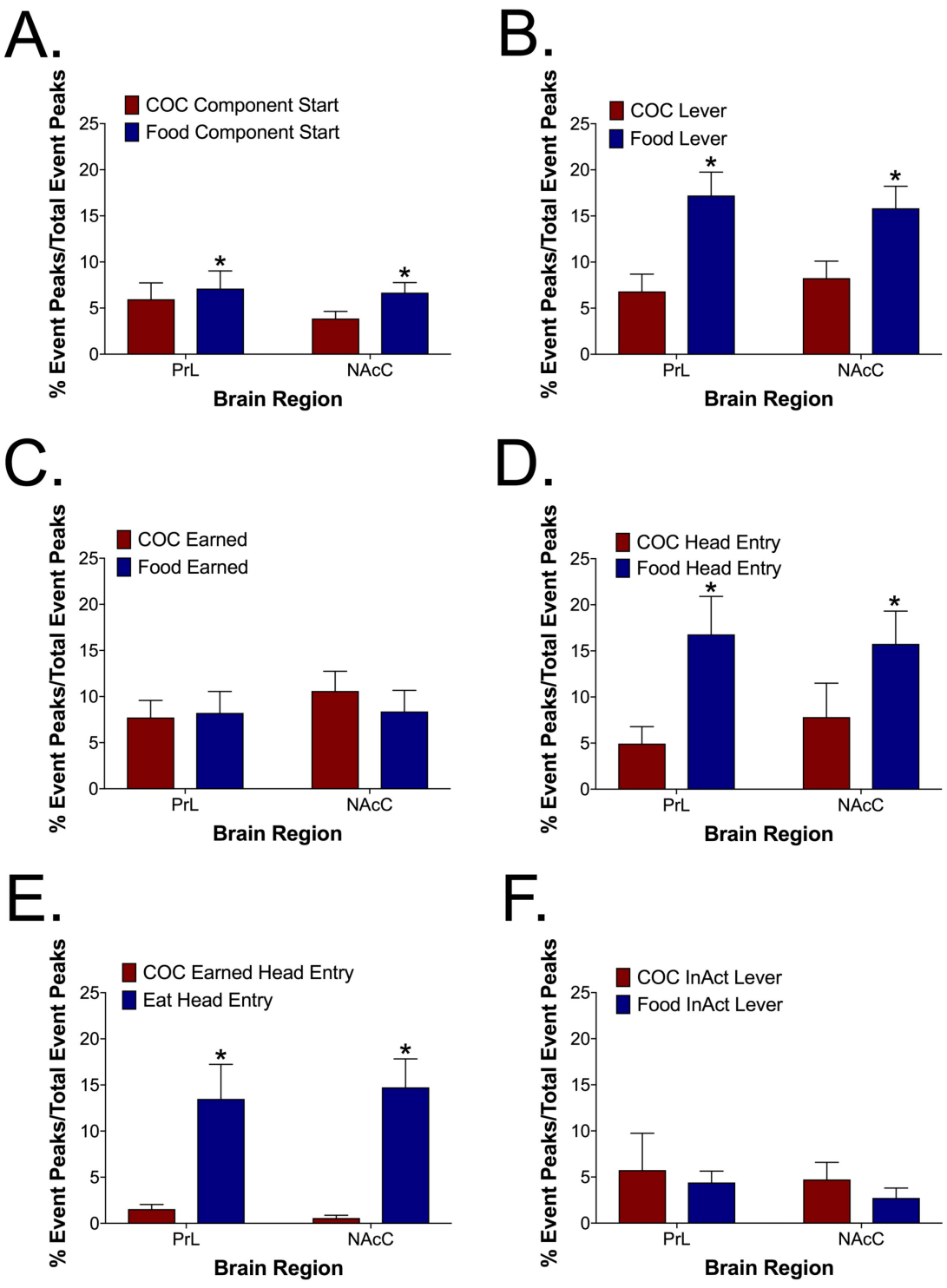

A.
COC Component Start
Food Component Start
% Event Peaks/Total Event Peaks
25
20
15
10
5
0
PrL
NAcC
Brain Region
B.
COC Lever
Food Lever
% Event Peaks/Total Event Peaks
Brain Region
C.
COC Earned
Food Earned
% Event Peaks/Total Event Peaks
Brain Region
D.
COC Head Entry
Food Head Entry
% Event Peaks/Total Event Peaks
Brain Region
E.
COC Earned Head Entry
Eat Head Entry
% Event Peaks/Total Event Peaks
Brain Region
F.
COC InAct Lever
Food InAct Lever
% Event Peaks/Total Event Peaks
Brain Region

Figure 2.11 Distribution of Glutamate Peaks To Behavioral Events

(A) The percentage of glutamate peaks that occurred was greater to the beginning of the food components compared to the beginning of the COC components. **(B)** The percentage of glutamate peaks that occurred was greater to responses on the food lever compared to responses on the COC lever. **(C)** There were no statistical differences in the percentage of glutamate peaks that occurred when COC was earned compared to when food was earned. **(D)** A greater percentage of glutamate peaks occurred to head entries into the food receptacle during food components compared to head entries during COC components. **(E)** A significantly greater percentage of glutamate peaks occurred to head entries related to eating compared to head entries after COC infusions. **(F)** No statistical differences were observed to inactive lever presses during the COC and food components. LME, $*p < 0.05$. Data represented as mean ± SEM.

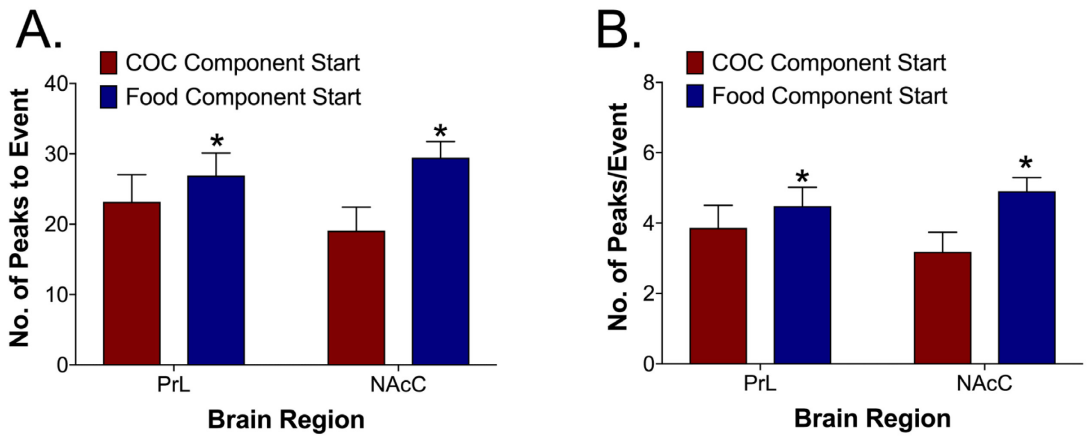
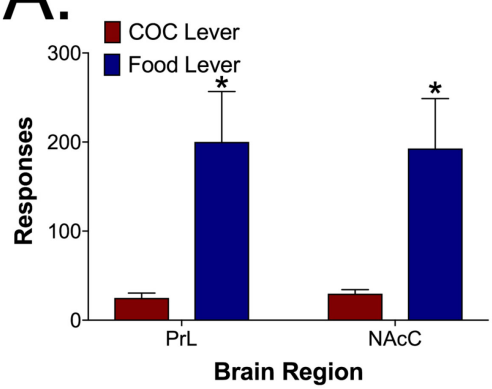


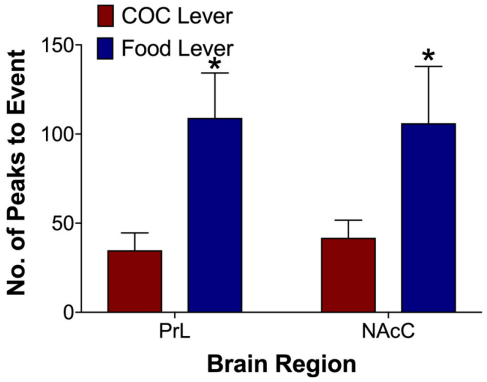
Figure 2.12 Number of Glutamate Peaks That Occurred To The Beginning of COC and Food Components

(A) Significantly more glutamate peaks occurred to the beginning of food components compared to the beginning of COC components. **(B)** Significantly more glutamate peaks per component occurred to the beginning of the food components compared to the beginning of the COC components. LME, $*p < 0.05$. Data represented as mean ± SEM.

Lever Responses

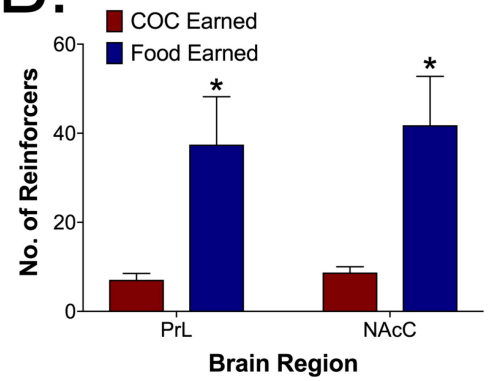
A.

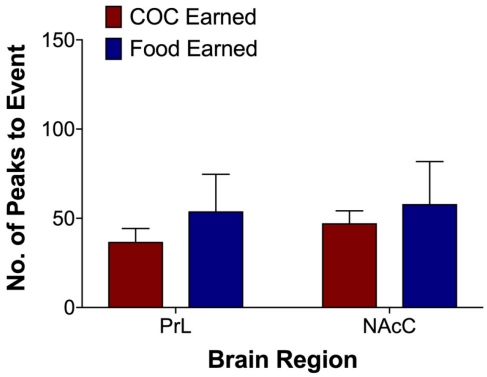




Reinforcer Earned

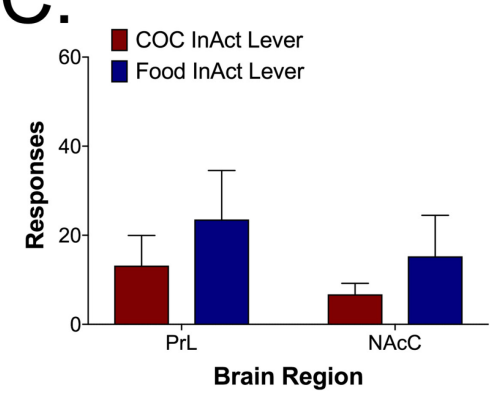
B.





Inactive Lever Responses

C.



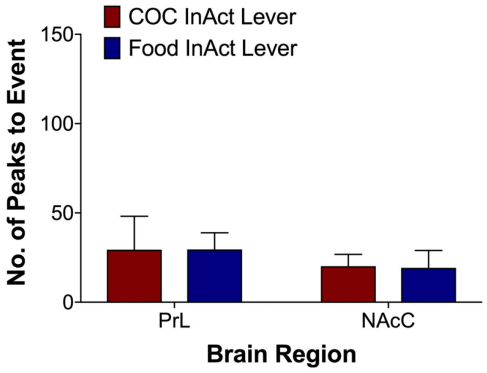
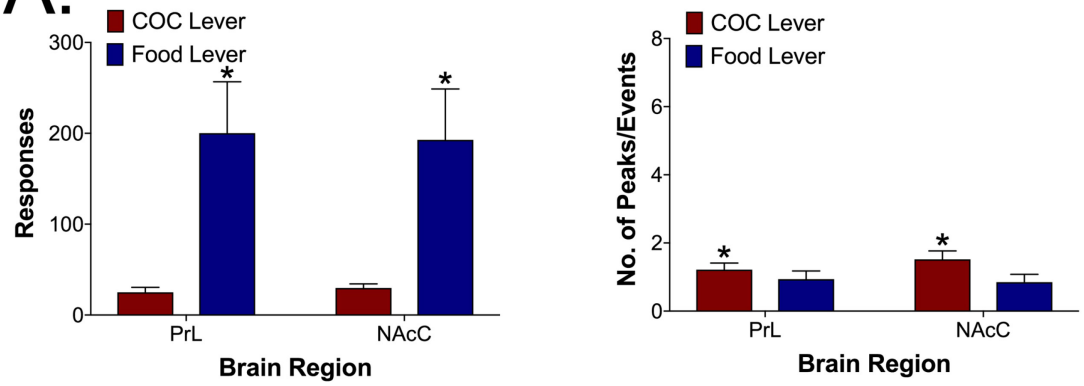


Figure 2.13 The Number of Glutamate Peaks That Occurred to Lever Presses, Reinforcers, & Inactive Lever Presses

(A) Significantly more food lever presses were observed compared to COC lever presses (left); a greater number of glutamate peaks occurred to food lever presses compared to COC lever presses (right). **(B)** Significantly more food reinforcers were earned compared to COC reinforcers (left); there were no differences in the number of glutamate peaks that occurred between COC and food reinforcers (right). **(C)** There were no differences in the number of COC or food inactive lever presses (left); there were no differences in the number of glutamate peaks that occurred between COC and food inactive lever presses (right). LME, $*p < 0.05$. Data represented as mean ± SEM.

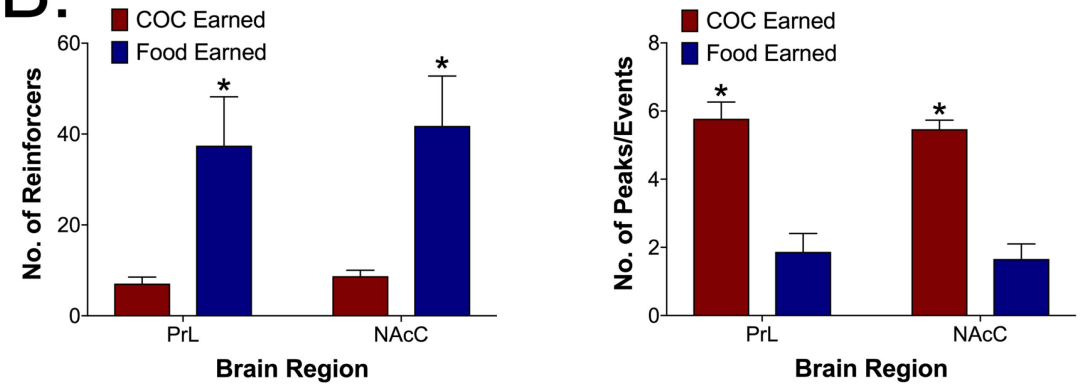
Lever Responses

A.



Reinforcer Earned

B.



Inactive Lever Responses

C.

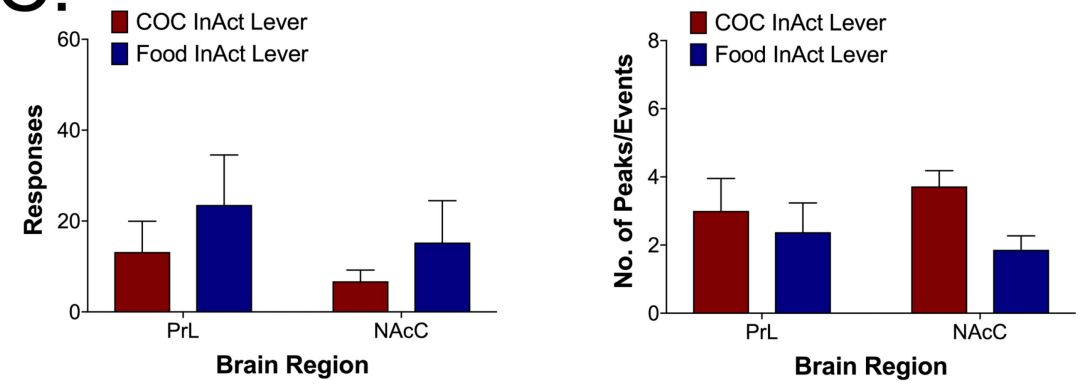


Figure 2.14 The Number of Glutamate Peaks That Occurred Per Behavioral Event for Lever Presses, Reinforcers, & Inactive Lever Presses

(A) A greater number of glutamate peaks occurred per COC lever press compared to per food lever press. **(B)** A greater number of glutamate peaks occurred per COC reinforcer compared to per food reinforcer. **(C)** There were no differences in the number of glutamate peaks that occurred per inactive lever press. LME, $*p < 0.05$. Data represented as mean ± SEM.

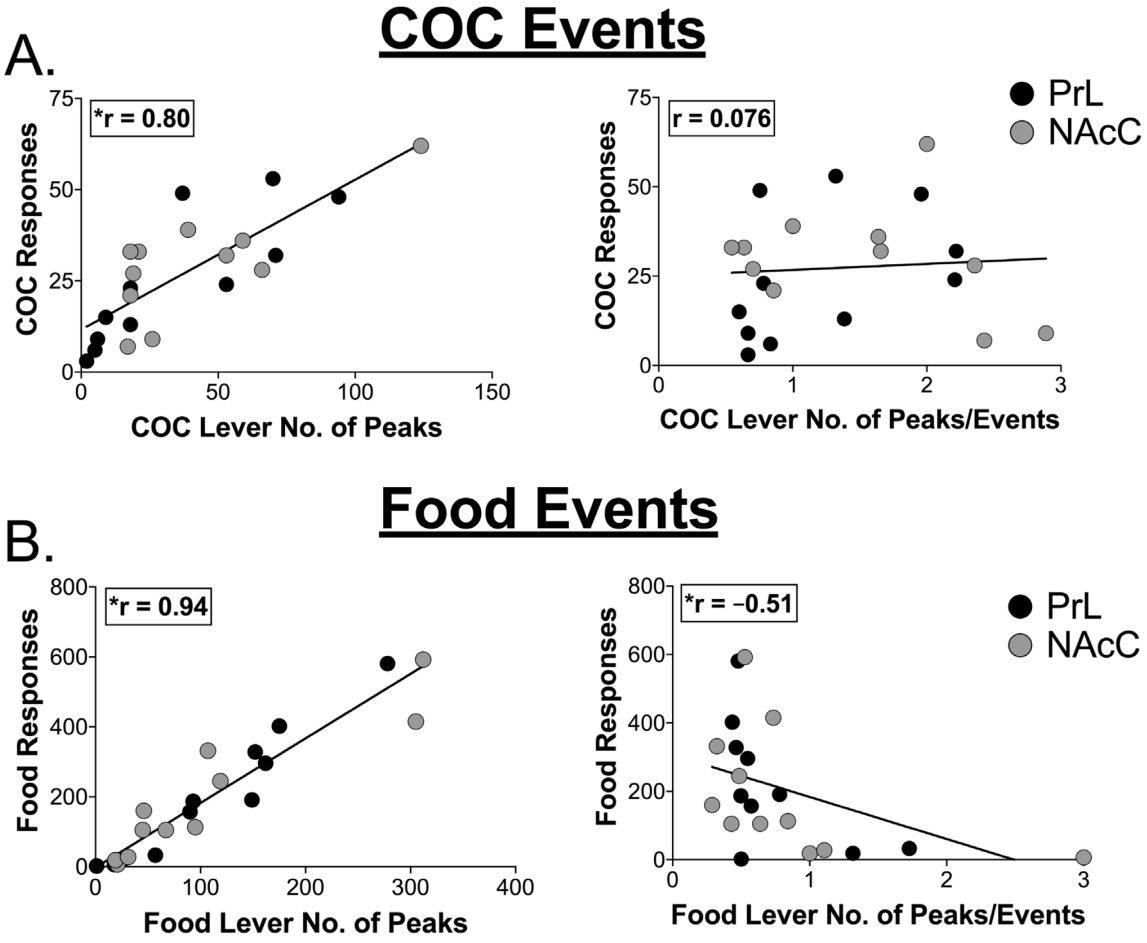


Figure 2.15 Correlations Between The Number of Glutamate Peaks & The Number of Lever Presses That Occurred for COC and Food Responses

(A) There was a significant positive correlation between the number of glutamate peaks observed and the number of COC events that occurred (left); there was no correlation between the number of glutamate peaks observed per COC lever press and the number of COC lever presses that occurred (right). **(B)** There was a significant positive correlation between the number of glutamate peaks that were observed to food lever presses and the number of food lever presses that occurred (left); there was a significant negative correlation between the number of glutamate peaks observed per food lever press and the number of food lever presses that occurred (right). Note the difference in scale between COC and food. Linear regression, $*p < 0.05$.

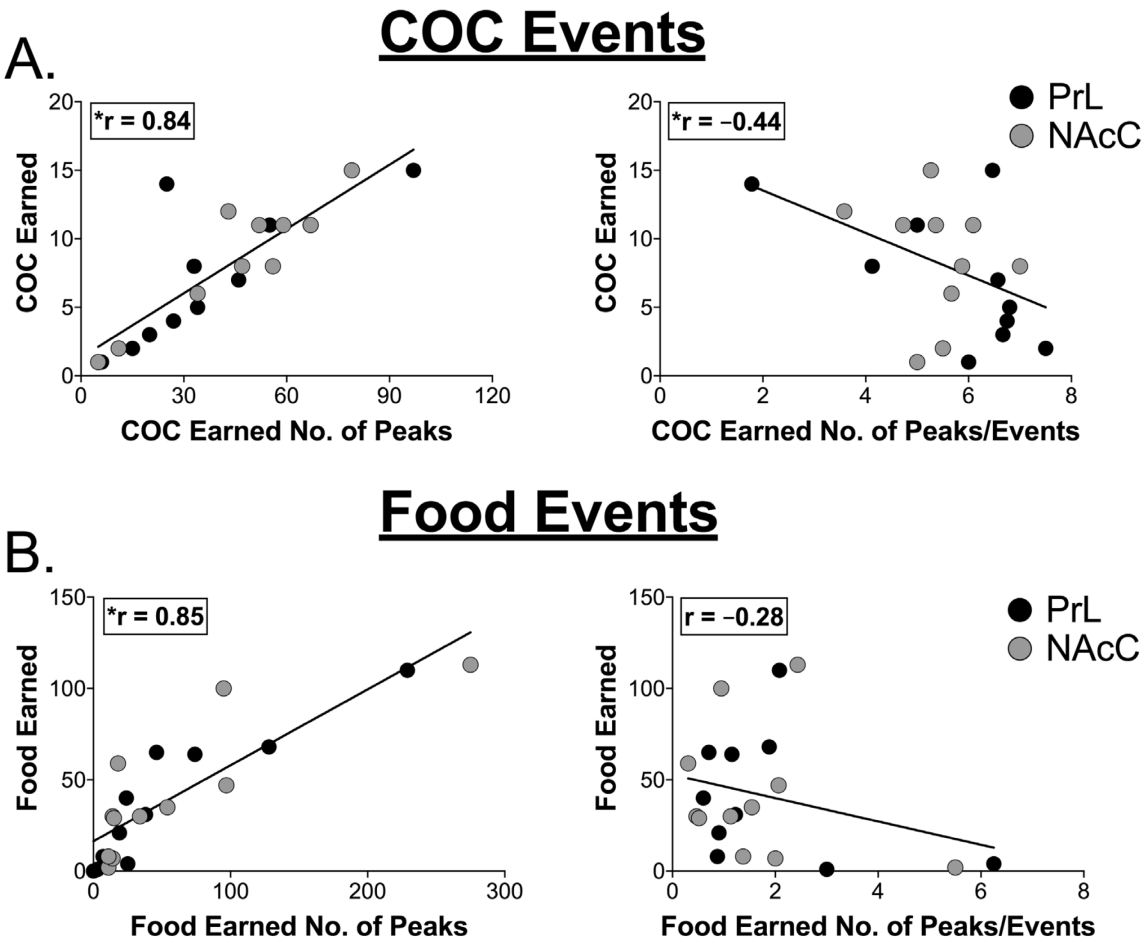


Figure 2.16 The Number of Glutamate Peaks That Occurred to COC and Food Reinforcers

(A) The number of glutamate peaks observed to COC reinforcers was positively correlated with the number of COC reinforcers earned (left); the number of glutamate peaks that occurred per COC reinforcer was significantly negatively correlated with the number of COC reinforcers that occurred (right). **(B)** The number of glutamate peaks observed was significantly positively correlated with the number of food reinforcers earned (left); there was no correlation between the number of glutamate peaks observed per food reinforcer and the number of food reinforcers earned (right). Note the difference in scale between COC and food. Linear regression, $*p < 0.05$.

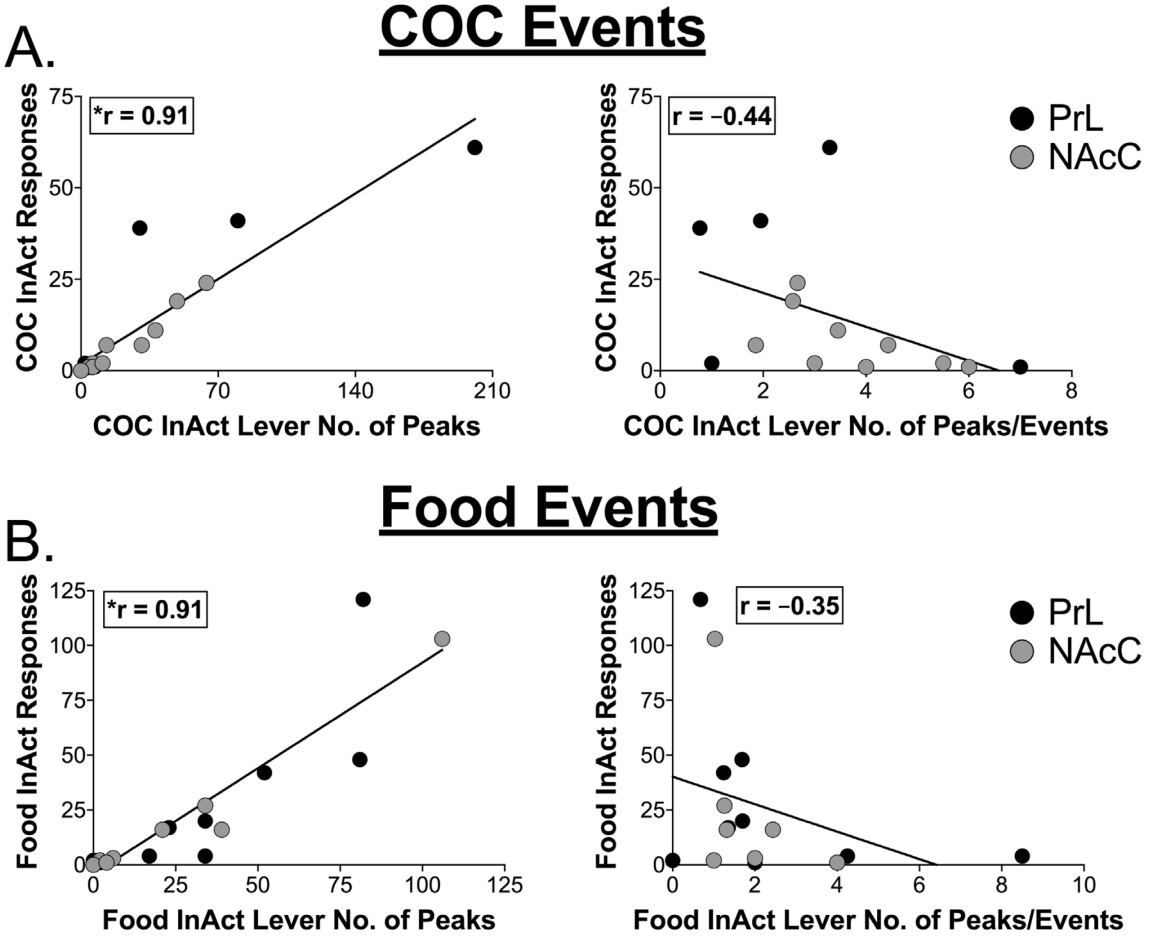
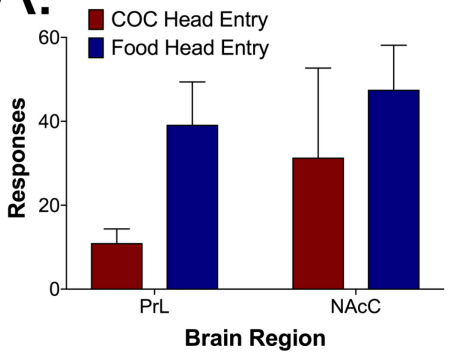


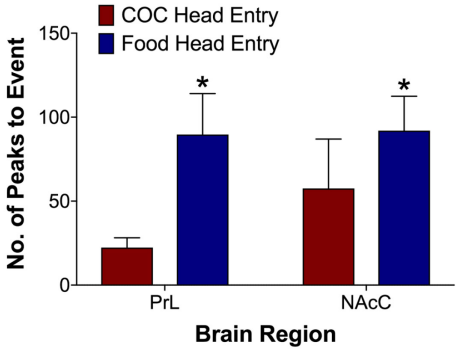
Figure 2.17 The Number of Glutamate Peaks That Occurred to COC and Food Inactive Lever Presses

(A) The number of glutamate peaks observed to COC inactive lever presses was positively correlated with the number of COC inactive lever presses that occurred (left); the number of glutamate peaks observed per COC inactive lever press was not correlated with the number of COC inactive lever presses that occurred (right). **(B)** The number of glutamate peaks observed was significantly positively correlated with the number of food inactive lever presses that occurred (left); there was no correlation between the number of glutamate peaks observed per food inactive lever press and the number of food inactive lever presses that occurred (right). Note the difference in scale between COC and food. Linear regression, $*p < 0.05$.

Head Entry Responses

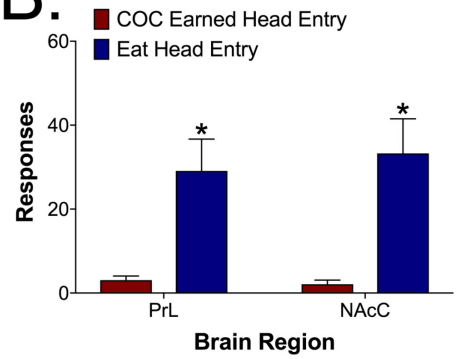
A.

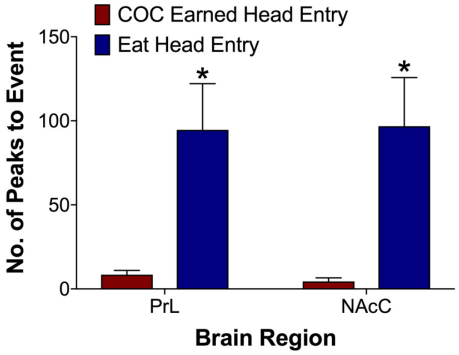




Reinforcer Head Entry Responses

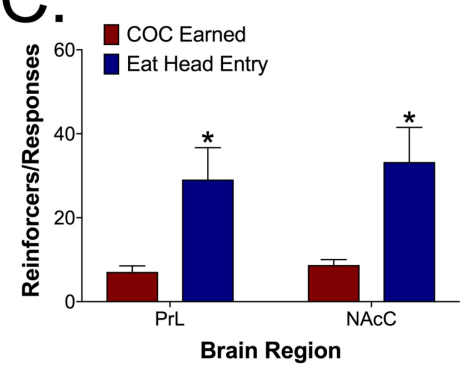
B.





Reinforcer Consumption

C.



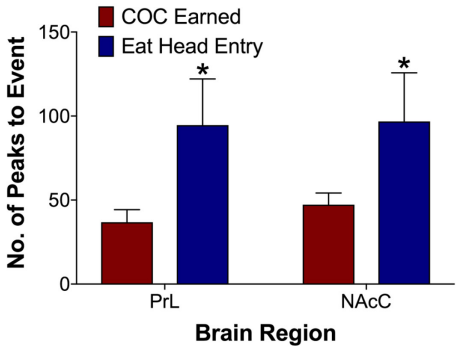
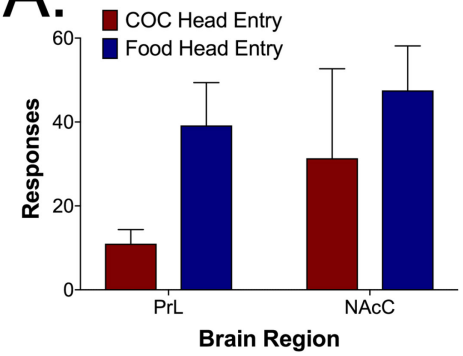


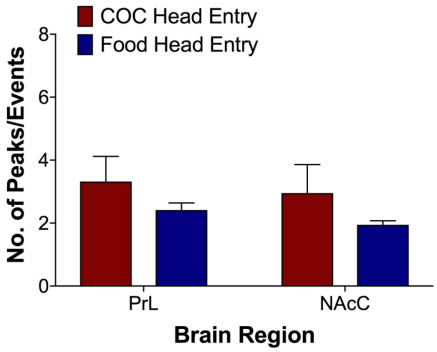
Figure 2.18 The Number of Glutamate Peaks That Occurred to Head Entries, Head Entries After Reinforcers, & COC Earned vs. Eating

(A) No differences were observed between COC and food head entry responses (left); the number of glutamate peaks that occurred was greater for food head entries compared to COC head entries (right). **(B)** Significantly more head entry responses occurred after a food pellet was delivered compared to when a COC infusion was earned (left); the number of glutamate peaks that occurred to head entries after a pellet was delivered was greater than head entries during/after a COC infusion (right). **(C)** Significantly more head entries occurred after a food pellet was delivered compared to the number of COC reinforcers earned (left); significantly more glutamate peaks were observed to eating responses than to COC infusions earned (right). LME, $*p < 0.05$. Data represented as mean ± SEM.

Head Entry Responses

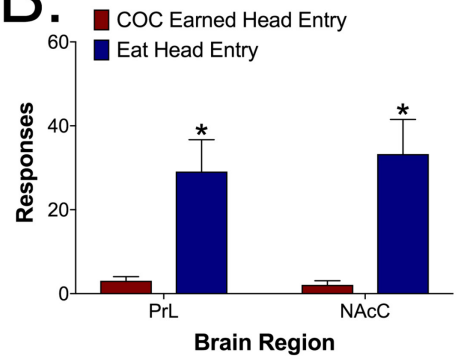
A.

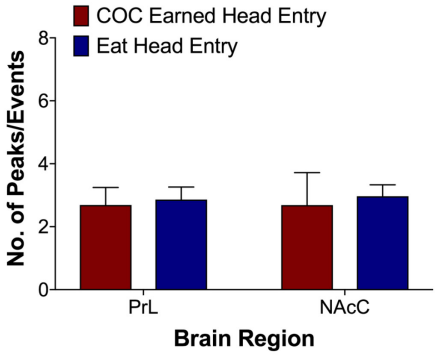




Reinforcer Head Entry Responses

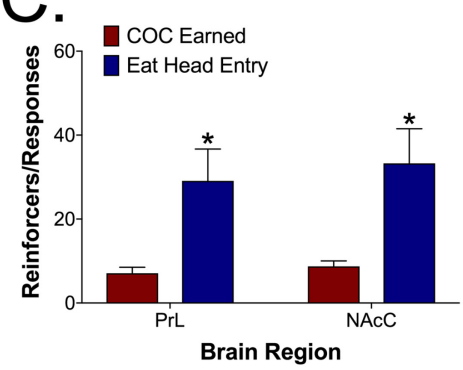
B.





Reinforcer Consumption

C.



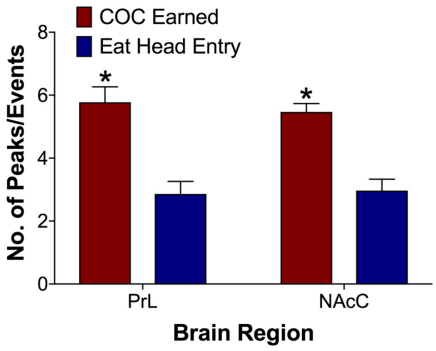


Figure 2.19 The Number of Glutamate Peaks That Occurred Per Behavioral Event for Head Entries, Head Entries After Reinforcers, & COC Earned vs. Eating

(A) No differences were observed for the number of glutamate peaks that occurred per head entry responses (right). **(B)** No differences were observed in the number of glutamate peaks that occurred per head entries after reinforcer delivery (right). **(C)** More glutamate peaks were observed per COC reinforcer earned compared to eating responses (right). LME, $*p < 0.05$. Data represented as mean ± SEM.

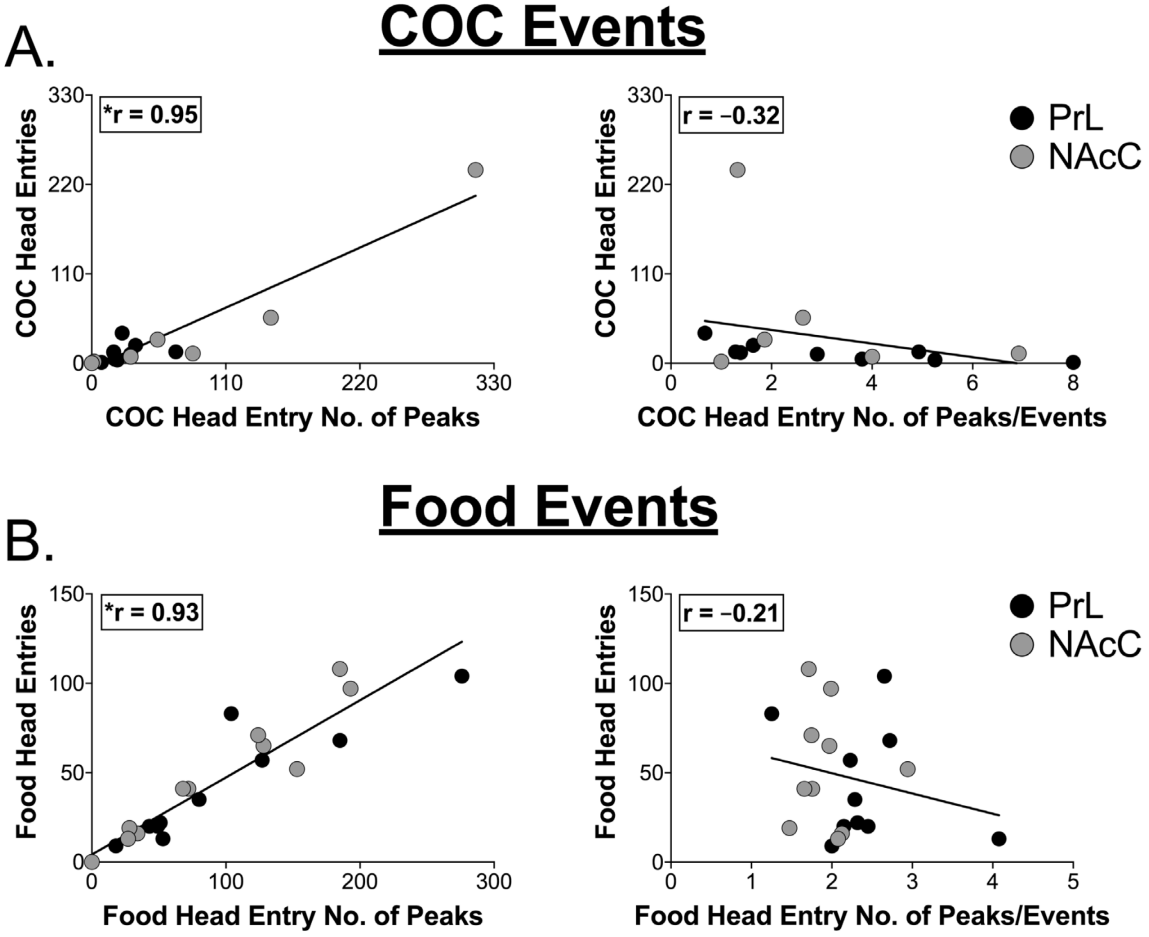


Figure 2.20 The Number of Glutamate Peaks That Occurred to COC and Food Head Entries During Components

(A) The number of glutamate peaks observed to COC head entries was positively correlated with the number of COC head entries that occurred (left); The number of glutamate peaks that occurred per COC head entry was not correlated with the number of COC head entries that occurred (right). **(B)** The number of glutamate peaks observed to food head entries was significantly positively correlated with the number of food head entries that occurred (left); there was no correlation between the number of glutamate peaks that occurred per food head entry and the number of food head entries that occurred (right). Note the difference in scale between COC and food. Linear regression, *$p < 0.05$.

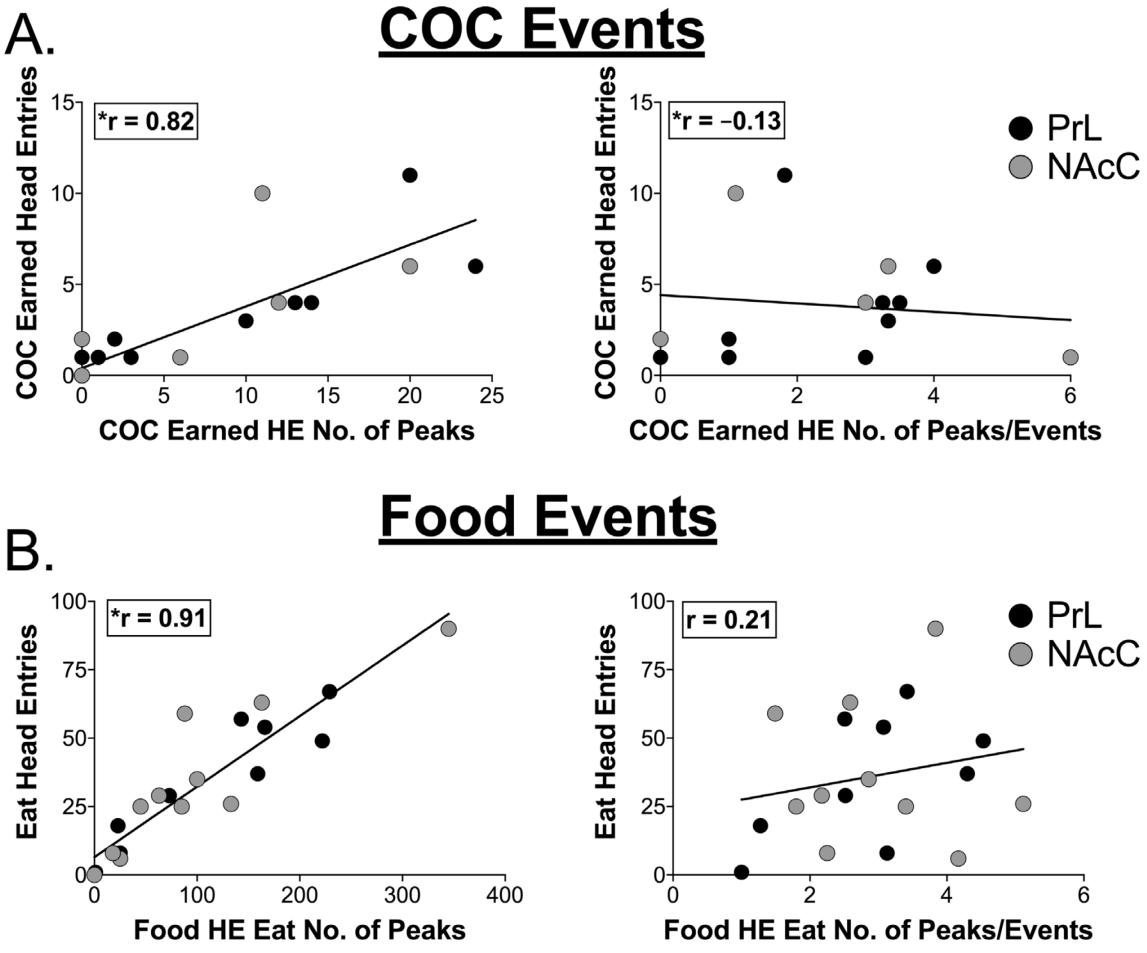
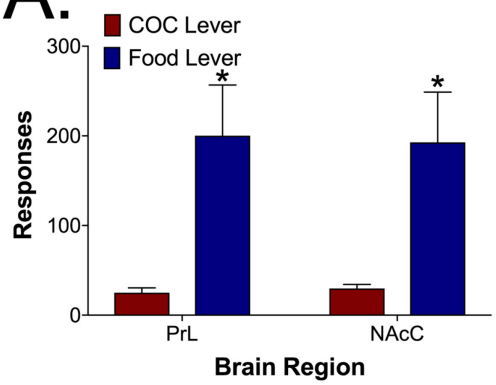


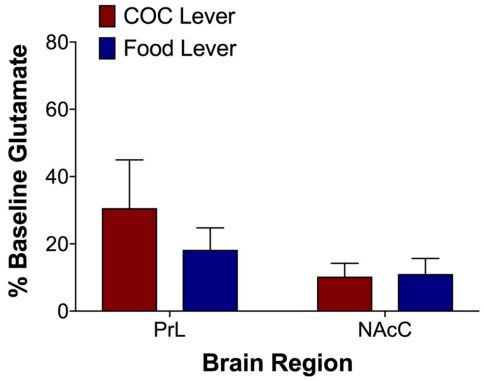
Figure 2.21 The Number of Glutamate Peaks That Occurred to COC and Food Head Entries After Reinforcer Delivery

(A) The number of glutamate peaks observed to COC head entries after COC infusions was positively correlated with the number of COC infusion head entries that occurred (left); The number of glutamate peaks that occurred per COC infusion head entry was not correlated with the number of COC infusion head entries that occurred (right). **(B)** The number of glutamate peaks to food pellet head entries was significantly positively correlated with the number of food pellet head entries that occurred (left); there was no correlation between the number of glutamate peaks that occurred per food pellet head entry and the number of food pellet head entries that occurred (right). Note the difference in scale between COC and food. Linear regression, $*p < 0.05$.

Lever Responses

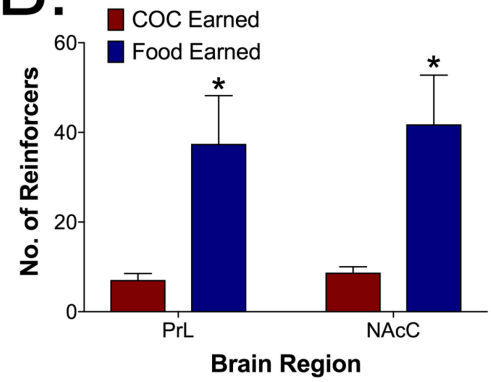
A.

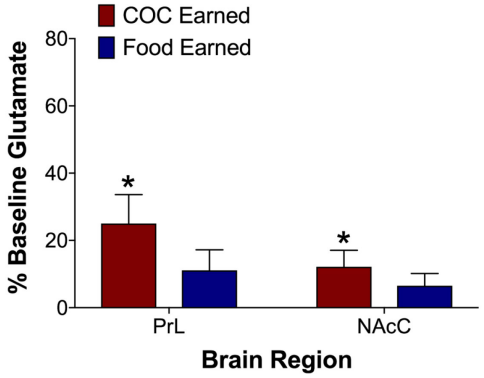




Reinforcer Earned

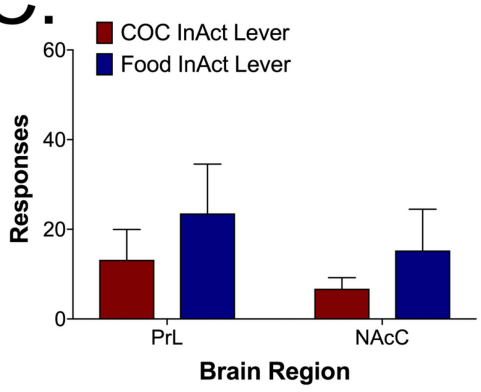
B.





Inactive Lever Responses

C.



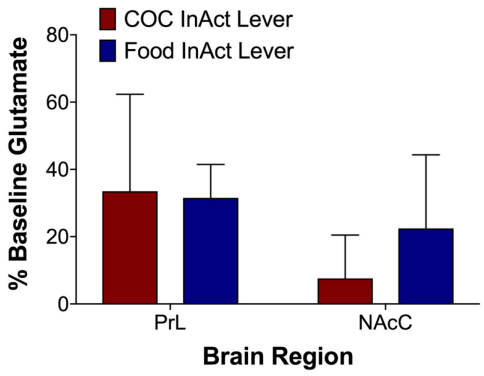
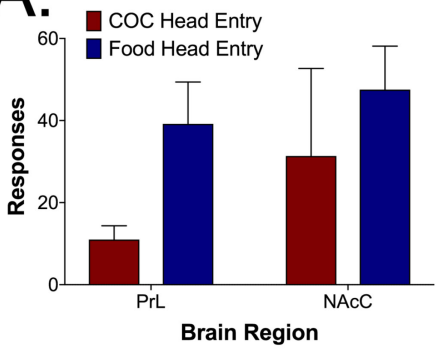


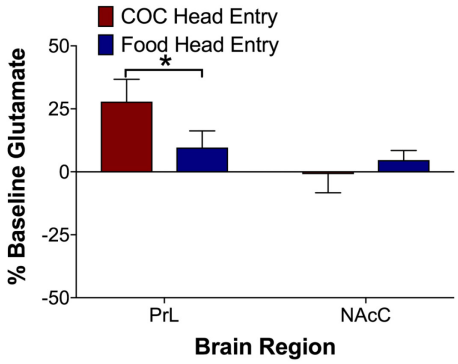
Figure 2.22 Percent Increase From Baseline For Glutamate Peaks to Lever Presses, Reinforcers, & Inactive Lever Presses

(A) No percent baseline glutamate differences were observed in relation to lever presses (right). **(B)** A greater percent increase from baseline was seen when the COC reinforcer was earned compared to the food reinforcer (right). **(C)** No percent baseline glutamate differences were observed in relation to inactive lever presses (right). LME, $*p < 0.05$. Data represented as mean ± SEM.

Head Entry Responses

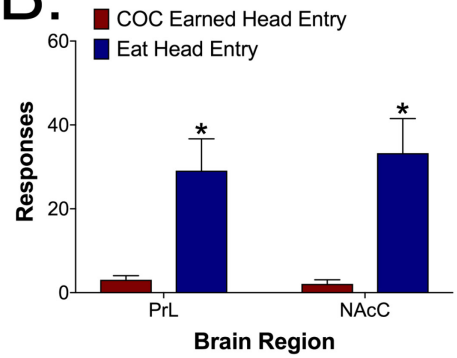
A.

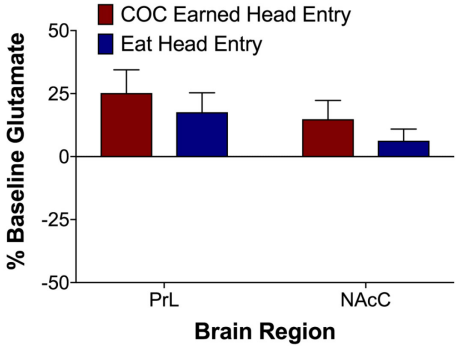




Reinforcer Head Entry Responses

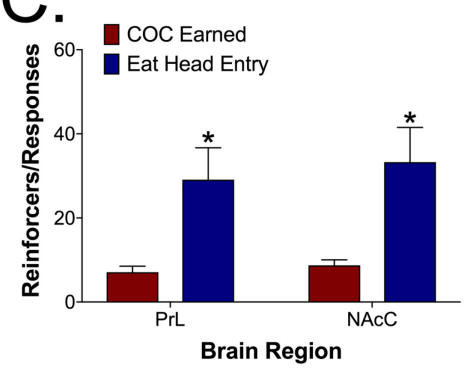
B.





Reinforcer Consumption

C.



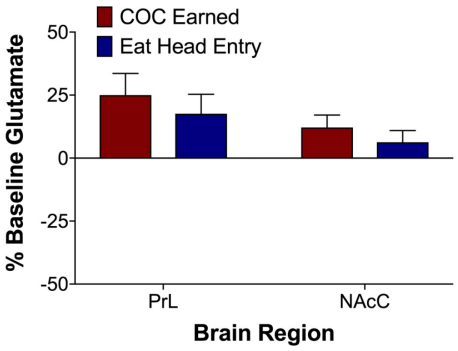


Figure 2.23 Percent Increase From Baseline For Glutamate Peaks to Head Entries, Eat/Infusion Head Entries, & COC Earned/Eat Head Entries

(A) A greater increase in the glutamate percent baseline was observed to COC head entries compared to food head entries in the PrL (right). **(B)** No percent baseline differences were observed to head entries after/during COC infusions or after a food pellet was delivered (right). **(C)** No differences in percent baseline glutamate measures were observed between COC earned and eat head entries (right). LME, $*p < 0.05$. Data represented as mean ± SEM.

CHAPTER 3

INTRODUCTION TO OXYGEN DYNAMICS AND DECISION-MAKING IN COCAINE-USE DISORDER

Choice Behavior in Cocaine-Use Disorder

In recent years, researchers have begun to use choice measures to assess the relative value of drug reinforcers in both animals and humans (Rush et al., 2010; Thomsen et al., 2013; Banks et al., 2015), promoting a translational understanding of mechanisms that govern drug-associated choice behavior. Furthermore, there is increasing evidence that the somewhat commonplace notion that substance abuse is governed by compulsive, habitual-like response relations that are insensitive to alternative consequences (Volkow and Morales, 2015) is incompatible with drug-associated choice behavior. For example, the current most effective behavioral and pharmacological treatments for human stimulant abuse disorders are based on offering either a non-pharmacological (contingency management; Schierenberg et al., 2012) or pharmacological (replacement therapy; Stoops and Rush, 2013) alternative to the abused stimulant, indicating sensitivity to alternative consequences. In preclinical models, conditions thought to produce compulsive, habit-like drug taking do not readily alter drug-associated choice behavior (Ahmed, 2010), and choice behavior is resistant to compulsive, habit-like response relations (Kosaki and Dickinson, 2010). Although it is clear that choice behavior is heavily linked to substance-use disorder and treatment sensitivity, the neurobehavioral mechanisms that govern drug-associated decision-making, including how the brain encodes and weighs valuations for both drug and non-drug reinforcers, is currently unknown.

Evidence suggests that both the limbic system and prefrontal areas of the brain are important in value-based decision-making (for review Kable & Glimcher, 2009). Specifically, the nucleus accumbens (NAc) and orbitofrontal cortex (OFC)

are particularly important in the creation and weighing of subjective value signals (for review Shultz, 1997; Padoa-Schioppa, 2007; O'Doherty et al., 2017). For example, electrophysiology recordings from the NAc show that different neuronal populations respond to natural rewards vs. cocaine when the two reinforcers are presented in isolation (Carelli, 2002). Further, choice data suggests that dopamine release in the NAc tracks choices that result in a higher magnitude reward (Sackett et al., 2017). Similarly, electrophysiology studies in non-human primates show that neurons in the OFC may represent both the 'offer value' and the 'chosen value' of two qualitatively different goods of varying magnitudes (Padoa-Schioppa, 2007). Further, choice data from rodents suggest that different neuronal populations in the OFC respond to saccharin vs. cocaine and that an increase in either of these populations firing rates precedes the chosen option (Guillem & Ahmed, 2017). Collectively, the aforementioned findings suggest that the NAc and the OFC are involved in value-based decision-making processes in general and in relation to choices involving drugs of abuse.

A general proxy for increased neuronal activity in a given brain region is increased oxygen consumption (Ogawa et al., 1992). Further, direct measures of oxygen in the rodent brain produce similar results as human studies using blood-oxygenated-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) thus showing translational viability (Francois et al., 2016; Li et al., 2016). For example, research from rodents where BOLD signals and oxygen measures were obtained simultaneously shows that these two measures are strongly correlated ($r = 0.8$; Lowry et al., 2010). Further, oxygen data collected in freely-moving rats shows that oxygen measures from the NAc are sensitive to reward receipt and changes in reward magnitude (Francois et al., 2012). Similarly, differential oxygen signaling is seen in the NAc and the prefrontal cortex to food predictive and non-predictive discriminative stimuli suggesting that oxygen measures are also sensitive to changes in reward-related events between brain regions (Francois et al., 2014). Thus, measuring oxygen should help further elucidate the role of certain brain regions in decision-making.

Herein decision-making processes are reviewed including mathematical models used to further understand choice behavior. Neurobiological mechanisms of choice are then discussed with a special focus on the role of the OFC. The use of oxygen as a measure of brain activity is then discussed. This chapter concludes by tying together decision-making processes with what is known about OFC function in cocaine abuse as well as briefly describing the proposed experiments.

Mechanisms of Choice Behavior: An Overview

A formal study of choice did not come about until Blaise Pascal published his work, *Pensées* (1670). However, it was not until Adam Smith published *The Wealth of Nations* (1776) that the field of study known as classical economics began. The ideas set forth in classical economics were then refined by Vilfredo Pareto (1906) whose work began the neoclassical revolution. Neoclassical economists such as Paul Samuleson (1938), von Neumann and Morgenstern (1944), and Hendrik Houthhakker (1950) further refined the ideas of early economists by making models of utility, choice, and preference more parsimonious. However, the later work of the psychologists Daniel Kahneman and Amos Tversky (1979) presented new ideas on choice behavior in an attempt to better explain discrepancies observed between neoclassical models and actual human decisions, ideas that later developed into the field of behavioral economics. Recently, the emerging field of neuroeconomics have used the principles of neoclassical and behavioral economics and taken them a step further by attempting to understand how the brain functions to make decisions.

Here the basics of what is known about how organisms make choices from an economic and psychological perspective are discussed. A specific focus is given to the idea of utility and the neoclassical framework (economic perspective) and the matching law (behavioral perspective) because these ideas serve as a foundation for this section of the dissertation. The neuroeconomic

view of choice is also discussed with a special focus on the role of the OFC in decision-making processes.

The Economic Perspective of Choice

There are many basic economic principles and economic theories geared toward understanding choice. To discuss all economic principles and theories is out of the scope of this dissertation. Thus, here I focus on two major economic components: utility and the general perspective of neoclassical economics.

Utility

Utility is defined as a hidden variable representing the amount of satisfaction a good or service generates (Glimcher, 2001). A utility function is a mathematical function that ranks alternatives according to their utility (Bernoulli, 1738). Historically, the utility function is represented as a monotonically increasing, decelerating function, although it is possible for this function to take on many forms (Glimcher, 2001). According to economic theory, utility cannot be directly measured considering it is a hidden variable (Bernoulli, 1738). Thus, at best, one may be able to say something about utility based on choices but not the other way around (Samuelson, 1938). Further, the utility of goods are considered ordinal in nature; thus, one can say that a certain good has more utility than another (based on preference) but cannot comment on how much more utility one good generates compared to another (Pareto, 1906). Nevertheless, utility is a basic component of economic theory and is a principle component of neoclassical economic models.

Neoclassical Models in Economics

Three major models are usually discussed when considering neoclassical economics: the Weak Axiom of Revealed Preferences (WARP), the Generalized Axiom of Revealed Preferences (GARP), and Expected Utility Theory. WARP, developed by Paul Samuelson in the 1930's, essentially states that if an economic agent chooses good A over good B and never chooses good B over good A (i.e. shows stable preference) then that subject can be described as behaving exactly as if a utility-like representation guided their choice (Samuelson, 1938; Glimcher, 2001; Glimcher et al., 2009). While this theory may

seem laughably simple it was critical in advancing neoclassical thought in that it made predictions about choice behavior and constrained the concept of utility (i.e. stated choice conditions in which you could construct an ordinal scale of utilities). Importantly, WARP created a more parsimonious framework for economics that could be used and extended upon to better predict a subject's choices.

In the 1940's Hendrik Houthhakker extended the ideas set forth in WARP by developing the GARP model. GARP assumes that choosers can never be satiated and posits that if a an economic agent prefers A over B and B over C then the same chooser indirectly prefers A over C (Houthhakker, 1950; Glimcher, 2001; Glimcher et al., 2009). Again, this model may seem extremely simple but it advanced existing theory in two major ways: (1) it allowed for economists to make choice predictions between pairs of objects that the subject had never been presented with based on previous choices and (2) showed that if a subject obeys the axioms of GARP it is the same as saying they have a monotonic utility function. Thus, GARP extended predictions to choice sets that subjects had yet to encounter and suggested the class of utility functions that were being used to guide these choices. However, there are still shortcomings of GARP, namely that it does not create a framework to understand choice under uncertainty.

Starting with essentially the same core features of GARP, von Newman and Morgenstern (1944) developed Expected Utility Theory in an attempt to describe choices under uncertainty. In developing their theory these economists added three additional pieces to the core axioms of GARP. First, these scholars defined the objects of probabilistic choice, which is formally called a "lottery" (von Newman and Morgenstern, 1944; Glimcher, 2001). In their terms, a lottery is composed of a probability and a value (e.g. 25% [probability] of gaining a single good [value]). Second, they proposed the continuity axiom, which states that if a chooser prefers A to B and B to C then preference should not change if there is a probability p of winning A to winning B with certainty plus C with a probability 1-p (von Newman and Morgenstern, 1944; Glimcher, 2001). The third axiom proposed was the independence axiom that states that if a chooser prefers some

probability of A to some probability of B then an irrelevant option added to both sides of the choice relation will not change preference (von Newman and Morgenstern, 1944; Glimcher, 2001). Expected Utility adds two major advances in understanding choices for those that follow its axioms: first, a chooser behaves as if they make choices by calculating the product of probability and utility; second, by observing the choices one makes between two goods at different probabilities it allows one to measure how much more one good is preferred over the other (e.g. if a chooser prefers 50% chance of A to 100% chance of B then they prefer A twice as much; Glimcher, 2001). This last point is important because it strengthens the cardinal notion of utility and mostly circumvents treating utilities as only ordinal objects (see Glimcher, 2001 for a discussion on this topic).

All three of these neoclassical theories have three major principles in common (principles that are seen in just about every aspect of modern economic thought). The first is that economic theories assume that subjects have stable preferences. Second, these theories assume that all economic agents are error-free choosers. Third, economists assume that choosers behave to maximize their utility given the behavior of others and thus yield equilibrium behavior (Santos & Chen, 2009). Thus, what these theories and their axioms propose is the idea of how a given economic agent should distribute their choices in a given setting. If said agent chooses in a way compliant with the axioms of these theories they are considered to be rational actors.

The Rational Decision-Maker

At a basic level an economic agent is considered to be rational if they show consistent and stable preferences between choice sets (Glimcher, 2001). The so-called "*homo economicus*" is assumed to take into account available information, probabilities of events, the potential costs and benefits in determining preference, and to consistently make choices that maximize their utility (for review Becker, 1976; Sen 2008; Blume & Easley, 2016). Specifically, two basic assumptions of the rational actor are that they have complete preferences (i.e. they either prefer A > B, B > A, or are indifferent [A = B]) and

show transitivity in their choices (i.e. if they prefer A > B > C then they will prefer A > C; for review Becker, 1976; Sen 2008; Blume & Easley, 2016). If an economic agent fails to comply with the axioms of the above theories or their assumptions then neoclassical economists view this agent as irrational in which case the above theories are falsified for that individual and cannot accurately describe their choice behavior (Glimcher, 2001; Santos & Chen, 2009). Even though the concept of the rational actor has taken the field of economics far, it is not without criticism on both philosophical and empirical grounds.

One weakness of the concept of the rational decision-maker is that if the subject does not follow the discussed axioms their choice behavior cannot be adequately described (for discussion see Glimcher, 2001). Thus, this leaves several situations in which choice behavior cannot be predicted. More importantly, empirical evidence suggests that, more often than not, economic agents do not exhibit rationality in their choices (see Santos & Chen, 2009 for a discussion of this topic). Thus, these issues suggested to scholars that new theories on decision-making needed to be developed in order to mitigate the aforementioned discrepancies. Generally speaking, it was scholars from the field of psychology that worked to this end.

The Psychological Perspectives of Choice

Upon the realization that consumers regularly violate standard economic assumptions about choice, scholars began to add concepts from psychology and sociology to current economic theory in an attempt to formulate new concepts to account for these violations. The study of decision-making from this perspective has become known as behavioral economics and is best exemplified by the work of Daniel Kahneman and Amos Tversky.

Another perspective on choice that sought to better describe the irrational behavior observed in economics is rooted in the field of behaviorism and is best exemplified by the work of Richard J. Herrnstein. Through laboratory observations, Herrnstein concluded that the relative rate of responding between two options was equal to the relative rate of reinforcement for those options, a concept he termed matching, and whose mathematical formulation has become

known as the matching law. Further, he described a theory that is an extension of the matching law termed melioration. Here the concepts inherent in behavioral economics are briefly discussed. However, most attention is given to the matching law and its extensions because of the central role it plays in this dissertation.

Behavioral Economics: Basic Concepts

Behavioral economics was born out of the idea that principles from psychology could improve the neoclassical models of choice behavior (Glimcher et al., 2009). Specifically, behavioral economics proposes models that impose limits on rational calculation, willpower, and self-interest and aims to codify those limits formally and explore their implications through experiments and mathematical theory (Glimcher et al., 2009). Three major components seem to make up the behavioral economic framework.

The first component of the behavioral economic framework was highlighted by Kahneman and Tversky (1979) with the development of prospect theory. Prospect theory sought to explain the discrepancies observed from expected utility theory concerning choices made under uncertainty. Prospect theory proposed to account for these discrepancies by: (1) framing the value of choices as either gains or losses and (2) framing these choices in a reference-dependent fashion. The implication from this theory is that decision makers naturally frame their choices as gains or losses from a particular reference point. The S-shaped form of this value function is such that the slope for gains is shallower than the slope for losses. Thus, a loss will decrease value more than an equal sized gain will increase value. The development of prospect theory eventually led to potential explanations for why choosers are loss-averse as well as helped to explain other phenomenon such as the reflection effect and the endowment effect (Kahneman & Tversky, 1979; Tversky & Kahneman, 1981; Kahneman et al., 1990).

The second component of the behavioral economic foundation deals with heuristics or the idea that people reduce the complex task of assessing probabilities and determining value to simple judgment rules (Tversky &

Kahneman, 1974). Generally, heuristics can work quite well; however, they can lead to systematic errors in judgments. An example is estimating distance based on the clarity of an object. Usually when an object is perceived as sharp it is thought to be closer than one that is seen as blurry. However, basing distance estimations simply off of how sharply an object appears can cause errors in judgment because several variables can affect an object's clarity (e.g. weather, light) thus causing an object to appear closer or further than it is in actuality. Considering this idea, it was thought that understanding how choosers use heuristics to guide their choices could provide another potential basis for better understanding decision-making.

The third component deals with social preferences or how choosers value choices when those choices affect other people (Glimcher at al., 2009). This interest in social preferences developed from the observation that self-interests hypotheses fail to predict choices when choosers are in strategic interactions (Cramerer & Fehr, 2006). Thus, it is thought that by understanding how choosers make choices when they are taking others' welfare in account could strengthen choice predictions in some arenas. Collectively, the goal of behavioral economics is to develop mathematical systems that take these components into account in order to explain empirical facts and make more accurate choice predictions.

The Matching Law

In 1961 Richard Herrnstein published his initial study providing evidence for matching. In this study Herrnstein gave pigeons choices between two concurrently available options with different variable interval schedules of reinforcement operating on each option. Herrnstein's independent variable was the rate of reinforcement delivery and his dependent variable was the number of responses for each option. He then compared the number of responses emitted and the number of reinforcers earned across each option. What he found was that the relative rate of responding for an alternative equaled the relative rate reinforcement obtained for that alternative, a relationship he termed matching. Herrnstein (1970) formally stated the aforementioned experimental results in

what become known as the matching law. The matching law follows the following form:

$$\frac{B_1}{B_1 + B_2} = \frac{R_1}{R_1 + R_2} \qquad (3)$$

where B_1 is the behavior (i.e. total responses) allocated to Alternative 1, B_2 is the behavior allocated to Alternative 2, R_1 is the number of reinforcers earned under Alternative 1, and R_2 is the number of reinforcers earned under Alternative 2.

Research shows that equation 3 does a good job of describing choice behavior when strict matching occurs. However, there are situations in which equation 3 does not adequately describe choice behavior (Baum, 1974,1979). One such situation is the occurrence of overmatching where an organism allocates relatively more behavior to the alternative that provides more reinforcement than would be predicted by equation 3 (Baum, 1979). Another example is undermatching, where relatively more behavior is allocated to the alternative that provides less reinforcement (Baum, 1974, 1979). Another example is bias, where the amount of behavior allocated to one alternative is higher or lower regardless of the rate of reinforcement for that alternative (Baum, 1974). Further, equation 3 was limited in describing choice behavior between options with varying reinforcer magnitudes and varying delays to reinforcement as well as in other choice scenarios (for review see McDowell, 2005). This fact prompted scholars to formulate an updated matching model in order to better describe choice behavior in the above situations. This model became known at the generalized matching law (Baum, 1974) and takes the form:

$$\frac{B_1}{B_2} = c\left(\frac{R_1}{R_2}\right)^a \qquad (4)$$

where B_1, B_2, R_1, and R_2 are the same as in equation 3, a is the slope, and c is the y-intercept. Note that the generalized matching law can be modified to the concatenated generalized matching law by adding the dimensions of reinforcer

magnitude, delay, and/or frequency of reinforcement in order to better describe choice situations in which these variables are manipulated (Baum & Rachlin, 1969; Rachlin, 1971; Killeen, 1972). Together, these two versions of the matching law have been shown to accurately describe choice behavior in a multitude of situations (see McDowell, 2005 for examples).

The matching law is an aggregate and descriptive model; thus, it can only describe choice behavior at the molar level and cannot characterize moment-by-moment choices (Corrado et al., 2009). This contrasts with the economic models discussed above, which are all normative, aggregate models that prescribe a pattern of behavior for the choices at hand (Corrado et al., 2009). However, the matching law and the economic models discussed above do share similarities in that they are both relativistic and that the matching law, in some scholar's opinions (and this is contentiously debated), is consistent with utility maximization (see Herrnstein, 2000 for a discussion of this topic). Nevertheless, there is evidence that the matching law and its extensions better describes choice behavior that is considered irrational by neoclassical economic theory (Herrnstein, 2000).

In summary, the matching law is a is a descriptive, aggregate model that characterizes choice behavior by focusing on the consequences of one act relative to another and how choices are allocated based on these consequences. However, even though this model is generally thought of as aggregate, with a few simple extensions, the matching law can be extended to describe molecular (local) choice behavior (e.g. Herrnstein & Prelec, 1991). This is a point discussed in the next section.

Melioration: The Molecular Mechanism of Matching

Melioration can be thought of as the dynamic, choice-by-choice process that leads to matching behavior on an aggregate molar scale (Herrnstein, 2000). In essence melioration is thought to be the mechanism that gives rise to matching (Commons et al., 1982). Simply stated, melioration is the concept that, given a choice between two options, the option with the higher rate of local reinforcement will be chosen (Herrnstein & Vaughan, 1980). For example, given

two alternatives, if B_1 earns a higher rate of reinforcement than B_2 behavior will shift toward B_1. If the time distribution between B_1 and B_2 earn equal rates of reinforcement then equilibrium (matching) has been reached. However, if B_1 earns more reinforcement at all time allocations, then at equilibrium B_1 replaces B_2 (Commons et al., 1982). Formally this relationship can be expressed as:

$$R_D = \frac{R_1}{t_1} - \frac{R_2}{t_2} \qquad (5)$$

where R_D is the difference between local reinforcement rates for the behavior of two alternatives, R_1 is the local reinforcement obtained from alternative 1, R_2 is the local reinforcement earned from alternative 2, t_1 is the time allocated to alternative 1, and t_2 is the time allocated to alternative 2. Note that when $R_D > 0$ time allocation shifts to t_1, when $R_D < 0$ time allocation shifts towards t_2, and when $R_D = 0$ equilibrium is reached. In the scenario where $R_D = 0$ then:

$$\frac{R_1}{t_1} = \frac{R_2}{t_2} \qquad (6)$$

thus, equation 4 is the matching law for time allocation between the two alternatives (Commons et al., 1982).

Theoretically, melioration and maximization theory from economics are similar in several respects. Both are hedonic, utilitarian theories to the extent that behavior is assumed to be driven by psychological consequences. Further, both theories involve adapting choice behavior in such a way that betters the state of the chooser (Commons et al., 1982; Herrnstein, 1990). However, the theories differ in that melioration calls for responding only to the difference in local reinforcement between options whereas maximization requires the selection of the largest aggregation of reinforcement across choices (Commons et al., 1982; Herrnstein, 1990). Evidence also suggests that melioration does a better job of predicting behavior particularly of the type considered irrational from a

neoclassical economist's point of view (Herrnstein, 1990; Herrnstein, 2000). That being said, as with all theories, melioration does have its limitations.

One limitation of melioration, as with many behavioral psychology theories, is that most studies employ animals as experimental subjects in a controlled laboratory setting (Herrnstein & Heyman 1979; Herrnstein & Vaughan, 1980; Mazur, 1981; Vaughan, 1981). Thus, it is possible that the process of melioration does not generalize to humans behaving in the marketplace. However, as Kagel et al. (1995) discussed, it is likely that a theory that does not accurately make predictions in more simplistic choice situations will not generalize to more complex choice situations. Thus, by experimenting with animals one can, at the very least, rule out inadequate theories. That being said, there is evidence that human behavior is consistent with the theory of melioration and matching (Tunney & Shanks, 2002). Thus, by showing that a theory holds up across many different species, it is likely that that the theory describes a basic process inherent to all organisms (Kagel et al., 1995).

There are also psychophysical limitations to melioration. Considering melioration assumes that an organism compares local rates of reinforcement it requires the organism to transform objective local rates into subjective local rates (Commons et al., 1982). The parameters of this transformation are not known; thus, there may be between-subject variability in this transformation that result in different equilibrium states even though the organism is meliorating and matching (Commons et al., 1982). In turn, an absence of knowledge about this transformation process could decrease the predictability of this theory.

There are also limitations on the ability of melioration to describe choice behavior across choices that result in different classes of reinforcement (Commons et al., 1982). With a few exceptions (e.g. Miller 1976) melioration has mostly been studied using reinforcers of single classes, which has prompted some scientists to suggest that it cannot be extended to describe choice situations across different types of commodities (Hursh, 1978; Rachlin et al., 1981). However, recently, there have been experiments showing that matching behavior holds when choice options include different classes of reinforcers (e.g.

Hutsell et al., 2015; Beckmann et al., 2019) suggesting that this theory likely will generalize to these types of choice situations.

In summary, melioration is thought to be the dynamic process that leads to matching behavior on an aggregate scale. From a theoretical perspective melioration has fundamental similarities and differences with maximization theory. Specifically, melioration is able to describe behavior in certain choice situations that neoclassical economic theories fail to describe. This has led to the general idea (supported by laboratory evidence) that organism allocate their behavior optimally when doing so also satisfies melioration and matching and that when matching and optimization make divergent predictions, behavior is often closer to matching than it is to maximization (Herrnstein, 1990).

Neuroeconomics: Decision-Making and The Brain

Neuroeconomics is an emerging field that uses principles from economics, psychology, and neuroscience in order to better understand and predict economic decisions (Zak, 2004; Glimcher et al., 2009). Neuroeconomic studies have been conducted in order to better understand decision-making under uncertainty, during games, and in the realm of finance (just to name a few; Glimcher et al., 2009; Frydman & Cramerer, 2016). Generally, all of this research aims to answer three basic questions: (1) how does the brain assign value to the objects of choice?, (2) how does the brain compare these valuations?, and (3) how is this value information used to produce choices? (Glimcher, 2011). What follows are the basics of what are known about these three processes. This section ends with a specific discussion of the role of the OFC in choice behavior considering the scope of this dissertation.

How Might The Brain Encode Value?

Evidence suggests that the brain utilizes different valuations systems depending on the type of options being considered (Rangel, 2009). The three valuation systems generally discussed are those that calculate Pavlovian valuations, habit valuations, and goal valuations (Balleine et al., 2009). Pavlovian valuations are those that are computed in situations where outcomes occur independent of any action, habit valuations are those computed in stimulus-

response situations, and goal valuations are those computed in situations where we are concerned with the outcome of an action (Balleine et al., 2009). All of these valuations systems have specific advantages and play a role in economic decisions (Rangel, 2009); however, to date most research has focused on goal-directed actions and their valuations (Kable & Glimcher, 2009). Due to the larger body of research and the scope of this dissertation, only goal-directed valuations and actions will be generally discussed.

The best way to begin to consider the concept of goal-directed value (or valuation in general) at the level of the brain is to start with a simple thought experiment: how would you choose between one apple and four apples? If it is assumed that more is better than less then, on a neural level, the magnitude of these commodities just need to be represented and compared (Levy & Glimcher, 2012). However, what if you were faced with choosing between a few fluid ounces of water and two apples? In this case, one cannot just simply compare the magnitude between two commodities in order to make a decision but instead has to take into account the commodity types, their magnitudes, as well as several different attributes (taste, health benefits, metabolic state, etc.; Levy & Glimcher, 2012). Theoretically, all of the attributes for a given commodity have to be transformed into a single value signal and the value signals between commodities have to be represented on a common scale in order for comparisons to be made and choices initiated (Glimcher, 2011; Levy & Glimcher, 2012).

Work has shown that the medial prefrontal cortex (mPFC) and the ventral striatum encode the subjective value of both immediate and delayed rewards on a common scale (Kable & Glimcher, 2007). Evidence has also shown that activity in the mPFC and striatum correlate with choices associated with gains and losses (Tom et al., 2007). Note that similar findings have also been observed in choices involving risk and ambiguity (Levy et al., 2009). Evidence also suggests that neurons in the OFC encode the subjective value of goods on a common scale (Padoa-Schioppa & Assad, 2006) whereas the striatum may be more involved in encoding the subjective value of actions (Lau & Glimcher, 2008). This

difference in goods- vs. action-based valuations has raised the question of how goods-based subjective value and action-based subjective value are used by the brain to guide choice. There is evidence that these two valuations are interconnected (Horwitz et al., 2004); however, this sentiment remains controversial and has yet to be resolved (Padoa-Schioppa & Assad, 2006; Padoa-Schioppa & Assad, 2008). Nevertheless, regardless of the controversy on how specific valuations are interconnected (including the role of specific brain regions), there is general agreement that dopamine signaling is involved in value encoding (Balleine et al., 2009; Schultz, 2009)

In his seminal paper, Wolfram Schultz and colleagues (1997) showed that dopamine signaling in the midbrain reflected information about the value of rewards through what he called 'reward-prediction errors'. In fact, data has shown that the firing rate of dopamine cells is linearly correlated with model derived reward prediction errors (Bayer & Glimcher, 2005). This evidence suggested that the dopamine system might be responsible for encoding a teaching signal that can be used to learn the subjective value of actions based on past experience (Schultz, 2009). For example, in studies where conditioned cues predict rewards of different magnitudes or probabilities, the dopaminergic response scales with magnitude or probability (Fiorillo et al., 2003; Tobler et al., 2005). Further, dopamine neurons project to brain regions shown to be important in evaluating choices (e.g. Haber, 2003). Thus, taken together, research suggests that the dopamine system is critical for learning the subjective value of goods and actions (Kable & Glimcher, 2009). However, how dopamine signaling and 'reward-prediction errors' work in different choice scenarios and in different brain regions in relation to value is not fully understood (Balleine et al., 2009).

To summarize, it is generally agreed upon that value has to be computed and compared on a common scale in order for choices to be made. Evidence suggests that the dopamine system and brain regions such as the striatum and frontal cortex are important in encoding subjective value. However, it is not clear yet how all of these aspects coherently and cohesively function together to encode value.

How Might The Brain Compare Values and Initiate Choice?

Theoretically, in order for a choice to be made, values must be compared and the highest value selected. It has been shown both behaviorally and through economic models, that this process is somewhat stochastic in nature (McFadden, 1974). For example, if two objects of choice have similar subjective values the object with the least value is occasionally selected. This begs the question of how, in the brain, values are represented, compared, and passed on to motor circuits to initiate choice. Some of the most interesting work attempting to answer this question has come from studying the primate saccadic system in perceptual choice.

Evidence in monkeys shows that, when presented with two options in different locations, activity in the superior colliculus increases in the area of the topographic map associated with the location of the more valued option while the activity in all other areas decreases (Glimcher & Sparks, 1992). This suggests a winner-take-all mechanism for action selection in this area (Van Gisbergen et al., 1981). Further, when the two options were of equal value there was only weak activity in both areas (Glimcher & Sparks, 1992). Subsequent studies have shown that activity in these two movement sites, before a period of burst of activity, was graded. For example, if the probability that a saccade would yield reinforcement was increased or decreased then firing in this area would increase or decrease, respectively, before any action was taken (Basso & Wurtz, 1998; Dorris & Munoz, 1998). This observation led to further research where the activity of the lateral intraparietal cortex (LIP; an area upstream of the superior colliculus) was recorded when monkeys were given a choice between two options that varied in the magnitude and probability of reinforcement.

Researchers found that activity in area LIP, before the burst of collicular activity discussed above, was a linear function of both probability and magnitude (Platt & Glimcher, 1999). This work led to the idea that the fronto-parietal network of saccade control areas formed a topographic map of value for each saccade (Kable & Glimcher, 2009). Thus, the choice mechanism for saccades is thought be a competitive process where the area of the topographic map associated with

the most neural activity initiates the choice for that option. Further work has concluded that firing rates in these brain areas encode the subjective value for a particular saccade relative to other saccade options (Dorris & Glimcher, 2004). These data suggest that firing rates in the fronto-parietal regions are not 'menu invariant' like those in the OFC and striatum. Thus, the OFC and striatum may encode absolute subjective values whereas the fronto-parietal areas rescale these absolute values (presumably through some normalization mechanism) in order to magnify the difference between the two options before a choice is initiated (Kable & Glimcher, 2009 for discussion).

Evidence also supports the idea that activity in area LIP carries stochastic information about the likelihood that a given saccade would yield reinforcement (Shadlen et al., 1996; Shadlen & Newsome, 2001). Further it has been shown that firing rates in area LIP increase as more information about whether or not a given option will be reinforced increases. However, this increase in activity seems to be bounded (Roitman & Shadlen, 2002; Churchland et al., 2008); thus, once firing rates reach a certain threshold, a choice is initiated. Research has shown that this threshold represents a value threshold for movement selection (i.e. choice initiation; Kiani et al., 2008). Thus, when the value of any saccade reaches the value threshold, that saccade is immediately initiated. This line of work has also shown that the intrinsic stochasticity of this neural system gives rise to the stochasticity observed in choice behavior (Shadlen et al., 1996; Shadlen & Newsome, 2001).

Together this research highlights two types of choice mechanism depending on the type of choice situation. In reaction-time types of scenarios it appears that the threshold mechanism predominates such that when a given value is reached, a choice is initiated. However, in economic-style scenarios (where reaction time is not a factor) the winner-take-all mechanism seems to predominate. Note that there are choice situations where both of these mechanisms are at work (Lo & Wang, 2006, Wong & Wang, 2006; Wang, 2008). However, exactly how these different systems work to compare value and initiate choices is not fully understood at this time.

The Role of The OFC in Valuation and Choice

The OFC was first thought as being important in decision-making processes when it was observed that people with OFC lesions perform abnormally in gambling tasks and have choice deficits in simple preference tasks (Rahman et al., 1999; Fellows & Farah, 2007). Further, evidence from imaging studies showed that the OFC was more active in situations that involved choice oppose to those that did not (Arana et al., 2003). Early electrophysiology studies also showed that neuron firing patterns in the OFC were sensitive to qualitative differences between goods, motivational states (e.g. hunger, satiety, etc.), and to the magnitude between goods (Thorpe et al., 1983; Rolls et al., 1989; Wallis, 2007). Further, lesion studies have shown that decreased OFC function is associated with increased violations in transitivity and causes a decreased sensitivity to reinforcer devaluation (Camille et al., 2011; West et al., 2011). Studies have also collected evidence suggesting that neurons in the OFC encode the subjective value of goods during economic choice.

In one experiment, monkeys had a choice between two different juices of varying magnitudes and the subjective values of the options were calculated from their choices (Padoa-Schioppa & Assad, 2006). It was found that when the two choices were presented, OFC neurons fired at a rate proportional to the value of either of the of the two goods (what the authors termed the 'offer value'). When a choice was made, OFC neurons fired at a rate proportional to the relative value of the two goods ('chosen value'). These researchers also found OFC neurons that responded to the type of good earned. The 'chosen value' was also found to be independent of the visuo-motor contingencies of the choice, further suggesting that these neurons are encoding the subjective value of the chosen juice.

Neurons in the OFC also seem to fire in a 'menu invariant' way (i.e. neurons fire similarly regardless of the items being compared in the choice set) when a monkey is making choices between different pairs of goods (Padoa-Schioppa & Assad, 2008). This 'menu invariance' is suggested to be a potential reason for the observation of transitive choices and is consistent with the

independence required of utility-like representations (Houthakker, 1950; Platt & Padoa-Schioppa, 2009). Thus, the fact that OFC neurons appear to be 'menu invariant' provides stronger evidence that the OFC may be encoding subjective values (Kable & Glimcher, 2009).

There is also evidence from human BOLD fMRI studies that the OFC encodes the subjective value of delayed monetary reinforcers, probabilistic reinforcers, and qualitatively different goods (FitzGerald et al., 2009; Levy et al., 2009; Peters & Buchel, 2009). Further, human studies have also suggested that the OFC encodes the subjective value of qualitatively different goods by transforming value into a common scale (Smith et al., 2010; Levy & Glimcher, 2011). Recent research also suggests that value comparisons may also take place in the OFC.

Evidence from computational modeling suggests that neurons in the OFC are capable of performing value comparisons for goods-based decisions (Rustichini & Padoa-Schioppa, 2015). Specifically, the model showed that a biophysically compatible neural network comprised of 'offer value', 'chosen value', and good-specific cells can generate binary decisions. This model also reproduced experimental observations such as choice hysteresis. Further, there is evidence that the variability of OFC neurons can account for the variability observed in choice behavior (Conen & Padoa-Schioppa, 2015). Recent evidence also suggests that the subjective value of goods and the subjective value of the actions necessary to obtain them are integrated in the OFC (Cai & Padoa-Schioppa, 2014). Taken together, there is a myriad of evidence suggesting that the OFC likely has an important role in value encoding and the comparison of value between goods. However, there are studies that make the role of the OFC a bit less clear.

With regard to the encoding of value, it is not clear whether or not the OFC encodes values in a cardinal fashion (e.g. Padoa-Schioppa & Assad, 2008) or in an ordinal fashion (e.g. Tremblay & Schultz, 1999). Further, some evidence suggests that the inactivation of the OFC does not affect economic choice (Gardner et al., 2017). Some have even suggested that the OFC has more to do

with representing the current state of a choice task at any point in time as oppose to having anything to do with the encoding of value per se (Wilson et al., 2014; Sharpe et al., 2019). It is worth noting that some of these discrepancies are suggested to be due to differences in OFC structure/function between animal models (Feierstein et al., 2006). Nevertheless, the exact role of the OFC in subjective value encoding, value comparison, and choice is currently not clear.

Brain Oxygen Dynamics As A Measure of Neural Activity

As seen above it should be clear that measuring neuronal activity is imperative in order to understand how value, value comparisons, and choices are represented at the level of the brain. Further, the use of BOLD fMRI, a measure based on oxygen, is used readily in human choice experiments in attempt to dissect the function of brain regions during choice. It is also possible to measure oxygen directly in animal models using implantable biosensors (see below). Using oxygen measures in order to say something about neural activity is central to this section of the dissertation. Thus, different modes of measuring oxygen, how they relate to each other, and how they relate to neural activity are briefly reviewed. Experiments showing that oxygen measures are sensitive to brain changes in reward-related behavior are also discussed.

Brain Oxygen and Neural Activity: Are They Related?

Interestingly, the brain comprises 2% of the body's weight but is responsible for using 25% of the body's energy (Zhang & Raichle, 2010). The brain uses the majority of its allotted energy primarily to restore membrane potential after neurons fire (Attwell & ladecola, 2002). However, the brain maintains a small energy reserve; thus, the vascular system is the primary source of metabolic substrates (Pellerin, 2008). Considering this fact, it is thought that hemodynamic responses in the brain are related to cerebral energy metabolism (Attwell & ladecola, 2002).

The hemodynamic response consists of changes in blood flow, blood volume, and oxygenation (Lowry et al., 2010). During neural activity, an increase

in oxygen usage is followed by a larger increase in cerebral blood flow and increased blood volume (Fox & Raichle, 1986; Malonek et al., 1997). Evidence suggests that there is a linear relationship between cerebral blood flow, integrated synaptic activity, and neuronal firing rates (Mathiesen et al., 1998; Ngai et al., 1999; Smith et al., 2002). However, the exact functional relationship between neural activity and the hemodynamic response is a point of contention (Sheth et al., 2004).

The BOLD Signal and Functional Magnetic Resonance

The blood-oxygen-level-dependent (BOLD) magnetic resonance signal is used in order to obtain functional imaging of the brain (Attwell & ladecola, 2002). The BOLD signal is based on oxygenation changes that occur due to the mismatch between cerebral blood flow and neuronal oxidative metabolism (Ogawa et al., 1992). Specifically, the signal reflects the loss of oxygen from hemoglobin, causing its iron to become paramagnetic, which affects the magnetic field of surrounding water molecules (Ogawa et al., 1990). Increases in cerebral blood flow decrease paramagnetic deoxyhemoglobin and produce a positive BOLD signal (Buxton & Frank, 1997). Conversely, a decrease in cerebral blood flow and an increase in deoxyhemoglobin (presumably due to increased oxidative metabolism) produces a negative BOLD signal (Duong et al., 2000).

BOLD functional magnetic resonance imaging (fMRI) has become a staple in neuroscience as a non-invasive way of mapping regional activity of the brain while certain tasks are being performed. BOLD fMRI has spatial resolution at the micron level and can be used on animals as well as humans (Jones et al., 2005). However, one major limitation of BOLD fMRI is that it restricts the mobility of the participant thus limiting experimental design techniques (this issue is particularly problematic in animals studies; Lowry et al., 2010). Thus, other techniques that can measure oxygen in animals could produce more fruitful research.

Electrochemical Measures of Oxygen

Oxygen is a reactive, electron-accepting species whose properties allow it to be measured using constant potential amperometry (Li et al., 2016). By

applying a constant potential of -0.6 V vs. reference, oxygen can be reduced at the surface of an electrode by a one-step (A) or two-step (B and C) process:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O_2 \qquad (A)$$

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \qquad (B)$$

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \qquad (C)$$

during this reduction process, electrons are transferred to oxygen producing a negative current proportional to the concentration of oxygen at the surface of the electrode (Hitchman, 1978; Li et al., 2016; Ledo et al., 2017).

A basic two-electrode unit is used to measure oxygen in the brain consisting of the measuring electrode and a reference electrode (O'Neill et al., 1998; Ledo et al., 2016). The measuring electrode is placed in the brain region of interest and is where oxygen reduction takes place. The reference electrode is placed superficially between the dura mater and the brain and acts to balance the current passing through the measuring electrode (O'Neill et al., 1998; Ledo et al., 2017). The two-electrode unit is attached to a potentiostat that ensures the desired potential of -0.6 V is applied (O'Neill et al., 1998; Ledo et al., 2017). Both carbon fiber electrodes and platinum surface electrodes can be used to measure oxygen in the brain (Bolger & Lowry, 2005; Kealy et al., 2013; Ledo et al., 2017). These electrodes have high spatial (μm) and temporal resolution (ms) along with high sensitivities, low limits of detection, and a linearity of $R^2 > 0.9$ (Bolger et al., 2011; Ledo et al., 2017). These electrodes can be used to monitor oxygen dynamics in the brains of feely-moving animals (Francois et al., 2016; Solis et al., 2017). This allows for oxygen dynamics to be measured as animals exhibit different behavior (McHugh et al., 2012; Francois et al., 2016).

The BOLD Signal and Electrochemical Measures: Translational Efficacy?

As mentioned above BOLD fMRI is primarily used in human decision-making research as well as some animal research. However, a primary limitation

in using BOLD fMRI, especially in animal research, is the restriction this methodology imposes on experimental design. As discussed, *in vivo* electrochemistry has the capacity to alleviate these experimental design restrictions; however, this begs the question as to whether electrochemical oxygen measures are consistent with BOLD fMRI measures? If not then performing electrochemical oxygen measures in animal models is a futile endeavor if our goal is to advance knowledge about human processes. Fortunately, electrochemistry and BOLD fMRI are shown to produce similar results.

Evidence suggests oxygen measures from electrochemistry and BOLD fMRI measures are highly correlated (Lowry et al., 2010). Specifically, these authors took electrochemical measures and fMRI measures simultaneously in rats while manipulating available oxygen. They found that the responses to the two measures were strongly correlated ($r = 0.79$). Further, research has shown that reward related signals measured in the NAc of rats share characteristics to similar studies in humans using BOLD fMRI (Knutson & Cooper, 2005; Francois et al., 2012). Data has also shown that ketamine produces the same effect on brain oxygen as measured by electrochemistry in rats and by BOLD fMRI in humans (Francois et al., 2016). Further, environmental cues were shown to produce similar effects in the PFC wheter measured in rats using electrochemistry or in humans using BOLD fMRI (Howe et al., 2013). Taken together, these data suggest that using *in vivo* electrochemistry in rodents has translational efficacy in elucidating mechanisms of human brain function.

Is Oximetry Capable of Measuring Changes in Reward Processing?

As mentioned above there is evidence that measuring oxygen dynamics in animals has translational efficacy. However, considering the scope of this dissertation, it is important to assess if oxygen amperometry measures (oximetry) are capable of detecting changes in reward sensitivity.

Research shows that oxygen increases in the NAc following a lever press for a food reinforcer (Francois et al., 2012). Further, these researchers also found that oxygen signals were sensitive to changes in reinforcer magnitude and

motivational state. Oxygen measures in rats also show similarities to human fMRI data where increases in oxygen to the presentation of a positively associated conditioned stimulus are attenuated by ketamine administration (Francois et al., 2016). Evidence has also shown that oxygen in the NAc increases to the presentation of arousing stimuli (Solis et al., 2017). Further, an increase in oxygen is observed in the PFC when rats correctly discriminate between actions that result in reinforcement (Howe et al., 2013). In another study, an increase in NAc oxygen was seen to responses for an option that resulted in reinforcement whereas an increase in infralimbic cortex oxygen was seen for responses that did not result in reinforcement (Francois et al., 2014). Oxygen measures from the amygdala also show a greater increase to a conditioned stimulus paired with a foot shock compared to a neutral conditioned stimulus (McHugh et al., 2014). Thus, taken together, there is ample evidence to suggest that oximetry in animals is translatable to humans and capable of detecting changes in the reward system.

Connecting The Dots: Decision-Making, The Brain, and Cocaine Abuse

As mentioned in the beginning of this chapter, the somewhat commonplace notion that substance abuse is governed by compulsive, habitual-like response relations that are insensitive to alternative consequences (Volkow and Morales, 2015) is incompatible with drug-associated choice behavior. Evidence for this is seen by the fact that the current most effective behavioral and pharmacological treatments for human substance-use disorders are based on offering either a non-pharmacological (e.g. contingency management) or pharmacological (e.g. replacement therapy) alternative to the abused drug; thus, indicating sensitivity to alternative consequences (Schierenberg et al., 2012; Stoops and Rush, 2013). Further, in preclinical models, conditions thought to produce compulsive, habit-like drug taking do not readily alter drug-associated choice behavior (Ahmed, 2010), and choice behavior is resistant to compulsive, habit-like response relations (Kosaki and Dickinson, 2010). Thus, these data

suggest that in order to produce more effective treatments for substance-use disorder, it is critical to understand the neurobehavioral mechanisms of drug-associated choice.

Cocaine-Related Choice in Humans

The concepts from behavioral economics, discussed in length earlier, provide a framework for better understanding substance-use disorder especially in regard to the value of a commodity, the substitutability of qualitatively different reinforcers, and the idea of opportunity cost (Rachlin et al., 1976, 1980; Hursh, 1980; Hursh et al., 2005; Bickel et al., 2014). For example, Bickel et al. (1995) reanalyzed several data sets showing a multitude of substitutes exist for many different drugs of abuse across species. Further, contingency management, a program where abstinence is reinforced, decreases abuse behavior for several different drugs including cocaine (Higgins et al., 1993; Schierenberg et al., 2012). The decrease in abuse behavior seen in contingency management is presumably through increasing the opportunity cost of using drugs and by also allowing the participant to come into contact with other reinforcers that may substitute for the drug of abuse (Bickel et al., 2014). Similarly, agonist replacement therapy can function to decrease cocaine abuse in that the pharmacotherapy itself can substitute for the abused drug (Collins et al., 2006; Stoops & Rush, 2013). Note that animal research shows similar results to human work suggesting translational efficacy. Thus, more invasive experiments can be conducted in animals to explore the neurobehavioral mechanisms of drug choice

Cocaine-Related Choice in Animal Models

Early work in rats showed that presenting an animal with a concurrently available non-drug option could decrease the amount of cocaine an animal would administer (e.g. Carroll et al., 1989). Further, it has been shown that the opportunity to earn a sweet solution in a cocaine autoshaping procedure can delay or prohibit drug-taking behavior (Carroll & Lac, 1993). Sweet solutions have also been shown to decrease cocaine choice (Lenoir et al., 2007; Cantin et al., 2010; Madsen & Ahmed, 2015). Research has also shown that increasing the magnitude of an alternative reinforcer or increasing the work requirement needed

to earn cocaine can separately decrease cocaine choice (Nader & Woolverton, 1991; Thomsen et al., 2013). Further, exercise on a running wheel can also compete to decrease cocaine-taking behavior (Cosgrove et al., 2002). Evidence also suggests that context, current intoxication state, extended access to drug, and previous choices can influence drug-related choice behavior (Lenoir et al., 2013; Vandaele et al., 2016; Kearns et al., 2017). Contingency management has also been replicated in animals (e.g. LeSage, 2009) creating an exciting opportunity to further explore the neurobehavioral mechanisms of this treatment. Taken together, these data suggest that environmental manipulations can function to decrease cocaine-taking behavior either through substitution or opportunity cost.

There is also non-clinical evidence that pharmaceuticals can decrease cocaine-taking behavior via substitution. For example, evidence suggests that chronic d-amphetamine can decrease choices for cocaine (Thomsen et al., 2013). Similarly, evidence also shows that diazepam can selectively decrease choices for cocaine (Augier et al., 2011). Further, acute aripiprazole has also been shown to decrease cocaine choice behavior (Thomsen et al., 2008). This research not only suggests that there is potential to find pharmacological treatments for cocaine abuse through animal models but also creates an opportunity to explore the neurobiology of drug-related choice behavior (Ahmed et al., 2013).

Cocaine-Related Choice Behavior and The Brain: Focus on the OFC

Evidence suggests that abnormalities exist in the OFC of those with substance abuse issues (Rogers et al., 1999; London et al., 2000). For example, cocaine abusers show a decrease in baseline OFC activity after acute withdrawal and after long periods of abstinence (Volkow & Fowler, 2000). However, when cocaine abusers are shown drug-related cues they show hyperactivity in the OFC that positively correlates with the magnitude of their craving (Maas et al., 1998). Interestingly, those with substance abuse issues are also shown to behave similarly on behavioral tasks as those with OFC lesions further supporting that

changes in OFC function occur in drug abuse (Chudasama & Robbins, 2003; Schoenbaum et al., 2003; Schoenbaum & Setlow, 2004).

Evidence from animals has also shown that stimulant self-administration causes a decrease in dendritic spine density in the OFC suggesting decreased synaptic plasticity after drug exposure (Crombag et al., 2004). Further, work from rats and monkeys suggest that cocaine administration decreases the ability of subjects to use the value of a predicted outcome to guide their behavior as seen in experiments using discrimination reversals (Jentsch et al., 2002; Schoenbaum et al., 2006). Further, evidence from rats suggest that cocaine causes a decreased ability to learn reinforcer devaluations which is an effect also seen in rats with OFC lesions (Schoenbaum & Setlow, 2005). Recent work suggests that there are different neurons in the OFC that fire before drug and non-drug choices are made, suggesting that these neurons may encode values for the different options further suggesting a role for the OFC in value-based decision-making involving drugs of abuse (Guillem & Ahmed, 2017).

Overall, research suggests that cocaine affects OFC function leading to issues with assigning and comparing the value of goods and outcomes. The effect cocaine has on the OFC is thought to make it difficult for those with substance abuse to incorporate predictive information into their decision-making leading these individuals to continue to seek drugs even though it often leads to negative consequences (Schoenbaum et al., 2006). Thus, further studying the role of the OFC in drug-related decision-making will lead to a better understanding of substance abuse and create avenues for potential treatments to be discovered.

Limitations of Current Research

While there is evidence that the OFC is involved in value-based choice behavior between qualitatively different goods, no work has assessed oxygen dynamics during choices involving drug vs. non-drug alternatives. Further, in most drug vs. non-drug choice procedures the dose of drug is held constant (e.g. Lenoir et al., 2007; Cantin et al., 2010; Madsen & Ahmed, 2015) making relative value assessments more difficult. Another issue in studying drug-related

decision-making in free-choice procedures is that these procedures do not control for the positive feedback function between choices and experienced reinforcement (e.g. Ahmed et al., 2013). Considering that differential histories with drug reinforcers can cause neural adaptations and changes in associated value (Nestler, 2001; Kalivas & O'Brien, 2008), separating out effects from drug intake vs. preference becomes difficult. Further, free-choice procedures allow for disproportionate experience with each alternative. For example, repeated choices for one option causes an overall loss for the other option; thus, differences in choice histories can cause systematic biases making changes in the value of one alternative not easily detectable due to insufficient experience with each alternative (McCarthy & Davidson, 1979, 1981; Johnstone & Alsop, 1999). Thus, in order to adequately study choice behavior one must use a procedure that controls for the positive feedback function between choices and experienced reinforcement as well as reinforcement history.

Overview of Experiment 2

Experiment 2 aimed to further investigate the role of the OFC during value-based decision-making for drug and non-drug commodities. This was accomplished by concurrently measuring oxygen dynamics in the OFC during a novel choice procedure that controlled for both the feedback function between choices and experienced reinforcement as well as reinforcement history. Thus, this procedure allowed for the disassociation of drug intake from preference effects. The overall hypothesis for the proposed experiments was that oxygen would increase in the OFC as a function of increasing preference for either commodity.

Additionally, experiment 2 examined how increasing the magnitude of the food reinforcer changed cocaine relative subjective value and oxygen dynamics in the OFC. Considering that magnitude manipulations have been shown to change preference and brain signaling (Nader & Woolverton, 1991; Sackett et al., 2017) it was hypothesized that increasing the magnitude of the food

reinforcer would decrease the relative subjective value of cocaine. Additionally, it was hypothesized that OFC oxygen would track this change in relative subjective value.

CHAPTER 4

EXPERIMENT 2: EXPLORING THE ROLE OF THE ORBITOFRONTAL CORTEX IN DRUG-RELATED DECISION-MAKING USING OXIMETRY IN FREELY-MOVING RATS

Introduction

Evidence suggests that the OFC has a critical role in value-based decision-making (Padoa-Schioppa & Assad, 2006; Murray et al., 2007; Cai & Padoa-Schioppa, 2015). Further, there is evidence that cocaine use and abuse can affect OFC function potentially causing errors in value-based decision-making (Volkow & Fowler, 2000; Crombag et al., 2004; Schoenbaum & Setlow, 2005; Guillem & Ahmed, 2017). However, the exact role of the OFC in decision-making, especially during drug vs. non-drug choice, is not fully understood. Factors that have impeded this knowledge in non-clinical studies are the use of choice procedures that: (1) do not control for the positive feedback function between choices and experience reinforcement (e.g. Ahmed et al., 2013), (2) that do not control for reinforcement history (see McCarthy & Davidson, 1979, 1981; Johnstone & Alsop, 1999 for discussion), and (3) that do not change the magnitude of the drug or non-drug option (e.g. Lenoir et al., 2007; Cantin et al., 2010; Madsen & Ahmed, 2015). All of these issues make dissociating the effects of preference from intake difficult as well as making the relative subjective value of reinforcers hard to determine (see Beckmann et al., 2019 for discussion). This study was designed to address these issues by measuring OFC oxygen (via oximetry) in freely-moving rats behaving in a novel drug vs. food choice procedure that controls for the positive feedback function, reinforcer history, and that better assesses the relative subjective value of the two reinforcers by comparing varying magnitudes of each.

Materials and Methods

Animals

Six adult male Sprague-Dawley rats (Harlan, Inc.; Indianapolis, IN, USA) weighing approximately 250-300 g were used for experimentation. Rats were individually housed in a temperature-controlled environment on a 12:12 h light:dark cycle with lights on at 0600 h. All rats were acclimated to the colony room and handled a week before any experimentation began. All rats had *ad libitum* access to food and water during the experiment proper (with the exception of specific manipulations; see below). All experimental protocols were conducted according to the 2010 *NIH Guide for the Care and Use of Laboratory Animals* (8th addition) and were approved by The Institutional Animal Care and Use Committee at the University of Kentucky.

Drugs

Cocaine hydrochloride (COC; National Institute on Drug Abuse, Bethesda, MD) was prepared in 0.9% sterile saline for self-administration. COC was self-administered in a range from 0-1.0 mg/kg/infusion based on weight.

Apparatus

Experiments were conducted in operant conditioning chambers (ENV-008, Med Associates) housed within a sound-attenuating compartment (ENV-018M, Med Associates). Each chamber was connected to a computer (SG-502, Med Associates) and ran using MED-PC. Each operant chamber contained a 5.1 cm x 5.1 cm recessed food receptacle (ENV-200R2MA) on the front response panel with two retractable levers on either side (ENV-122CM; 6 cm above metal rod floor). Above each lever was one white cue light (ENV-221M; mounted 4.1 cm above each lever). A Sonalert tone (ENV-223 AM) was located above the top left cue light and another Sonalert tone (ENV-223 HAM) was located above the top right cue light. A house light (ENV-227M) was placed 17 cm above the metal floor in the middle of the back wall. Food pellets (45 mg, Dustless Precision Pellets; Bio Serv) were delivered via a dispenser (ENV-203M-45) placed behind the food receptacle. COC was self-administered via a syringe pump (PHM-100)

located outside of the sound-attenuating chamber. COC was pumped through a watertight swivel attached via tygon tubing to a back-mounted cannula.

Oxygen Biosensor

Microelectrode Array Preparation

Oxygen biosensors were prepared in the same fashion as described in Chapter 2 with the exception that these electrodes did not need to be coated with enzymes or plated with mPD in order to increase sensitivity or selectivity.

In Vitro Calibration

Amperometric recordings were collected at 100 Hz using the FAST16 mkIII electrochemical recording system (Fast Analytical Sensing Technology, Quanteon, LLC, Nicholasville, KY). Before *in vivo* implantation, all electrodes underwent an *in vitro* calibration to determine sensitivity (slope, nA/μM), limit of detection (in μM, signal-to-noise = 3), and linearity ($R^2 \geq 0.9$). Calibrations took place in 20 mL of 0.05 M, pH 7.4 PBS Lite at 37°C. The system was purged with nitrogen and 3 additions of 4.95 μM oxygen was used to generate the calibration curve. These methods were adapted from Ledo et al. (2017).

In Vivo Implantation

Oxygen biosensor implantation was the same as discussed in Chapter 2 with the exception that the electrodes were implanted in the OFC (AP: +3.7 mm; ML: ±1.7 mm; DV: -3.8 mm [from brain surface]) (Paxinos and Watson, 2009). Oxygen biosensor implantation occurred once stable baseline (1 pellet) choice behavior was reached, defined as no statistical difference in a or s parameters (see below) over a three-day period after rats learned to discriminate COC doses.

Electrochemical Recordings

Oxygen measurements were performed at -0.6 V potential vs. Ag/AgCl reference (able to reduce [measure] oxygen) using the FAST-16 mkIII recording system equipped with a low noise 4-channel Rat Hat amplifier (Quanteon, LLC, Nicholasville, KY) system connected through a low-noise commutator (Plastics One, Inc., Roanoke, VA). Setting the potential to -0.6 V vs. Ag/AgCl reference

allowed oxygen to be measured at the platinum surfaces of the electrode through a one-step reaction (Ledo et al., 2017) (Figure 4.1).

Establishing Procedures

Rats were first trained to retrieve food pellets from the food receptacle for two consecutive days. Following magazine shaping, rats were trained to lever press (left and right, randomly presented) on a fixed-ratio 1 (FR1) schedule of reinforcement; completion of the FR1 resulted in lever retraction and delivery of a food pellet. Each session consisted of 15 left- and 15 right-lever trials. Rats were incrementally moved from an FR1 to a terminal FR3 over 6 days. After stable responding on an FR3, an orienting response was added to the response chain. Specifically, each trial was signaled by illumination of the house light and a contingent head entry response into the food receptacle caused house light offset and either left or right lever extension. Completion of the FR3 requirement caused lever retraction and food pellet delivery. Each session consisted of 15 left- and 15 right-lever trials. Response-chain training lasted 3 days. These procedures were adapted from Beckmann et al. (2019).

Catheter Surgeries

Catheter surgeries were conducted in the same manner as described in Chapter 2.

Drug Self-Administration Training

After recovery, animals self-administered COC (1.0 mg/kg/infusion) via completion of a single lever (left or right, counterbalanced) FR1 response. Drug infusions (0.1 mL over 5.9 seconds) were paired with a cue light conditioned stimulus. Sessions lasted 1 hour and the FR requirement was increased to an FR3 over a 6-day period. After stable responding on an FR3, rats were moved on to lever discrimination training where the house light signaled the beginning of the trial. A contingent head entry response caused house light offset and the extension of either the left or right lever. Fulfillment of the FR3 response requirement caused lever offset and either a drug infusion or a food pellet (counterbalanced; the drug lever will remain the same as in previous training with the opposite lever being the food lever). Both the drug infusion and food pellet

were paired with a cue light (5.9 seconds) over the respective lever. Sessions ended when 5 of each reinforcer was earned. Rats were trained on this procedure for 5 days. This training was adapted from Beckmann et al. (2019)

Choice Procedure: Controlled Reinforcer Ratio Schedule

After lever discrimination training rats were placed on the controlled reinforcer ratio (CRR) choice procedure (Stubbs and Pliskoff, 1969; McCarthy & Davison, 1984; Beckmann et al., 2019) for COC vs. food. Importantly, experiments from our lab (e.g. Beckmann et al., 2019) have shown that this procedure allows for the dissociation of reinforcer intake from preference by controlling for the distribution of reinforcers experienced. Thus, this procedure removes the potential confounding of differential reinforcer experience with preference by keeping the drug- and food-taking histories of all animals the same. Specifically, each session was divided into 5 blocks where the animal could choose between a single 45 mg food pellet and COC (0, 0.032, 0.1, 0.32, and 1 mg/kg/infusion; determined by pump time; infusions increase as a function of block). Food pellet delivery was accompanied by lever retraction and cue light onset (5.9 seconds) over the respective lever for all blocks. COC infusions were accompanied with lever retraction and cue light illumination over the respective lever for the duration of the infusion (0, 0.189, 0.59, 1.89, and 5.9 seconds; increasing as a function of block). Each block was signaled by a tone pattern discriminative stimulus played for the duration of each trial block (1.8/0, 1.5/0.3, 0.9/0.9, 0.3/1.5, and 0/1.8 seconds of 40/29 kHz ratio). All blocks were separated by a 2-minute interblock interval (IBI) where all manipulanda were off. Each block was separated into 6 trials (3 food trials; 3 COC trials). The house light signaled the beginning of the trial. An orienting response into the food receptacle caused house light offset and the extension of both levers. Reinforcement was randomly set up for either food or COC in a given trial. Regardless of which lever the rat chose to respond on, the response requirement (FR3) for the randomly determined reinforcer had to be completed and the reinforcer earned (forced choice responses) in order to advance to the next trial. It is important to note that responses on the alternative lever that is not set up for reinforcement were

recorded as preferred (choice) responses. After completing the response requirement for the available reinforcer, the levers retracted and the cue light above the respective lever was illuminated as described above. If the rat changed over to respond on the alternate lever before the FR requirement was completed for the scheduled reinforcer the FR requirement was reset. Each trial was separated by a 20-second intertrial interval (ITI). A block ended after completion of all 6 trials. After stable responding on the choice procedure all rats were implanted with the oxygen biosensor (see above and Chapter 2).

Food Restriction and Magnitude Manipulations

After oxygen data were collected at choice baseline (i.e. when only 1 food pellet was delivered; *n* = 6) rats were then placed on the exact same choice procedure; however, this time each completed FR requirement on the food lever resulted in 4 food pellets (*n* = 3). After oxygen data were collected with the 4-pellet manipulation, animals were food restricted and put on the same 4-pellet choice procedure (4 food pellet + FR) and oxygen data was again collected (*n* = 3). After all data were collected, animals were euthanatized and the brains were extracted, flash frozen, and 40 μm slices were prepared using a cryostat. The slices were stained using Cresyl Violet (Sigma-Aldrich, St. Louis, MO) and were visualized to confirm biosensor placement into the OFC (Figure 4.2). Note that due to experimental issues one brain was visualized without the use of staining or a microscope.

Data Analysis

Choice data were expressed as percent choice for COC as follows:

$$Percent\ COC_{Choice} = \frac{COC_{Preferred}}{COC_{Preferred} + Food_{Preferred}} \times 100 \qquad (7)$$

where $COC_{Preferred}$ represents the total number of preferred responses on the COC lever (i.e. responses on the COC lever when COC was not available) and $Food_{Preferred}$ represents the total number of preferred responses on the food lever (i.e. responses the food lever when food was not available). Additionally, a

version of the generalized matching law (GML), a quantitative model relating differential reinforcer value to choice behavior, was applied to the data (Baum & Rachlin, 1969; Killeen, 1972; Beckmann et al., 2019). The form of the GML that was used is:

$$\frac{B_d}{B_d + B_f} = \frac{100}{1 + (a/x)^s} \qquad (8)$$

where B represents behavior (i.e. preferred responses) for either drug (d) or food (f), x represents the dose of COC available, a represents a free parameter that determines the dose at which the relative value of food and drug are equivalent (scaled in drug dose units; drug/food exchange rate), and s is the slope of the function that represents the sensitivity to the relative magnitude between drug and food. The GML was fit to the choice data using nonlinear mixed-effects (NLME) modeling using R Studio (Version 1.1.383) statistical software (Pinheiro et al., 2007) with subject as a random factor and the food manipulation (1 pellet, 4 pellet, and 4 pellet + FR) as a fixed, within-subject factor.

Brain oxygen data were analyzed using custom MATLAB®-based software (Quanteon LLC, Nicholasville, KY) and a custom-written MATLAB® (MathWorks, Inc., Natick, MA) program. Oxygen data and the location of respective behavioral events were extracted using the custom MATLAB®-based software. The oxygen signals related to the behavioral events were analyzed using the custom-written MATLAB® program. Specifically, an oxygen peak was defined as an event that was 5 standard deviations above the mean of the baseline (Gunaydin et al., 2014). The baseline was defined as the last 1-minute average of the IBI before a given block. For example, the baseline for block 1 was the average of the 1-minute interval before the block began, the baseline for block 2 was the average of the 1-minute interval before block 2 began, etc. Oxygen peaks were considered related to a given behavioral event if they occurred within a 20-second window of the behavioral event and were not interrupted by another type of behavioral event. Figure 4.3 and Figure 4.4 show example oxygen traces highlighting oxygen events considered to be peaks based on the previously

mentioned criteria. Measures assessed were the absolute maximum of the oxygen peak (μM), the maximum amplitude of the oxygen peak above baseline (μM), the percent increase of the oxygen event above baseline, the peak width (s), the peak prominence (μM), and the number of oxygen events that occurred to a given behavioral event. Statistical analyses were conducted with JMP Pro 12.0.0. statistical software using linear mixed-effects (LME) models (Gelman & Hill, 2007) with subject as a random factor and food manipulation, block (COC dose), and event type (e.g. COC vs. food) as fixed, within-subject factors. Note that there were some instances where event type was not a factor (such as in the total number of oxygen peaks that occurred per session or per block [see below]), in these cases the full model did not include event type as a factor.

Akaike information criterion (AIC) values were used to compare models; only statistics from the models that were most likely to describe the data are presented. Further, differences in AIC values (ΔAICs) were also calculated in order to assess the relative difference of information loss of all the other models compared to the best model. Evidence ratios for the best model relative to the second-best model were calculated from the ΔAICs (Burnham & Anderson, 2002; Burnham et al., 2011). The evidence ratios indicate the relative strength of the preferred model to the second-best model.

Where necessary linear regressions were performed and correlation coefficients, as well as if the slopes of the lines were statistically different than zero, were assessed. Any interactions were probed with contrasts (NLME) or the Tukey HSD (LME), and statistical significance was defined as $p < 0.05$.

Results

Choice Behavior During Recordings For All Manipulations

Figure 4.5 shows choice behavior (expressed as percent COC preference) during oxygen recordings for all manipulations. The version of the GML mentioned above (equation 8) was fit to the data points and analyzed via NLME. The model with the lowest AIC was the model that had the s parameter set as a

global parameter, which was 5.03 times more likely to describe the data than the model that allowed the s parameter to vary between animals. Specifically, it was found that, compared to the baseline condition (a = 0.29; s = 2.52), the 4-pellet + FR manipulation (a = 0.53; s = 2.52) significantly increased the drug/food exchange rate [$F(2,66) = 14.10$, $p < 0.0001$]. There were no differences between the 1-pllet (baseline condition) and the 4-pellet manipulation (a = 0.21; s = 2.52). However, contrasts revealed that the drug/food exchange rate for the 4-pellet manipulation was significantly decreased compared to the 4-pellet + FR manipulation [$F(1,66) = 27.90$, $p < 0.0001$].

Overall Behavioral Responses Per Trial

Figure 4.6 shows the overall responses (all forced and preferred lever responses for COC and food) as a function of block (COC dose) for all manipulations. There were no statistical difference between the 1-pellet, 4-pellet, and 4-pellet + FR manipulations as a function of block [$F(2,6.59) = 4.72$, $p = 0.054$]. Note that all AIC values were approximately the same ($\Delta AIC < 0.6$) thus the statistic reported was from the full model.

Behavioral Responses Per Preferred COC and Food Trials

Figure 4.7 shows all COC and food responses (forced choice and preferred choice lever presses) per preferred choice trial for the 1-pellet (Figure 4.7A), 4-pellet (Figure 4.7B), and 4-pellet + FR (Figure 4.7C) manipulations. Specifically, there was a block x event type interaction where the number of COC responses per preferred trial increased as a function of block and the number of food response per preferred trial decreased as a function of block [$F(1,4.83) = 20.89$, $p = 0.007$]. There was also a manipulation x event type interaction where food responses for the 4-pellet + FR manipulation were significantly greater than food response for the 1-pellet and 4-pellet conditions and COC responses for the 4-pellet + FR condition [$F(2,6.99) = 6.16$, $p = 0.03$]. These statistics came from the full model, which had the lowest AIC and was 84.35 times more likely to describe the data compared to the model that included only block and event type as factors.

Behavioral Responses To Head Entries, Preferred Choices, & Head Entries After Reinforcer Delivery

Figure 4.8 shows behavioral responses to head entries, preferred responses, and head entries after reinforcer delivery. Specifically, Figure 4.8A shows a main effect of event type where the number of head entries that occurred during trials were significantly greater than the number of head entries that occurred to initiate trials [$F(1,3.66) = 9.44$, $p = 0.04$]. This statistic came from the full model (block x manipulation x event type), which had the lowest AIC and was 1.14×10^9 times more likely to describe the data compared to the model that included manipulation and event type as factors. It is worth noting that this finding is not surprising considering that the number of head entries to initiate trials was procedurally bounded (6 initiations/block) whereas all other head entries were not.

Figure 4.8B shows that there was a block (COC dose) x event type interaction where COC preferred responses increased as a function of COC dose and food preferred response decreased as a function COC dose [$F(1,4.83) = 25.10$, $p = 0.005$]. There was also a manipulation x event type interaction where food preferred responses for the 4-pellet + FR condition were significantly greater than all other preferred responses across conditions [$F(2,6.92) = 5.86$, $p = 0.03$]. These statistics came from the full model, which had the lowest AIC and was 6.5×10^5 times more likely to describe the data compared to the model that included block and event type as factors.

Figure 4.8C shows a main effect of event type where more head entry responses occurred after a food pellet was delivered ('eating' responses) compared to head entry responses during/after a COC infusion [$F(1,4.45) = 39.25$, $p = 0.002$]. This statistic came from the model that included block and event type as factors, which had the lowest AIC and was 3.89×10^6 times more likely to describe the data compared to the full model.

Total Number of Oxygen Peaks That Occurred in A Choice Session

Figure 4.9 shows the total number of oxygen peaks that occurred overall and to behavioral events over choice sessions between manipulations. There

were no statistical difference in the total number of oxygen peaks that occurred between manipulations (Figure 4.7A) [$F(2,3.97) = 0.25$, $p = 0.79$]. There were also no differences in the number of oxygen peaks that occurred to behavioral events (Figure 4.9B) [$F(2,4.40) = 4.80$, $p = 0.08$] or in the percentage of oxygen peaks that occurred to behavioral events [$F(2,3.80) = 4.44$, $p = 0.10$] (Figure 4.9C) in a session across manipulations.

Total Number of Oxygen Peaks That Occurred in A Choice Session Per Block

Figure 4.10 shows the total number of oxygen peaks that occurred overall and to behavioral events per block across manipulations. There were no differences observed in the total number of peaks that occurred per block (Figure 4.10A) [$F(2,3.93) = 0.52$, $p = 0.63$]. This statistic came from the full model (block x manipulation), which had the lowest AIC and was 1.5×10^6 times more likely to describe the data compared to the model that included only manipulation as a factor.

Figure 4.10B highlights that there was a main effect of block with the number of oxygen peaks occurring to events decreasing as a function of block [$F(1,3.50) = 12.27$, $p = 0.031$]. This statistic came from the full model, which had the lowest AIC and was 3.70×10^3 times more likely to describe the data than the model with only manipulation as a factor.

Figure 4.10C shows that there were no differences in the percent of oxygen peaks that occurred to events per block [$F(2,4.23) = 1.37$, $p = 0.35$]. This statistic came from the full model, which had the lowest AIC and was 796.32 times more likely to describe the date than the model with only manipulation as a factor.

Number of Oxygen Peaks That Occurred Per Preferred COC and Food Trial

Figure 4.11 shows the number of behavioral responses that occurred per COC and food preferred trials (left) and the number of oxygen peaks that occurred during COC and food preferred trials (right). Note that the behavior was analyzed in Figure 4.8 and is shown in Figure 4.11 only for purposes of comparison. Specifically, there were no statistical differences that occurred per

COC or food preferred trials between the 1-pellet (Figure 4.11A), 4-pellet (Figure 4.11B), and 4-pellet + FR (Figure 4.11C) conditions [$F(2,6.60) = 0.30$, $p = 0.75$].

Correlations Between The Number of Oxygen Peaks and The Number of Responses That Occurred Per Preferred COC and Food Trials

Figure 4.12 shows correlations between the number of oxygen peaks that occurred per preferred COC and food trial and the number of responses that occurred per COC and food preferred trial. Specifically, Figure 4.12A (left) shows that there was no correlation between the number of oxygen peaks that occurred per preferred COC trial and the number of responses that occurred per preferred COC trial for the 1-pellet condition ($r = 0.025$, $p < 0.90$). Figure 4.12A shows that there was a significant positive correlation between the number of oxygen peaks that occurred per COC trial and the number of behavioral responses per trial for the 4-pellet (middle; $r = 0.85$, $p < 0.0001$) and 4-pellet + FR (right; $r = 0.64$, $p = 0.01$) conditions.

Figure 4.12B (left) shows that the number of oxygen peaks that occurred per preferred food trial and the number of behavioral responses per preferred food trial were not correlated for the 1-pellet condition ($r = 0.25$, $p = 0.17$). Figure 4.12B shows that the number of behavioral responses per preferred food trial and the number of behavioral responses per preferred trial were significantly positively correlated for the 4-pellet (middle; $r = 0.68$, $p = 0.005$) and 4-pellet + FR conditions (right; $r = 0.92$, $p < 0.0001$).

Number of Oxygen Peaks That Occurred To Behavioral Events

Figure 4.13 shows the number of oxygen peaks that occurred to forced trials (where reinforcers could be earned on an FR3) across all manipulations. Specifically, Figure 4.13 shows that there were no differences in the number of oxygen peaks that occurred to forced COC choices compared to forced food choices across the 1-pellet (Figure 4.13A), 4-pellet (Figure 4.13B), and 4-pellet + FR (Figure 4.13C) manipulations [$F(1,3.72) = 6.81$, $p = 0.06$]. This statistic came from the model that included only event type as a factor, which had the lowest AIC and was 1.48 times more likely to describe the data compared to the model that used block and event type as factors.

Figure 4.14 shows the number of oxygen peaks that occurred when each reinforcer was earned. Specifically, the results show a main effect of event type where a greater number of oxygen peaks occurred to earning the COC reinforcer compared to earning the food reinforcer for the 1-pellet (Figure 4.14A), 4-pellet (Figure 4.14B), and 4-pellet + FR (Figure 4.14C) conditions [$F(1,4.55) = 30.20$, $p = 0.004$]. The results also showed a main effect of block where the number of oxygen peaks increased as a function of block (COC dose) for the 1-pellet (Figure 4.14A), 4-pellet (Figure 4.14B), and 4-pellet + FR (Figure 4.14C) conditions [$F(1,2.60) = 12.59$, $p = 0.04$]. The model with the lowest AIC was the full model; however, the ΔAIC between the best model and second best model was lower than 4. Thus, the simpler of the two models (block x event type as factors) was selected.

Figure 4.15 shows the number of preferred responses to COC and food as a function of block (left) and the number of oxygen peaks that occurred to the preferred responses as a function of block (right). Note that the behavioral data was analyzed in Figure 4.8 and is presented here only for purposes of comparison. Specifically, Figure 4.15 shows that there are no statistical differences in preferred responses across the 1-pellet (Figure 4.15A), 4-pellet (Figure 4.15B), and 4-pellet + FR (Figure 4.15C) conditions [$F(2,3.66) = 2.29$, $p = 0.23$]. This statistic came from the model that included block and manipulation as factors, which had the lowest AIC and was 1.92 times more likely to describe that data compared to the model that included block and event type as factors. Note that the best model and the second best model did not have a ΔAIC less than 4 and thus both models were equally as likely to describe the data. However, due to the fact that both models were of equal complexity, the model with the lowest AIC was chosen. Neither model showed significance.

Correlation Between The Number of Oxygen Peaks That Occurred to Preferred Choices and COC Preference

Figure 4.15 highlighted that no statistical differences were observed with the number of oxygen peaks that occurred to preferred choices. However, there were significant relationships between the number of oxygen peaks that occurred

to preferred responses and COC preference, which are highlighted in Figure 4.16. Specifically, Figure 4.16A (left) shows that the percentage of oxygen peaks that occurred to preferred COC choices relative to all preferred choices (expressed as a percentage by substituting the number of preferred responses in equation 7 with the number of oxygen peaks to preferred responses) was positively correlated with COC preference for the 1-pellet condition ($r = 0.63$, $p = 0.01$). Figure 4.16A (middle) shows that no correlation was observed between the number of oxygen peaks that occurred to COC preferred responses and COC preference for the 1-pellet condition ($r = 0.26$, $p = 0.30$). Figure 2.16A (right) shows that the number of oxygen peaks that occurred to preferred food choices was negatively correlated with COC preference for the 1-pellet condition ($r = -0.38$, $p = 0.04$).

Figure 2.16B (left) shows that the percentage of oxygen peaks that occurred to preferred COC choices relative to all preferred choices was not correlated with COC preference for the 4-pellet condition ($r = 0.76$, $p = 0.14$). Figure 2.16B (middle) shows that no correlation was observed between the number of oxygen peaks that occurred to COC preferred responses and COC preference for the 4-pellet condition ($r = 0.75$, $p = 0.05$). Figure 2.16B (right) shows that the number of oxygen peaks that occurred to preferred food choices was not correlated with COC preference for the 4-pellet condition ($r = -0.41$, $p = 0.16$).

Figure 2.16C (left) shows that the percentage of oxygen peaks that occurred to preferred COC choices relative to all preferred choices was positively correlated with COC preference for the 4-pellet + FR condition ($r = 0.99$, $p = 0.0001$). Figure 2.16C (middle) shows that the number of oxygen peaks that occurred to COC preferred responses was positively correlated with COC preference for the 4-pellet + FR condition ($r = 0.96$, $p = 0.002$). Figure 2.16C (right) shows that the number of oxygen peaks that occurred to preferred food choices was not correlated with COC preference for the 4-pellet + FR condition ($r = -0.37$, $p = 0.18$).

Percent of Oxygen Peaks to Events Per Block Relative to The Number That Occurred to Specific Event Over A Session

Figure 4.17 shows how the number of oxygen peaks that occurred to forced COC and food responses were distributed as a function of block relative to the number of oxygen peaks found to forced responses over a session. Specifically, no statistical differences were found between the 1-pellet (Figure 4.17A), 4-pellet (Figure 4.17B), and 4-pellet + FR conditions (Figure 4.17C) [$F(2,22.01) = 2.95$, $p = 0.07$]. This statistic came from the full model, which had the lowest AIC and was 512.86 times more likely to describe the data compared to the model that included block and manipulation as factors.

Figure 4.18 shows how the number of oxygen peaks that occurred to COC and food reinforcers was distributed as a function of block relative to the number of oxygen peaks found to each reinforcer over a session. Thus, each reinforcer is relative to the total found for that specific reinforcer in a session. Specifically, there was a main effect of block where the percentage of oxygen peaks increased as a function of block for the 1-pellet (Figure 4.18A), 4-pellet (Figure 4.18B), and 4-pellet + FR (Figure 4.18C) conditions [$F(1,3.47) = 12.21$, $p = 0.03$]. This statistic came from the full model, which had the lowest AIC and was 77.09 times more likely to describe the data compared to the model that included block and event type as factors.

Figure 4.19 shows how the number of oxygen peaks that occurred to preferred COC and food responses were distributed as a function of block relative to the number of oxygen peaks found to each preferred response over a session. Specifically, there was a block x event type interaction where the percentage of COC preferred responses increased and the percentage of food preferred responses decreased as a function of block for the 1-pellet (Figure 4.19A), 4-pellet (Figure 4.19B), and 4-pellet + FR conditions (Figure 4.19C) [$F(1,52.31) = 25.21$, $p < 0.0001$]. This statistic came from the full model, which had the lowest AIC and was 471.07 times more likely to describe the data compared to the model that included block and event type as factors.

Number of Oxygen Peaks That Occurred Per Preferred Choice Responses

Figure 4.20 shows the number of oxygen peaks that occurred per preferred response as a function of block for all manipulations. Specifically, no statistical differences were observed between the 1-pellet (Figure 4.20A), 4-pellet (Figure 4.20B), or 4-pellet + FR (Figure 4.20C) conditions [$F(1,5.51) = 2.41$ $p = 0.18$].

Figure 4.21 shows correlations between the number of oxygen peaks that occurred per preferred event (expressed as a percentage by substituting the number of preferred responses in equation 7 with the number of oxygen peaks that occurred per preferred response) and percent COC preference. Specifically, no correlations were observed in the 1-pellet (Figure 4.20A; r = 0.31, $p = 0.26$), 4-pellet (Figure 4.20B; r = 0.01, $p = 0.99$), or 4-pellet + FR (Figure 4.20C; r = 0.20, $p = 0.70$) conditions.

Discussion

The results from these experiments showed that the COC/food exchange rate (a) increased in the 4-pellet + FR condition compared to baseline (1-pellet condition). Conversely, the COC/food exchange rate decreased in the 4-pellet condition compared to the 4-pellet + FR condition. There was also a significant positive correlation between the number of oxygen peaks that occurred per preferred COC and food trial and the amount of behavior that occurred in each preferred trial in the 4-pellet and 4-pellet + FR conditions. There were no differences in the number of oxygen peaks that occurred to forced COC responses compared to forced food responses. However, a greater number of oxygen peaks were observed when COC was earned compared to when food was earned. There were no statistical differences observed in the number of oxygen peaks that occurred to COC and food preferred responses. However, the number of oxygen peaks that occurred during COC preferred responses were generally positively correlated with COC preference and the number of oxygen

peaks that occurred to preferred food responses was generally negatively correlated with COC preference.

Although not significant, it was surprising that the COC/food exchange rate decreased (an increase in drug preference) when 4 food pellets could be earned compared to the 1-pellet condition (Figure 4.5). This is contradictory based on previous work in monkeys that showed that increasing the magnitude of the food reinforcer decreased drug preference (Nader & Woolverton, 1991). However, the COC/food exchanged rate increased (decreased drug preference) when 4 pellets could be earned and rats were food restricted, a finding that is supported by previous work (Beckmann et al., 2019). These results suggest that drug preference increased in the 4-pellet condition because rats became sated on food pellets, which presumably increased the relative subjective value of COC. Importantly though, these data are in line with other published reports showing that environmental manipulations, including changing reinforcer magnitude, can affect drug-related decision making (Carroll & Lac, 1993; Nader & Woolverton, 1991; Schierenberg et al., 2012; Hutsell et al., 2015; Chow, 2018; Beckmann et al., 2019).

The number of oxygen peaks that occurred per COC and food preferred trials between the manipulations was not statistically different (Figure 4.11). However, the number of oxygen peaks that occurred to preferred COC trials was positively correlated with the number of response that occurred during preferred COC trials and the number of oxygen peaks that occurred during preferred food trials was positively correlated with the number of responses that occurred during preferred food trials for the 4-pellet and 4-pellet + FR conditions (Figure 4.12). Considering brain oxygen measures could be a proxy for neuronal activity (Mathiesen et al., 1989; Ngai et al., 1999; Smith et al., 2002) this finding is somewhat similar to other work that found that neuronal activity in the rat OFC was positively correlated with COC choice (Guillem & Ahmed, 2017). It is not clear why there was not a positive correlation in the 1-pellet condition. However, this finding is similar to that of Chow (2018) who found no correlation between the percent of COC cFos+ cells and COC preference when reinforcer history was

held constant. Considering the magnitude of food and the hunger state of the animal were the only differences between the 1-pellet condition and the others, it suggests that these factors may be driving the positive correlations observed. The fact that neuronal firing patterns in the OFC are shown to be sensitive to qualitative differences between goods, motivational states (e.g. hunger, satiety, etc.), and to the magnitude between goods lends support to this claim (Thorpe et al., 1983; Rolls et al., 1989; Wallis, 2007). That being said, more work will need to be conducted the better understand this relationship.

The number of oxygen peaks that occurred to COC forced choice responses was not statistically different compared to those observed to food forced choice responses (Figure 4.13); further, how oxygen peaks were distributed over blocks relative to each response type was also not different (Figure 4.17). That being said, the number of oxygen peaks to COC forced choices tended to be greater compared to forced food choices (or at least followed a similar pattern). Considering there were an equal number of forced COC and food trials per block, the forced choice responses were equal between the two commodities (with the exception of change over delays, which were minimal). Thus, there were generally a greater number of oxygen peaks seen to forced COC responses compared to forced food response even though the responses were approximately equal between response types, lending support to this effect being COC specific. This result is consistent with previous literature that showed that OFC activity during COC sampling was greater than the activity observed during food sampling (Guillem & Ahmed, 2017). Unexpectedly, the oxygen response to forced COC responding was especially obvious in the first block when the completion of the FR requirement resulted in no COC delivery. Considering the COC lever was associated with COC in the other blocks and all animals were well trained, the increase in the number of oxygen peaks observed in the first block could be due to cue reactivity to the COC lever considering that BOLD activity in the OFC has been associated with craving (Risinger et al., 2005). Likewise, the association between the COC lever and COC delivery could be why the number of oxygen peaks was generally greater to forced COC

responses overall (if we assume that drug-paired stimuli create more associative strength compared to food-paired stimuli, which is a possibility; see discussion in Batten & Beckmann et al., 2018). Note that only oxygen peaks associated with lever presses are included in the forced analysis. Thus, this measure should not have been influence by reinforcer delivery. That being said, after the first forced COC trial in the second block COC is affecting the brain. Considering acute COC administration has been shown to increase BOLD activity in the OFC (Kufahl et al., 2005) it is possible that the changes in the number of oxygen peaks observed (after the second block) are due to the pharmacological properties of COC. However, considering the differences observed in this study between forced response and reinforcer delivery, it suggest that the difference seen in forced COC lever presses may have more to do with cue associative strength oppose to COC pharmacology.

It is also worth noting that the number of peaks for the forced COC responses generally followed a U-shaped pattern (with this being most pronounced in the 4-pellet + FR condition). Although not perfectly comparable considering difference in the experimental paradigm, this is similar to the findings of Padoa-Shcioppa & Assad (2006) who found that certain neurons in the OFC tracked the value of the chosen offer (where the magnitude of juices B:A were manipulated) creating a similar U-shaped firing pattern. Specifically, in the Padoa-Shcioppa & Assad (2006) study the U-shaped pattern in neuronal firing was increased when choices were exclusively for one juice and decreased as choices neared equivalency. In this study, only the magnitude of COC was manipulated within a given session. However, it appears that the number of oxygen peaks for forced COC and food responding are closest together around the COC/food exchange rate for each manipulation (where food pellets and COC are considered equal) and becomes more disperse as one moves away from this point of COC/food equivalency. Considering this general trend, it may suggest that this represents some relative encoding of value. However, note that in this experiment the preference measure is independent of forced responses. Thus, oxygen changes in forced trials should not directly relate to choice preference

making these findings hard to compare to Padoa-Shcioppa & Assad (2006) as well as difficult to interpret.

Note that there was no difference in the number of oxygen peaks that occurred to forced food responses between pellet manipulations. This result is somewhat contradictory to previous research showing that activity in the OFC was greater for responses for food reinforcers of greater magnitude when they were presented independently (Guillem & Ahmed, 2017). Assuming that the food manipulations here could be considered similar to independent presentations (considering the food manipulations did not happen within session), one may have expected to find parallel increases in the intercept of the forced choice food responses as a function of food manipulation (where the intercept increased as a function of manipulation with the 1-pellet condition being the lowest, the 4-pellet condition being in the middle, and the 4-pellet + FR condition being the greatest). It is possible that this difference did not occur because the difference in magnitude in this study was only 4 times greater whereas in the Guillem & Ahmed (2017) study the magnitude difference was 5 times greater. Nevertheless, taken together with results from the literature, the forced choice findings, even though not significant, lend support to the idea that responses associated with COC increase OFC activity.

Statistical differences were observed when the reinforcer was earned (Figure 4.14). Namely, there was a main effect of reinforcer where the number of oxygen peaks was increased when COC was earned compared to when food was earned. Again, this is similar to results showing that neuronal activity increased in the OFC to actions associated with a COC reinforcer compared to a food reinforcer (Guillem & Ahmed, 2017). Further, the increased activity to COC reinforcer delivery observed here is also similar to studies showing increased OFC activity to drug-related cues and to acute COC administration (Kufahl et al., 2005; for review Dom et al., 2005; Risinger et al., 2005; for review Schoenbaum et al., 2006). Considering the number of reinforcers per block is held constant, this again suggests a dissociation between COC and food events. However, considering the drug was being delivered when the increase in oxygen peaks

were observed it is likely this increase in oxygen has more to do with the direct effects of COC oppose to changes in value per se. Interestingly, when looking at the percentage of oxygen peaks that occurred to each reinforcer per block relative to the number that happened over the whole session there is a general increase as a function of block (i.e. no longer an main effect of reinforcer; Figure 4.18). This effect seems to be driven mostly be an increase in the percentage of oxygen peaks that occur to food reinforcers with largest percentage of peaks being observed in the last block (when COC can be earned at its highest dose). It is not clear why this occurred. However, it is possible that the drug being present in the system caused a general increase in OFC activity overall which could account for the increase in the number of food peaks in the last block. Again, overall these data suggest that the oxygen changes to reinforcer delivery have more to do with direct drug effects than subjective value encoding.

Unlike most choice studies, here the major measure of choice preference is responses on the COC or food lever when reinforcement is not available. No differences were observed in the number of oxygen peaks to preferred choice responses (Figure 4.15). However, there was a block x event type interaction when looking at the percentage of oxygen peaks that occurred to each response per block relative to the session (Figure 4.19). Further, there were also significant correlations between the number of oxygen peaks observed to COC and food preferred responses and COC preference (Figure 4.16). Specifically, the percent of oxygen peaks observed to preferred COC responses (calculated by replacing the number of preferred responses in equation 7 with the number of oxygen peaks that occurred to preferred responses) was positively correlated with COC preference in the 1-pellet and 4-pellet + FR conditions (Figure 4.16A and Figure 4.16C). These correlation seem to be driven by a general positive correlation with the number of oxygen peaks that occur to COC preferred responses and COC preference and a general negative correlation between the number of oxygen peaks that occur to food preferred responses and COC preference (however, not all of these correlations were significantly different from zero). Note that no significant correlations were observed in the 4-pellet condition, although the

general trend is the same. It is unclear why the correlations in the 4-pellet condition were not significant; however, it likely due to an interaction between a low sample size and general brain stochasticity. Nevertheless, these results are similar to others found in the literature. For example, Guillem and Ahmed (2017) found that neuronal activity in the OFC was positively correlated with COC preference. However, Chow (2018) found no correlation between the percent COC cFos+ cells in the OFC and COC preference. Considering the procedure used here and that of Chow (2018) are the same it is unclear why these results differ. However, it could be due to the fact that the oxygen measures shown here were collected in real-time and are a bit more dynamic than cFos measures. Conversely, it could be that the cFos measure is a more accurate representation of neural activity than oxygen measures. Thus, the exact reason for the discrepancy between these two studies will have to be further explored. Nevertheless, this study, Chow (2018), and Beckman et al. (2019) all support the idea that preference is a relative measure determined by different reinforcement dimensions (e.g. frequency, magnitude) and not drug intake (Iglauer & Woods, 1974; Anderson et al., 2002).

In this experiment many of the behavioral responses were held constant between COC and food events including the number of forced responses and the number of reinforcers earned. Thus, with these measures the number of oxygen peaks found should, in theory, be less confounded by the number of behavioral responses because this was held constant. However, the number of preferred choice responses was free to vary and was susceptible to being confounded with the amount of behavior that occurred in a similar way as was discussed in Chapter 2. Thus, in order try and control for this issue the number of oxygen peaks that occurred per preferred response were divided by the number of preferred responses that occurred. When this was done no statistical differences or correlations were observed (Figure 4.20 & Figure 4.21). These data suggest that any differences seen to preferred responses was only observed because of the amount of the behavior that occurred. However, it is worth noting that there was not a 1:1 relationship between the number of oxygen peaks that occurred to

preferred responses and the number of preferred response that occurred suggesting that these results were not completely confounded. Further, there were a number of measures here where behavior was held constant and the number of oxygen peaks that occurred still differed in a meaningful direction lending credence to these data. This is especially so when looking at the preferred trial data (Figure 4.11 & Figure 4.12) because those data showed a similar trend and they included all oxygen peaks that happened in the trial not just those associated with behavior. Nevertheless, these results should be interpreted cautiously especially in the 4-pellet and 4-pellet + FR conditions considering the low sample size.

In summary, this study showed the feasibility of coupling oximetry and drug self-administration with freely-moving choice studies. The major findings of this study were that environmental manipulations (namely food magnitude changes) shift COC preference. Further, it was found that responses associated with COC and earning COC caused more oxygen activity in the OFC than events related to food. Further, there was evidence that OFC oxygen (as measured through the number of oxygen peaks that occurred) followed COC preference. Thus, overall these data suggest that the OFC may play a role in the subjective valuations that occur in drug-related decision-making.

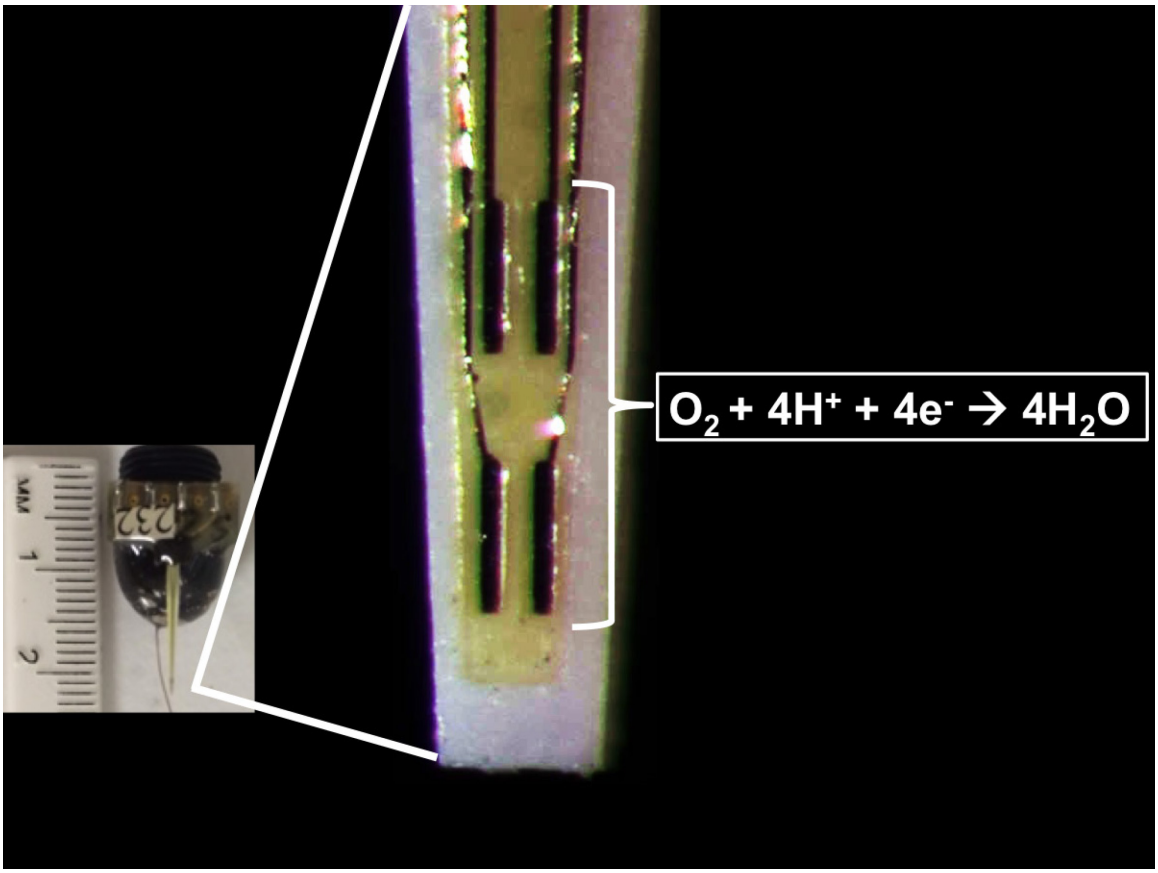


Figure 4.1 Oxygen Biosensor

The above figure shows an oxygen biosensor headcap (bottom left). At the tip of the electrode (enlarged picture) oxygen is reduced at all four platinum sites via the highlighted, one-step reaction.

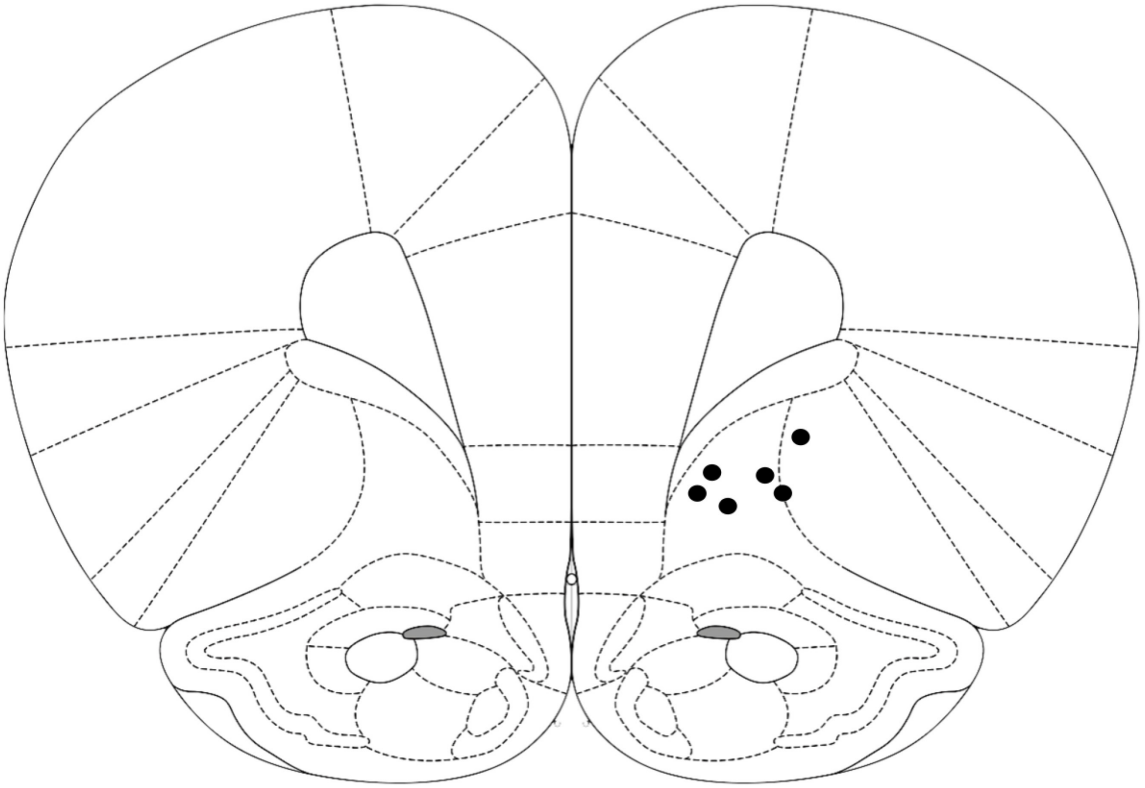


Figure 4.2 Oxygen Biosensor Placements

The circles represent the approximate placement of the tip of the oxygen biosensor for n = 6 subjects in the orbitofrontal cortex (OFC).

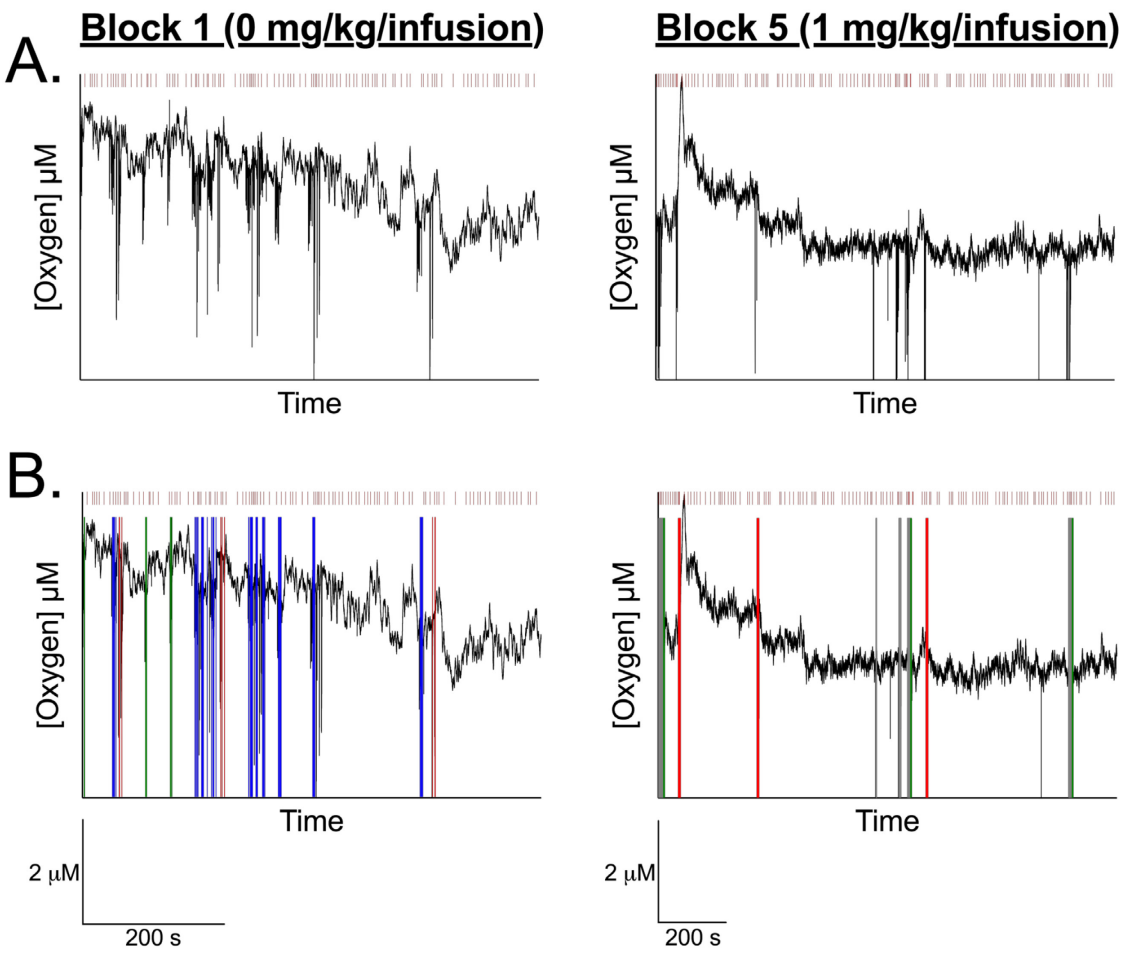


Figure 4.3 Oxygen Traces From The OFC During Block 1 and Block 5

(A) Example of an OFC oxygen trace from the 1-pellet condition during Block 1 (left) and Block 5 (right) with **(B)** lines representing behavioral events. The red-hatched lines above all traces represent the location of oxygen peaks based off criteria. The green lines represent forced food choices (lever presses and pellet delivery), the blue lines represent preferred choices for food, the red lines represent forced COC choices (lever presses and COC delivery), and the grey lines represent preferred COC choices. Note the difference in the x-axis scale bar between traces.

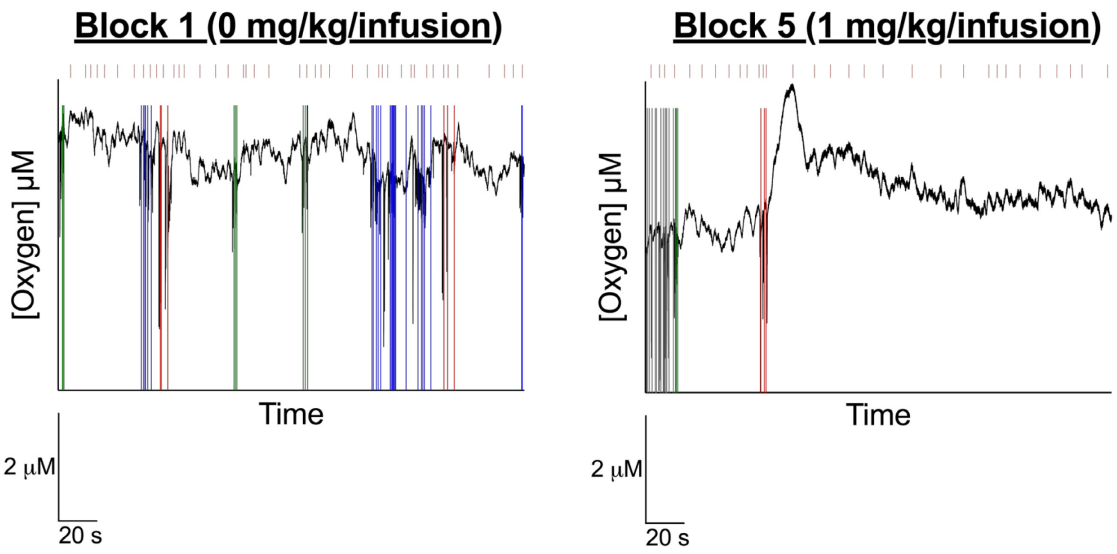


Figure 4.4 Magnified Oxygen Traces From The OFC During Block 1 and Block 5

OFC oxygen traces (magnified from Figure 4.3) from the 1-pellet condition during Block 1 (left) and Block 5 (right) with lines representing behavioral events. The red-hatched lines above both traces represent the location of oxygen peaks based off criteria. The green lines represent forced food choices (lever presses and pellet delivery), the blue lines represent preferred choices for food, the red lines represent forced COC choices (lever presses and COC delivery), and the grey lines represent preferred COC choices.

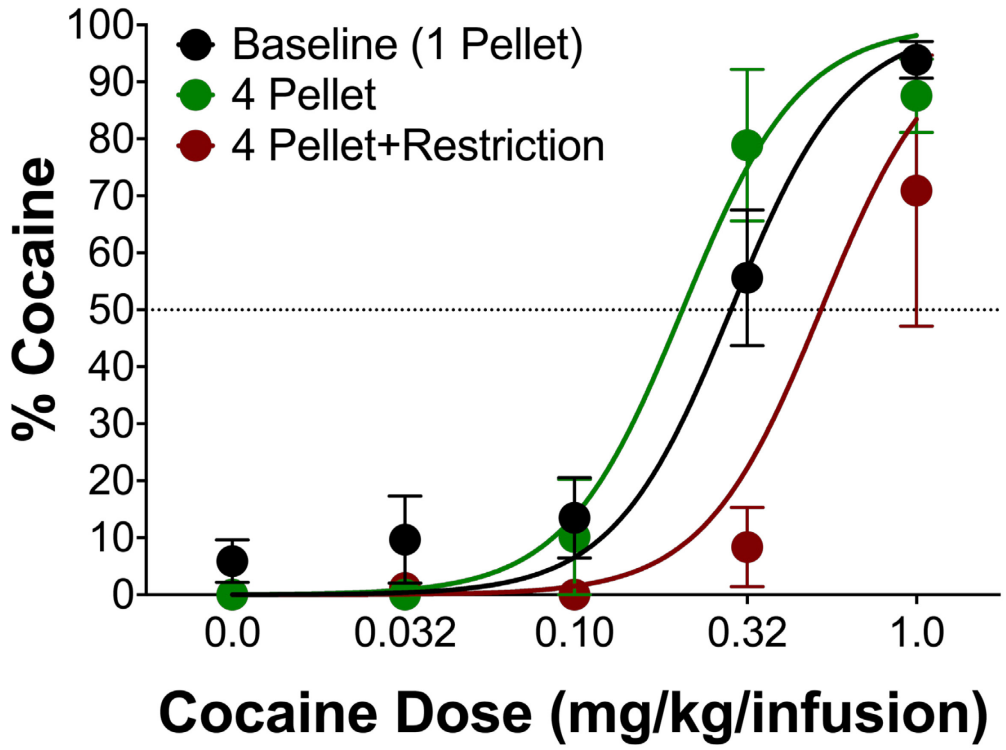


Figure 4.5 Choice Behavior For All Manipulations During Electrode Recordings

Compared to baseline (1 pellet; *a = 0.29; s = 2.52), 4-pellets + FR increased the drug/food exchange rate (*a = 0.53; s = 2.52). There were no differences between the 4-pellet condition and baseline (a = 0.21; s = 2.52). The 4-pellet drug/food exchange rate (*a = 0.21) was significantly decreased compared to the 4-pellet + FR condition. NLME, *$p < 0.05$. Data represented as mean ± SEM.

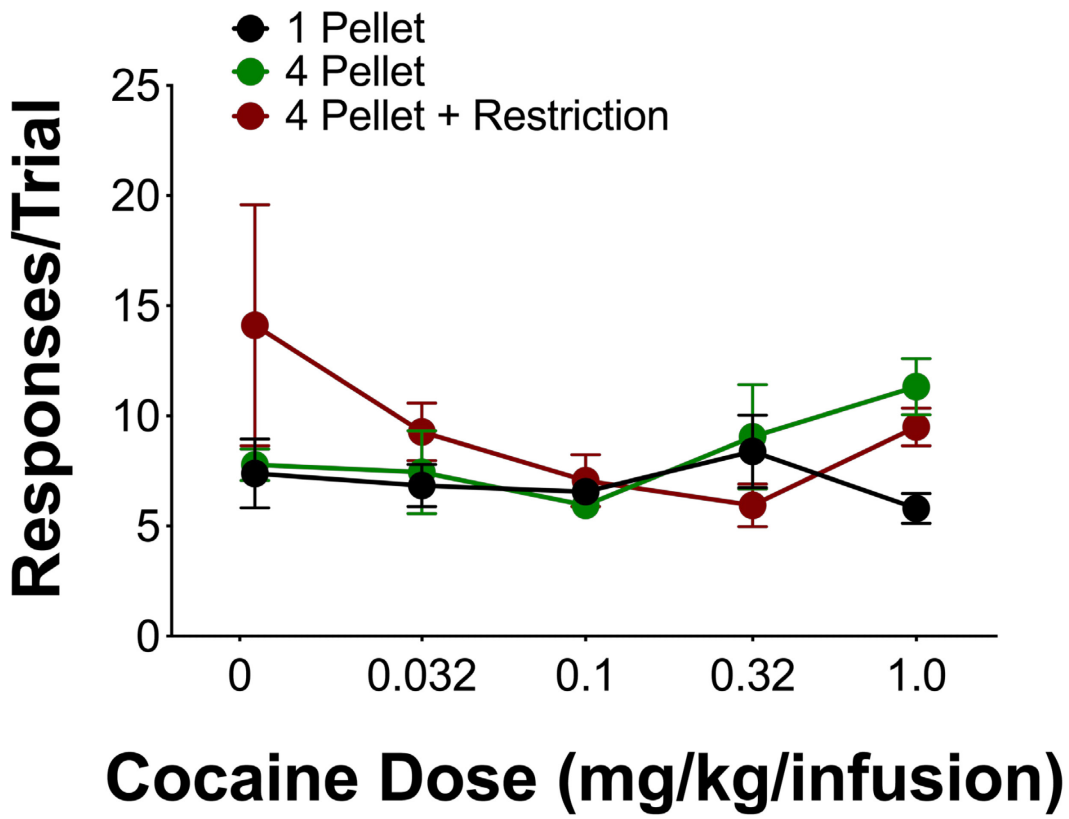


Figure 4.6 Total Number of Lever Responses Per Trial As A Function of Block

There were no significant differences in the number of lever responses per trial as function of block across manipulations. Data points represent the average forced and preferred lever responses across animals for a given block. LME, $p > 0.05$. Data represented as mean ± SEM.

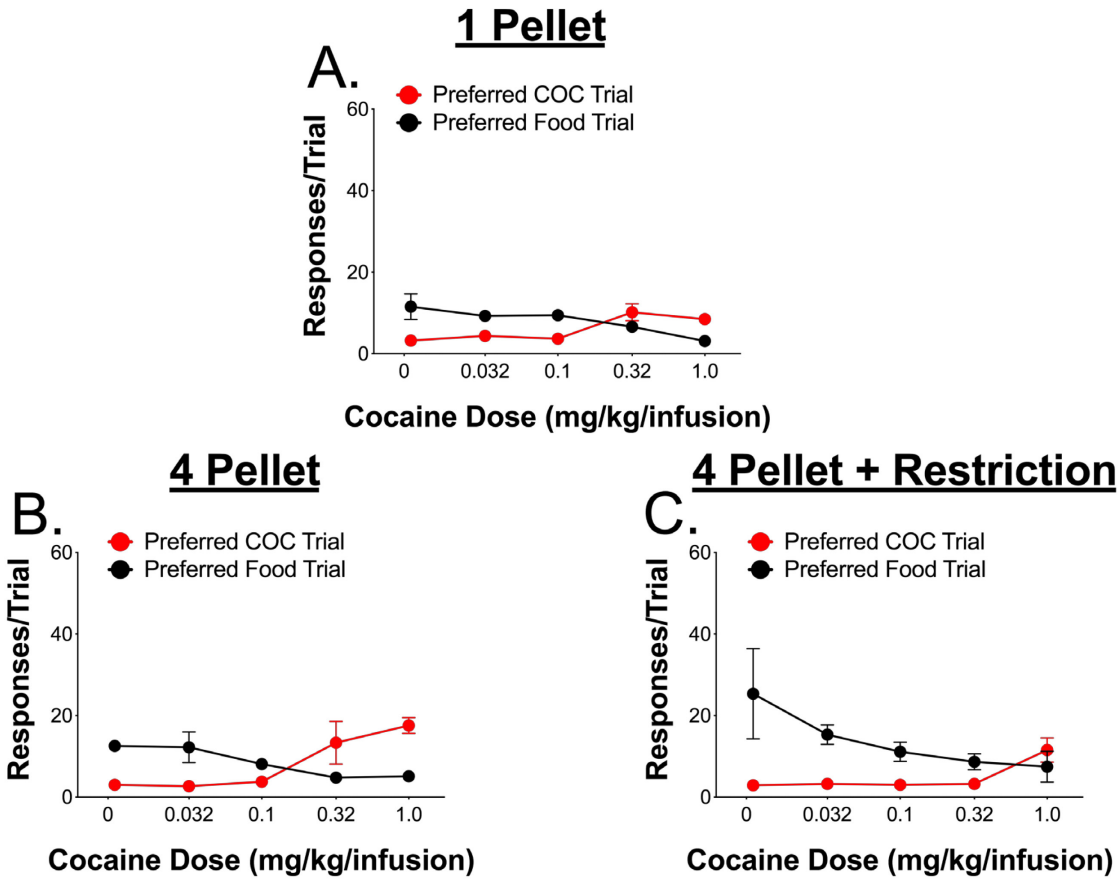


Figure 4.7 Number of Responses Per Preferred COC and Food Trial As A Function of Block

COC responses per preferred COC trial significantly increased and food responses per preferred food trial significantly deceased as a function of block for the **(A)** 1-pellet, **(B)** 4-pellet, and **(C)** 4-pellet + FR conditions. Food responses per preferred food trials for the 4-Pellet + FR condition are also significantly greater than food responses for the 1-pellet and 4-pellet conditions and COC responses for the 4-pellet + FR condition. . LME, $*p < 0.05$. Data represented as mean ± SEM.

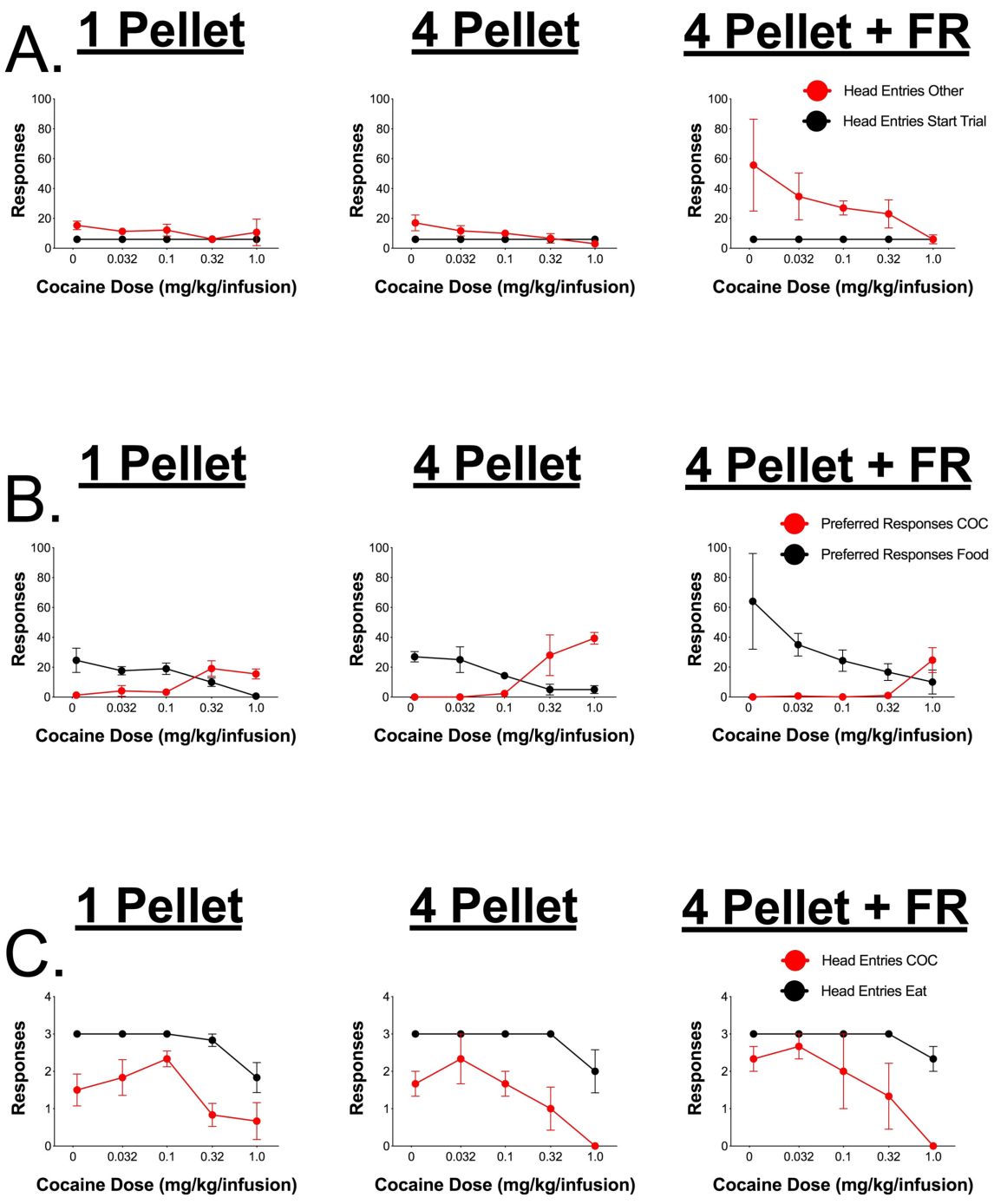

A.
1 Pellet
4 Pellet
4 Pellet + FR
Head Entries Other
Head Entries Start Trial
B.
1 Pellet
4 Pellet
4 Pellet + FR
Preferred Responses COC
Preferred Responses Food
C.
1 Pellet
4 Pellet
4 Pellet + FR
Head Entries COC
Head Entries Eat
Responses
Cocaine Dose (mg/kg/infusion)
0
0.032
0.1
0.32
1.0

Figure 4.8 Behavioral Responses To Head Entries, Preferred Choices, & Head Entries After Reinforcer Delivery

(A) More head entry responses were seen throughout the session compared to those that initiate the trial. Note that the number of head entries to initiate a trial was procedurally constrained (constant at 6/block) whereas all other head entries were unbounded. **(B)** COC and food preferred choice responses significantly changed (in different directions) as a function of block. The number of food responses in the 4-pellet + FR condition was also significantly greater than all other preferred responses across conditions. **(C)** More head entry responses were observed after food reinforcer delivery compared to after COC reinforcer delivery. LME, $*p < 0.05$. Data represented as mean ± SEM.

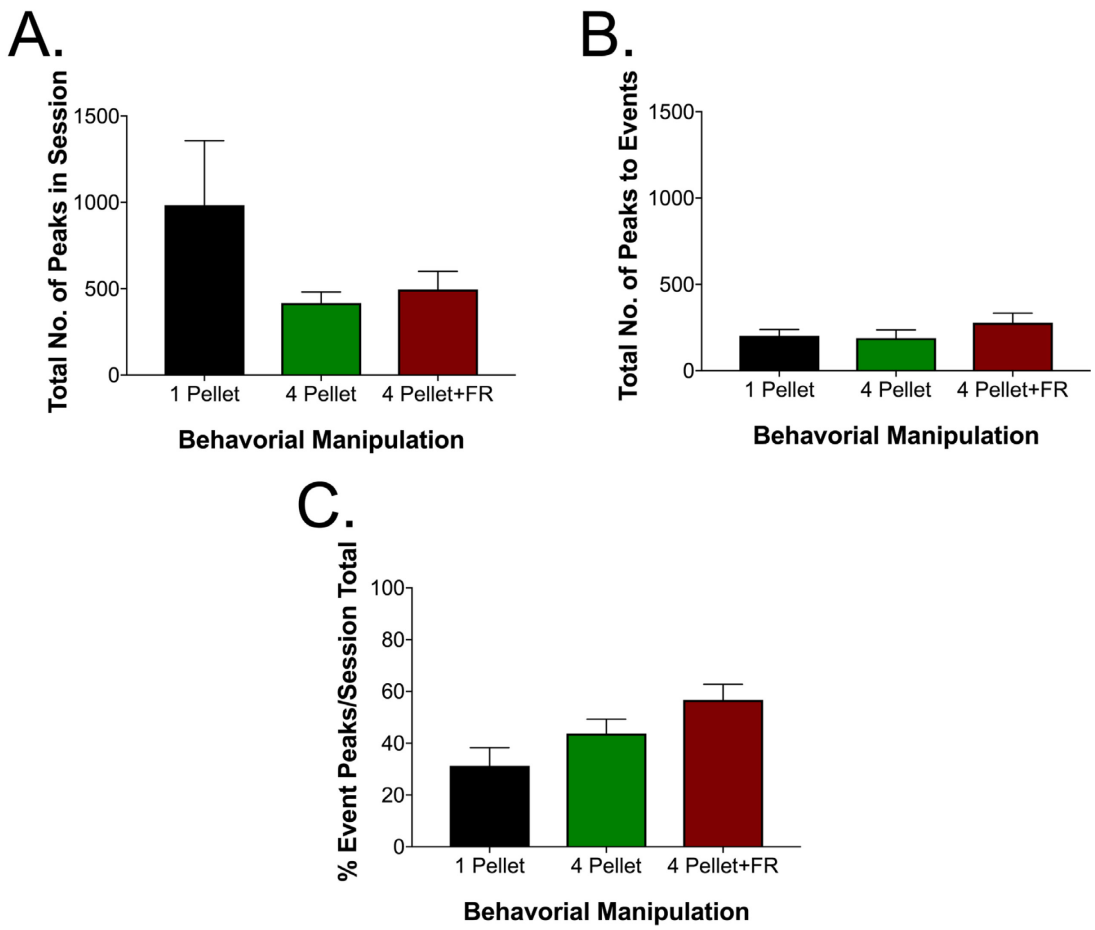


Figure 4.9 Total Number of Oxygen Peaks Found in Session and to Behavioral Events

(A) There were statistically no differences in the number of oxygen peaks that occurred overall between manipulations over a choice session. **(B)** No differences were observed between manipulations in the number of oxygen peaks that occurred to behavioral events in a session. **(C)** The percentage of oxygen peaks that occurred to behavioral events over a session was not different between manipulations. LME, $p > 0.05$. Data represented as mean ± SEM.

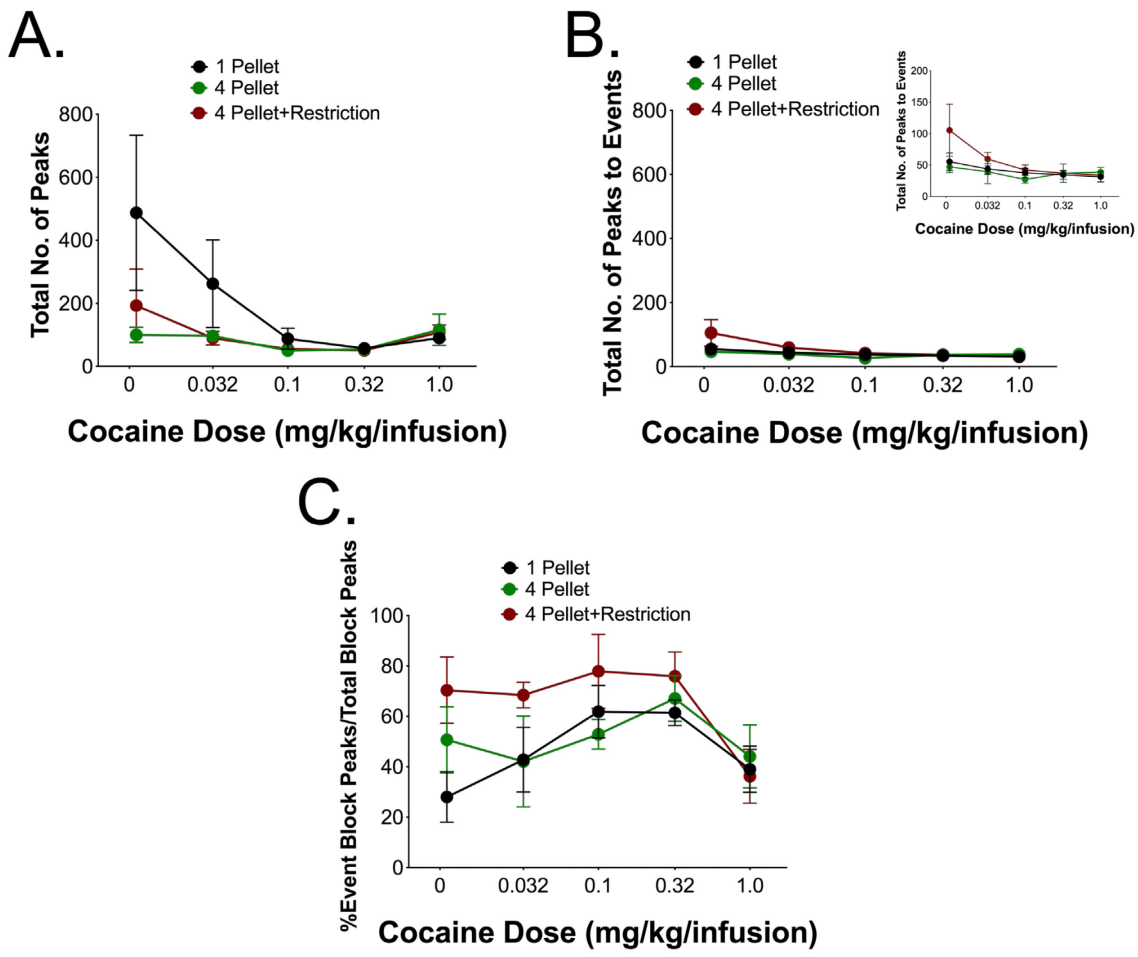
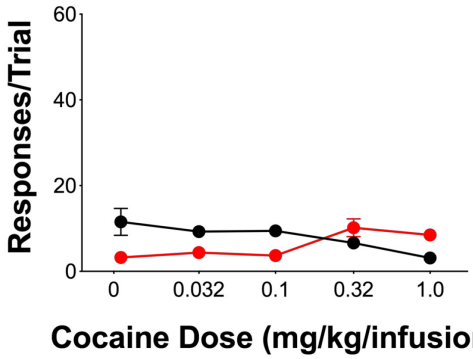


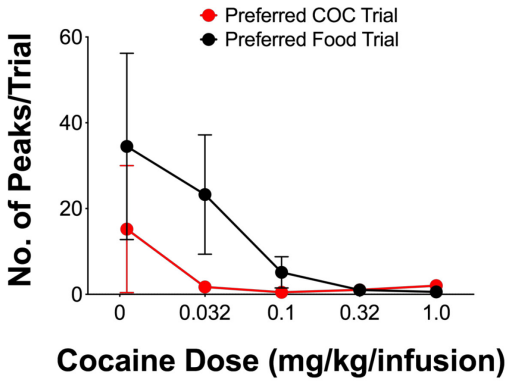
Figure 4.10 Total Number Peaks Found Per Block Overall & to Behavioral Events

(A) There were no differences observed to the overall number of peaks that occurred per block across manipulations. **(B)** The total number of oxygen peaks related to behavioral events significantly decreased as a function of block. Note that the inset is presented to highlight the block main effect. **(C)** No differences were observed in the percent of oxygen peaks related to behavioral events as a function of block. LME, $*p < 0.05$. Data represented as mean ± SEM.

A.

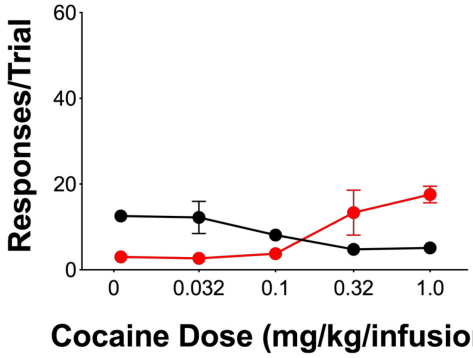
1 Pellet

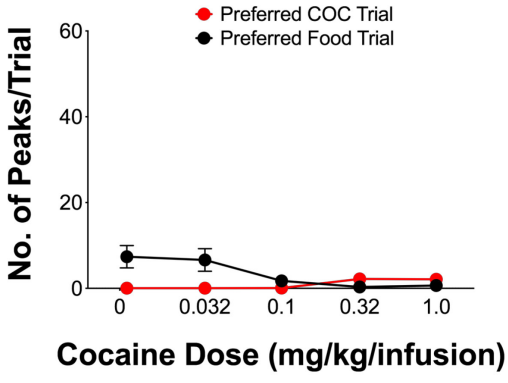




B.

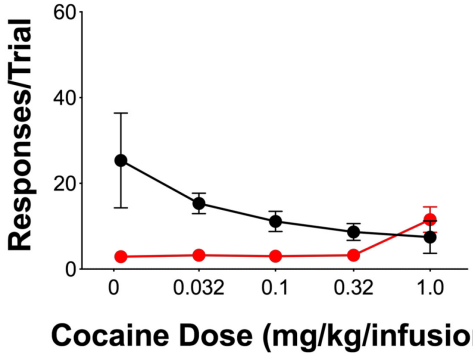
4 Pellet





C.

4 Pellet + Restriction



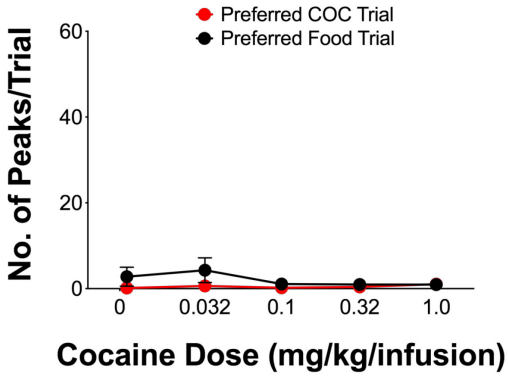
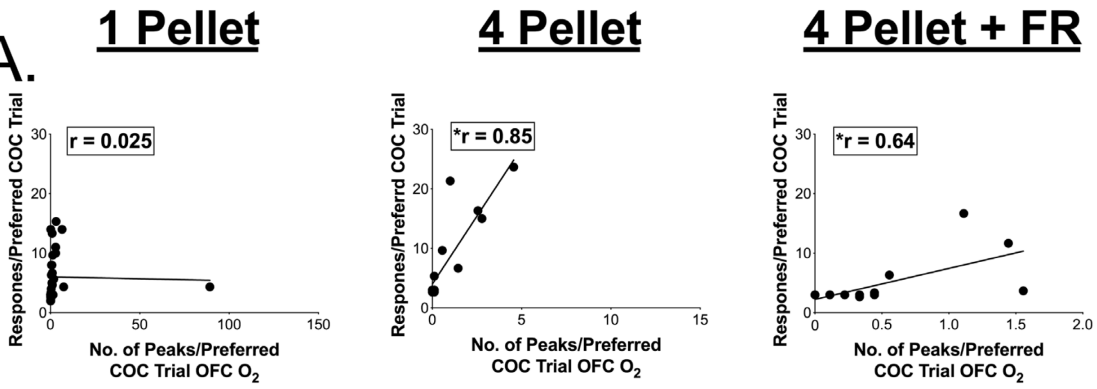


Figure 4.11 The Number of Oxygen Peaks That Occurred Per COC and Food Preferred Trials As A Function of Block

There were no differences in the number of oxygen peaks (regardless of behavioral events) that occurred per preferred COC or food trials for the **(A)** 1-pellet (right), **(B)** 4-pellet (right), and **(C)** 4-pellet + FR (right) conditions. LME, $p > 0.05$. Data represented as mean ± SEM.

A.


1 Pellet
4 Pellet
4 Pellet + FR
r = 0.025
*r = 0.85
*r = 0.64
Respones/Preferred COC Trial
Respones/Preferrd COC Trial
No. of Peaks/Preferred COC Trial OFC O2

B.

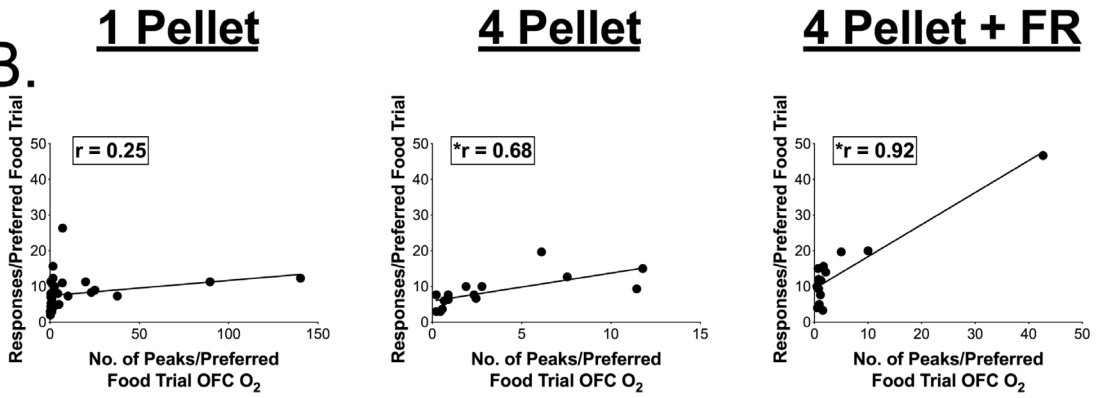

1 Pellet
4 Pellet
4 Pellet + FR
r = 0.25
*r = 0.68
*r = 0.92
Responses/Preferred Food Trial
No. of Peaks/Preferred Food Trial OFC O2

Figure 4.12 Correlation Between The Number of Oxygen Peaks That Occurred Per COC or Food Preferred Trial & The Number of Responses That Occurred Per Preferred Trial

(A) There was no correlation between the number of oxygen peaks that occurred per preferred COC trial and the number of responses that occurred per preferred COC trial for the 1-pellet condition (left). There was a significant positive correlation between the number of oxygen peaks that occurred per preferred COC trial and the number of responses that occurred per COC trial for the 4-pellet (middle) and 4-pellet + FR conditions (right). **(B)** There was no correlation between the number of oxygen peaks that occurred per preferred food trial and the number of responses that occurred per preferred food trial (left). There was a significant positive correlation between the number of oxygen peaks that occurred per preferred food trial and the number of responses that occurred per preferred food trial for the 4-pellet (middle) and 4-pellet + FR (right) conditions. Note the difference in the x-axis scale between measures. Linear regression, $*p < 0.05$.

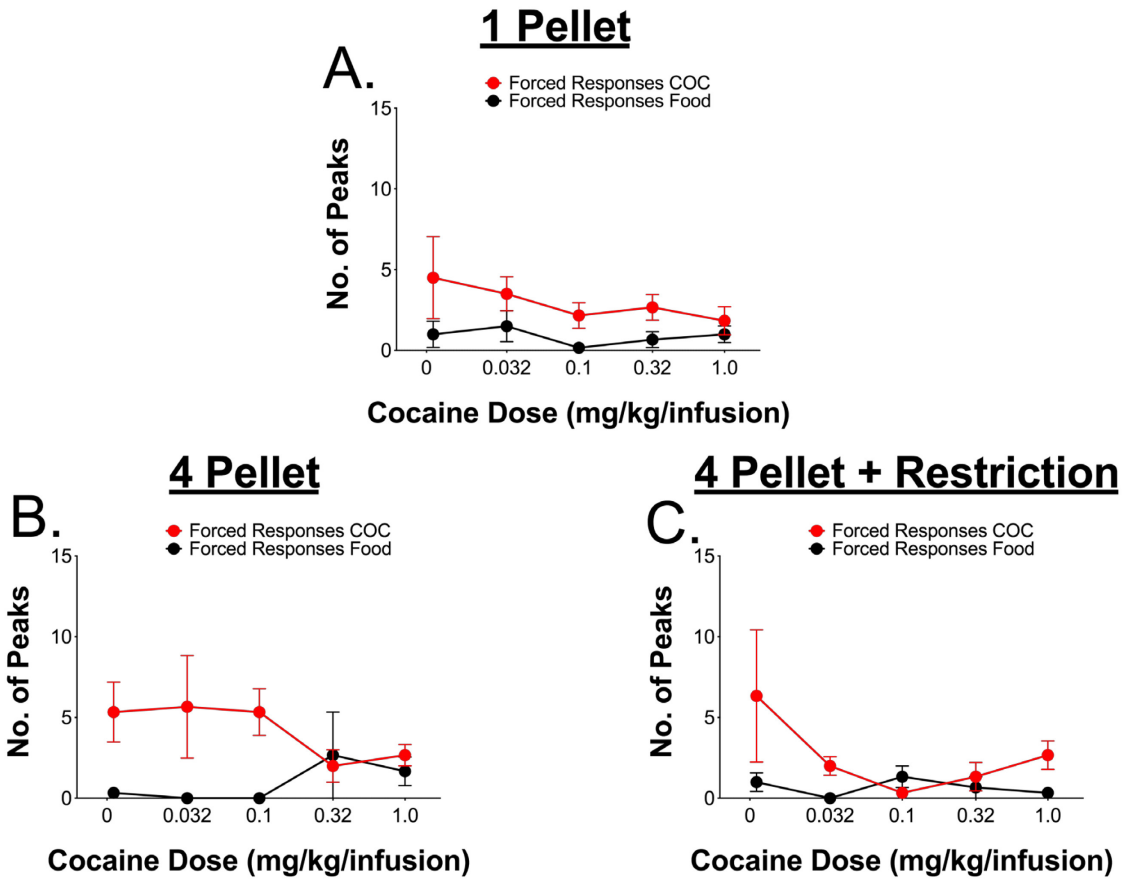


Figure 4.13 Number of Oxygen Peaks That Occurred To Forced Choice Responses

There were no differences in the number of oxygen peaks that occurred to the **(A)** 1-pellet, **(B)** 4-pellet, and **(C)** 4-pellet + FR conditions. However, the number of oxygen peaks to COC was generally greater. Note that no behavior is presented because there were 3 forced trials for each reinforcer (9 responses/block/reinforcer); thus, the behavior was constant across blocks. LME, $p > 0.05$. Data represented as mean ± SEM.

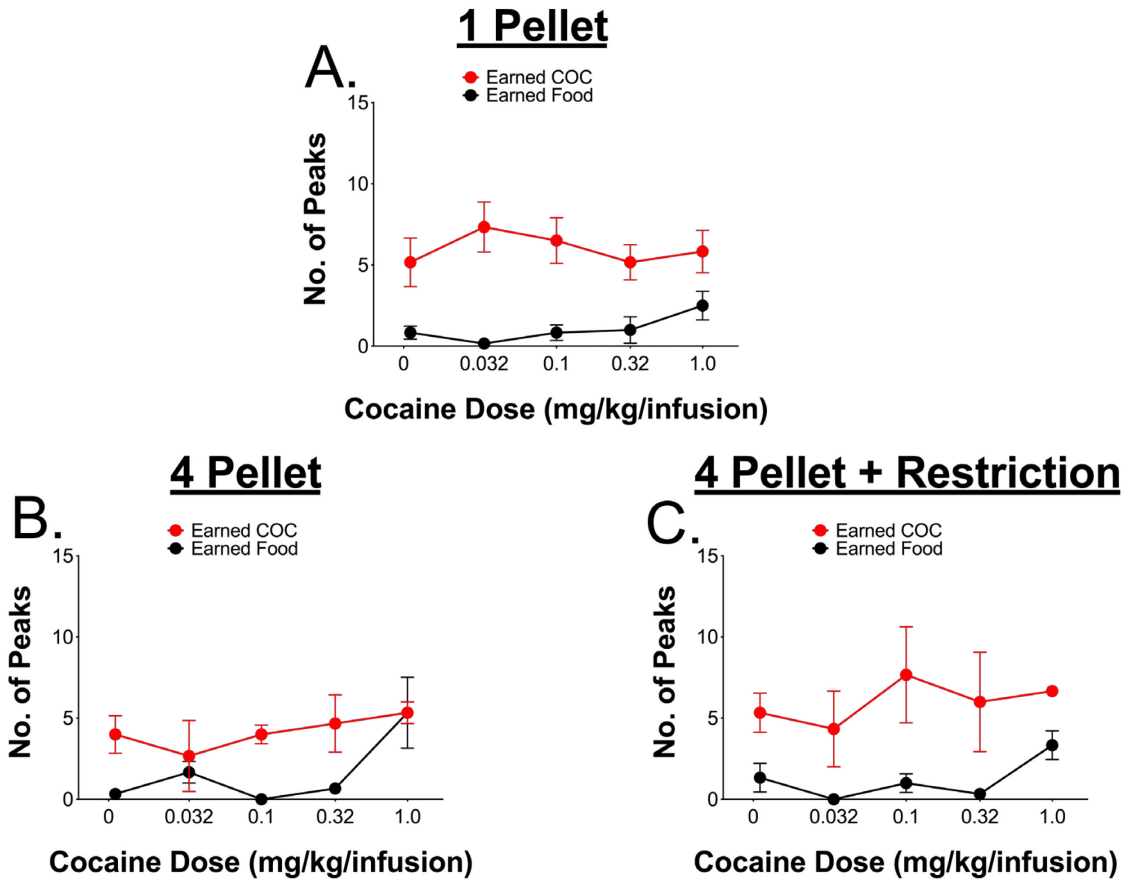
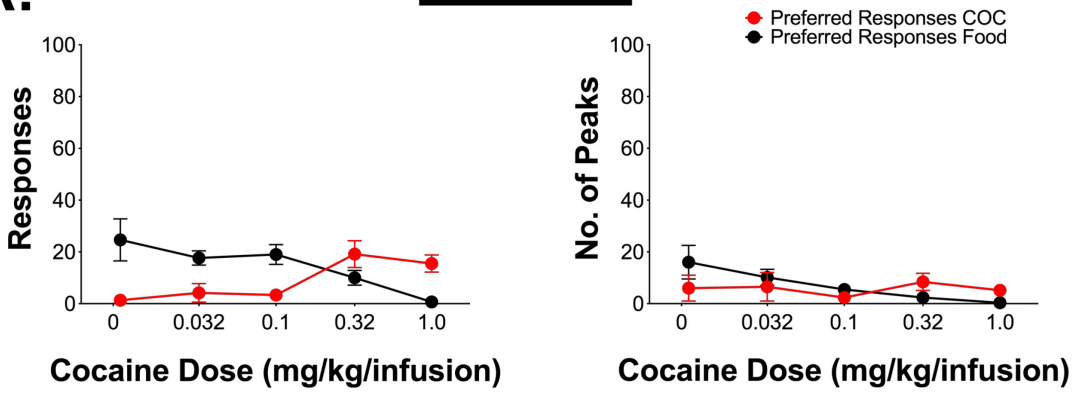


Figure 4.14 Number of Oxygen Peaks That Occurred to Reinforcer Delivery

More oxygen peaks occurred when COC was earned in the **(A)** 1-pellet, **(B)** 4-pellet, and **(C)** 4-pellet + FR conditions compared to when food was earned. Note that in block 1 (COC dose 0 mg/kg/infusion) no COC reinforcers were earned. Note that no behavior is presented because an equal number of reinforcers were earned in each block. LME, $*p < 0.05$. Data represented as mean ± SEM.

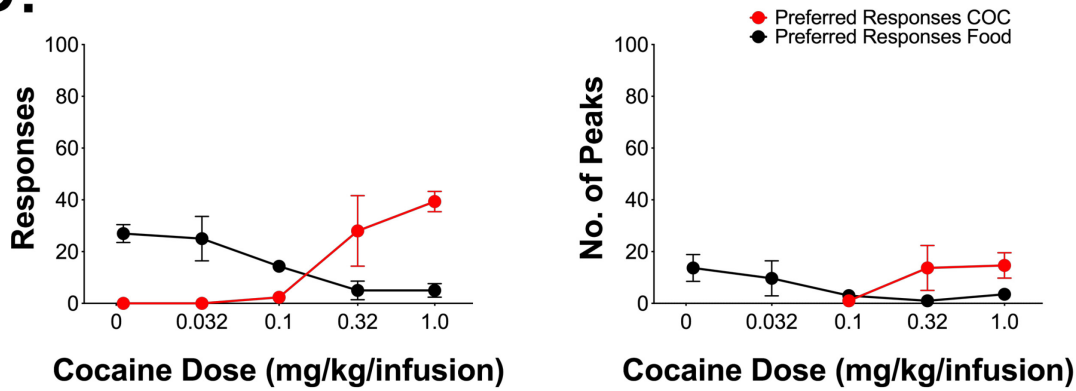
A.

1 Pellet



B.

4 Pellet



C.

4 Pellet + Restriction

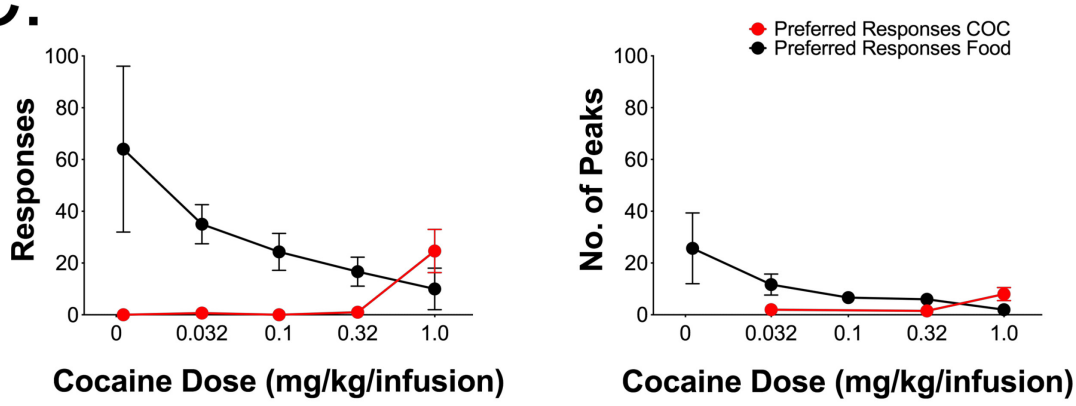
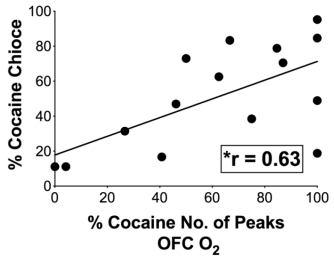


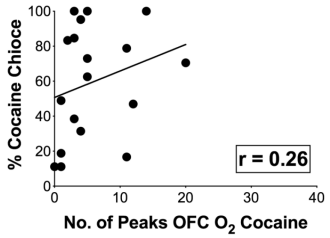
Figure 4.15 Number of Oxygen Peaks That Occurred to Preferred Choice Responses

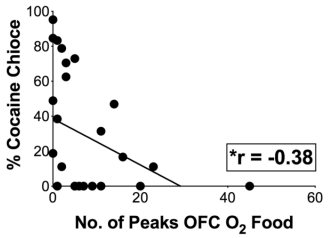
There were no differences in the number of oxygen peaks that occurred to preferred choice responses in the **(A)** 1-pellet (right), **(B)** 4-pellet (right), and **(C)** 4-pellet + FR (right) conditions. LME, $p > 0.05$. Data represented as mean ± SEM.

A.

1 Pellet

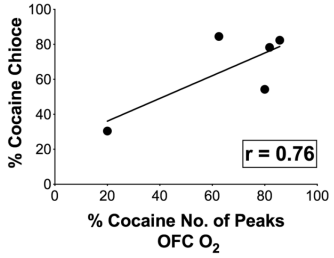


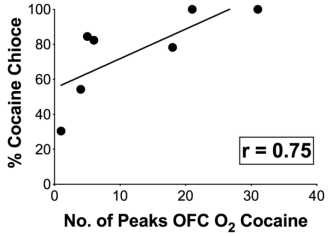


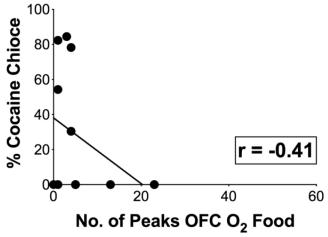


B.

4 Pellet

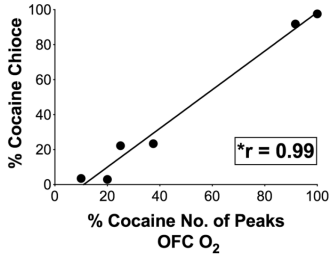


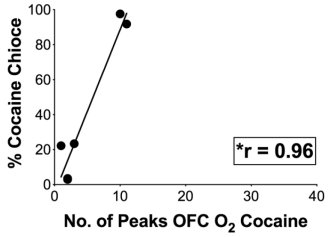




C.

4 Pellet + Restriction





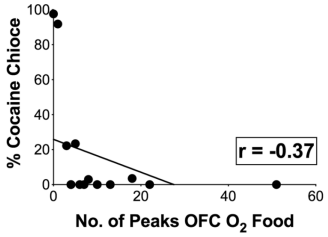


Figure 4.16 Correlation Between Number of Preferred Choice Responses & The Number of Oxygen Peaks That Occurred to Preferred Choice Responses

(A) The percentage of oxygen peaks that occurred to preferred COC choices relative to all preferred choices was positively correlated with COC preference for the 1-pellet condition (left). No correlation was observed between the number of oxygen peaks that occurred to COC preferred responses and COC preference for the 1-pellet condition (middle). The number of oxygen peaks that occurred to preferred food choices was negatively correlated with COC preference for the 1-pellet condition (right). **(B)** The percentage of oxygen peaks that occurred to preferred COC choices relative to all preferred choices was not correlated with COC preference for the 4-pellet condition (left). No correlation was observed between the number of oxygen peaks that occurred to COC preferred responses and COC preference for the 4-pellet condition (middle). The number of oxygen peaks that occurred to preferred food choices was not correlated with COC preference for the 4-pellet condition (right). **(C)** The percentage of oxygen peaks that occurred to preferred COC choices relative to all preferred choices was positively correlated with COC preference for the 4-pellet + FR condition (left). The number of oxygen peaks that occurred to COC preferred responses was positively correlated with COC preference for the 4-pellet + FR condition (middle). The number of oxygen peaks that occurred to preferred food choices was not correlated with COC preference for the 4-pellet + FR condition (right). Note the difference in the x-axis scale between measures. Linear regression, $*p < 0.05$.

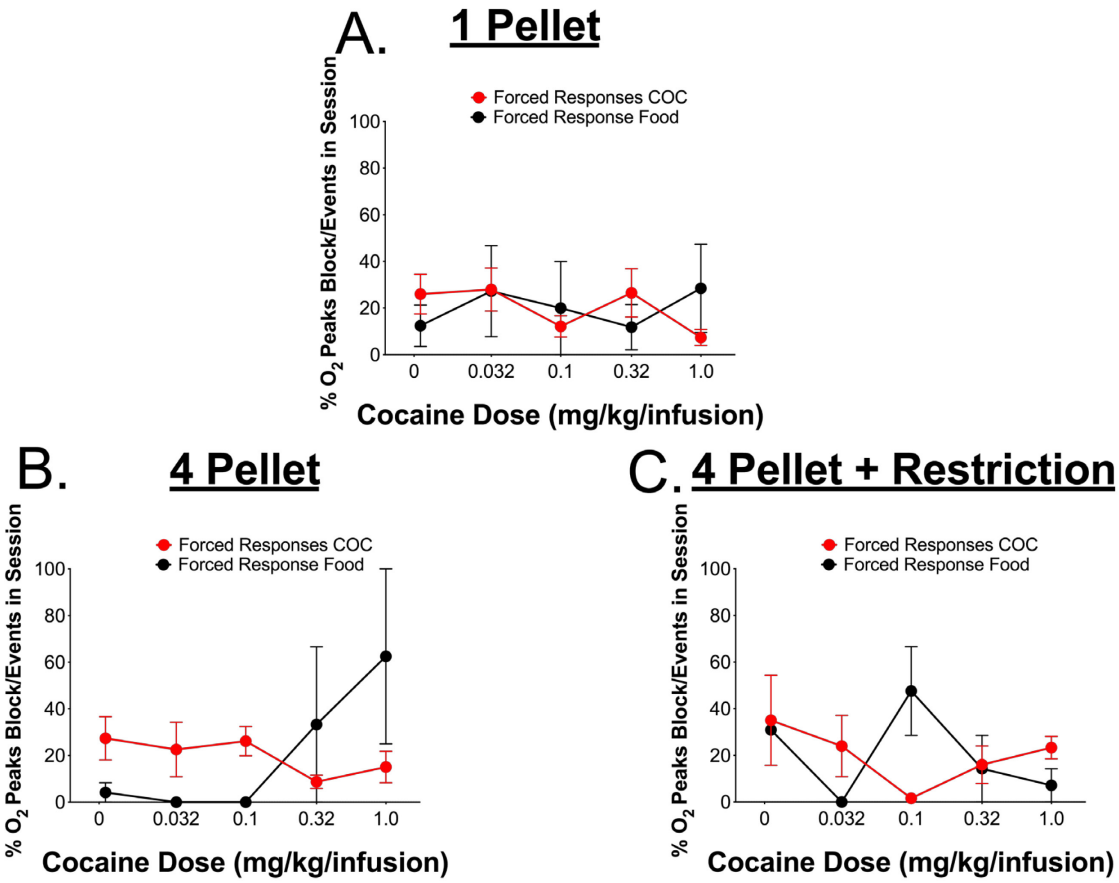


Figure 4.17 Percentage of Oxygen Peaks That Occurred to COC or Food Forced Choice Responses Per Block Relative to The Number of Oxygen Peaks That Occurred to COC or Food Forced Choice Responses in A Session

There were no significant differences observed in the percentage of oxygen peaks that occurred to COC or food forced choice responses per block relative to those found to COC or food forced responses over a session between the **(A)** 1-pellet, **(B)** 4-pellet, and **(C)** 4-pellet + FR conditions. LME, $p > 0.05$. Data represented as mean ± SEM.

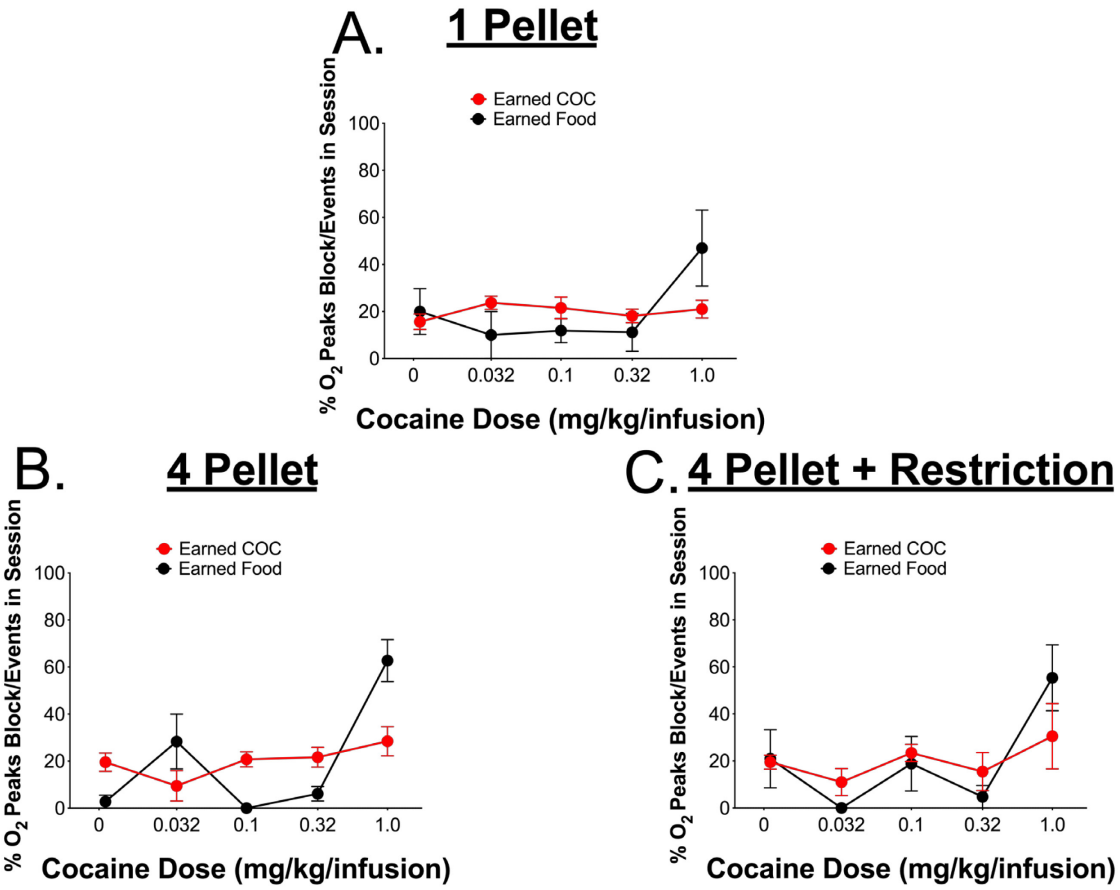
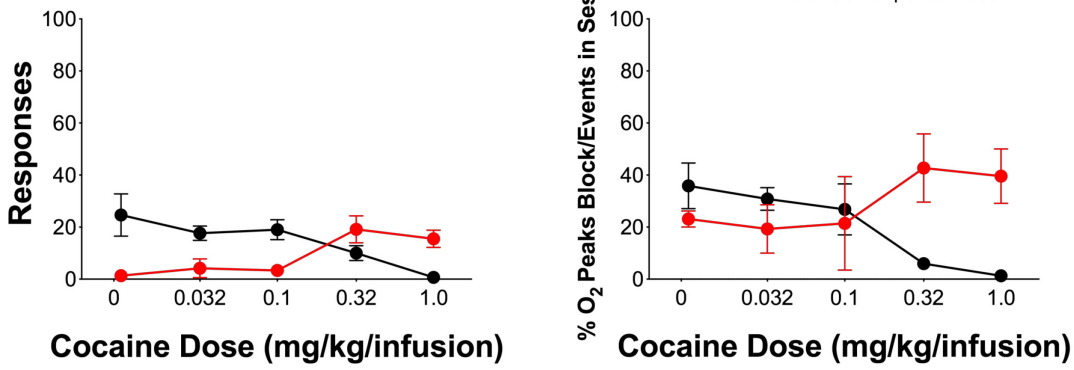


Figure 4.18 Percentage of Oxygen Peaks That Occurred to COC or Food Reinforcers Per Block Relative to The Number of Oxygen Peaks That Occurred to COC or Food Reinforcers in A Session

The percentage of oxygen peaks that occurred to COC or food reinforcer delivery per block relative to those found to COC or food reinforcers delivered over a session increased as a function of block in the **(A)** 1-pellet, **(B)** 4-pellet, and **(C)** 4-pellet + FR conditions. LME, $*p < 0.05$. Data represented as mean ± SEM.

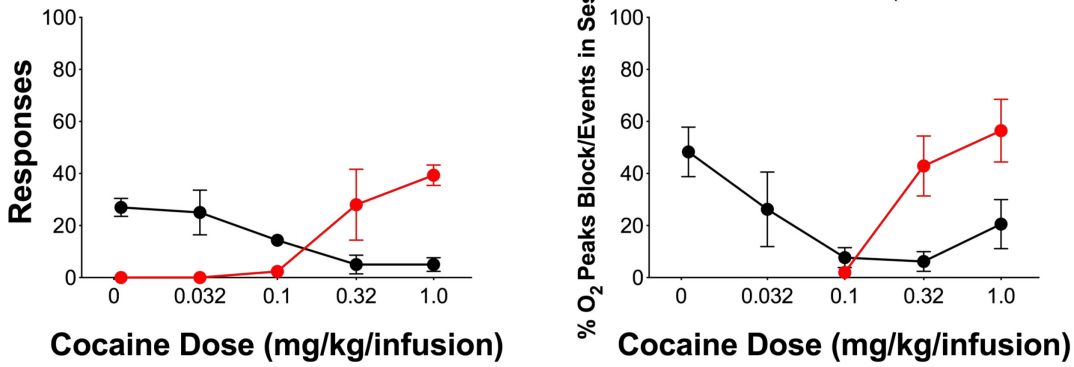
A.

1 Pellet



B.

4 Pellet



C.

4 Pellet + Restriction

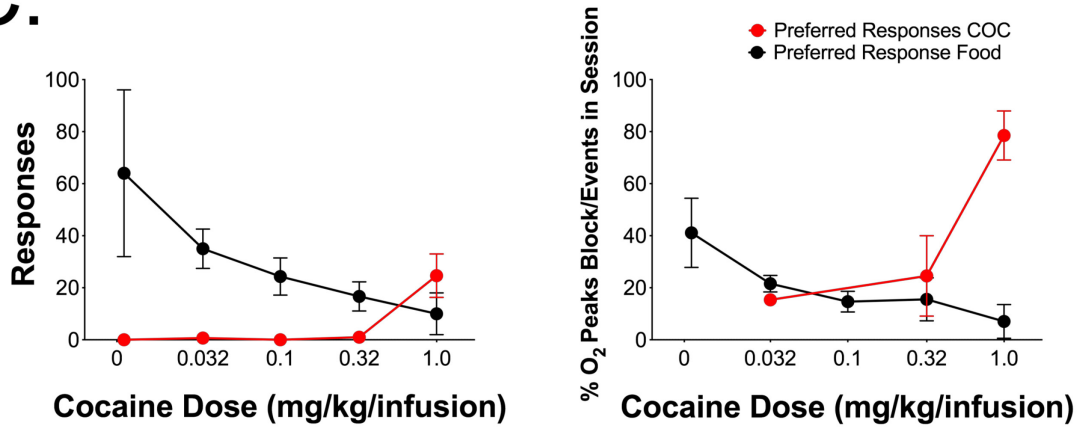
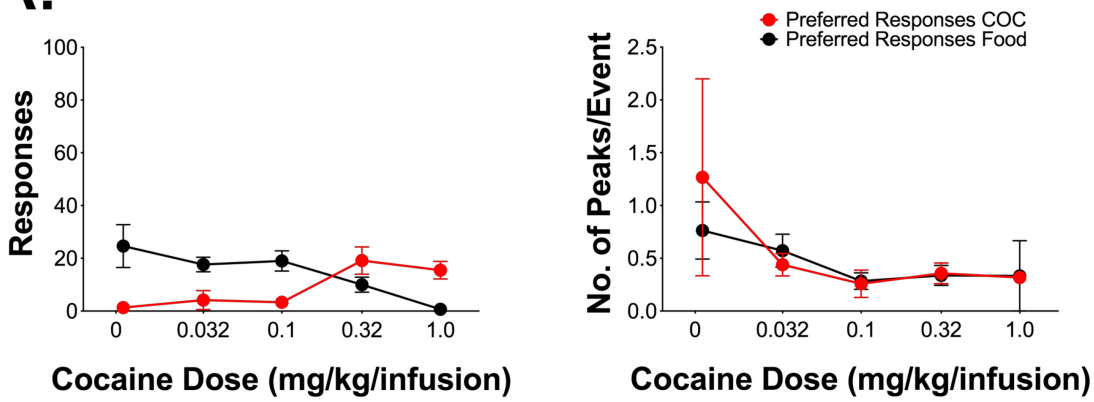


Figure 4.19 Percentage of Oxygen Peaks That Occurred to Preferred COC or Food Choices Per Block Relative to The Number of Oxygen Peaks That Occurred to Preferred COC or Food Choices in A Session

The percentage of oxygen peaks that occurred to preferred COC choices per block relative to those found to preferred COC choices over a session increased as a function of block in the **(A)** 1-pellet, **(B)** 4-pellet, and **(C)** 4-pellet + FR conditions. The percentage of oxygen peaks that occurred to preferred food choices per block relative to those found to preferred food choices over a session decreased as a function of block in the **(A)** 1-pellet, **(B)** 4-pellet, and **(C)** 4-pellet + FR conditions. LME, $*p < 0.05$. Data represented as mean ± SEM.

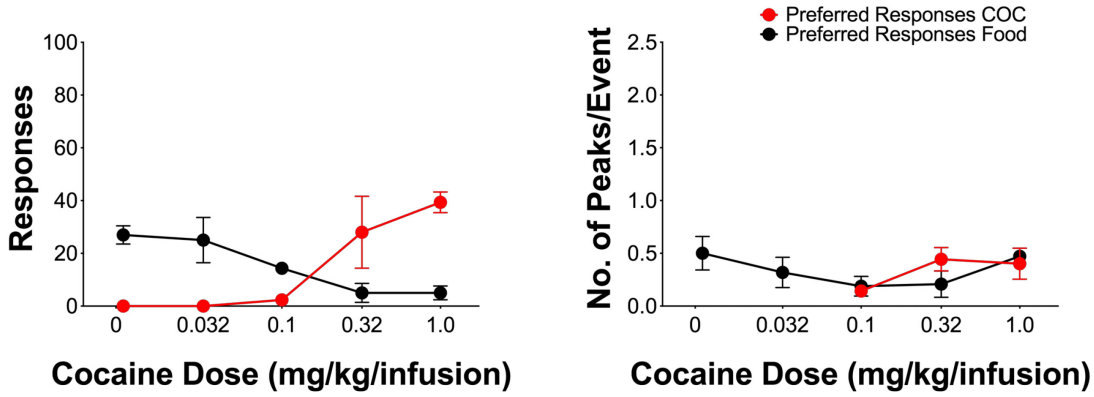
1 Pellet

A.



4 Pellet

B.



4 Pellet + Restriction

C.

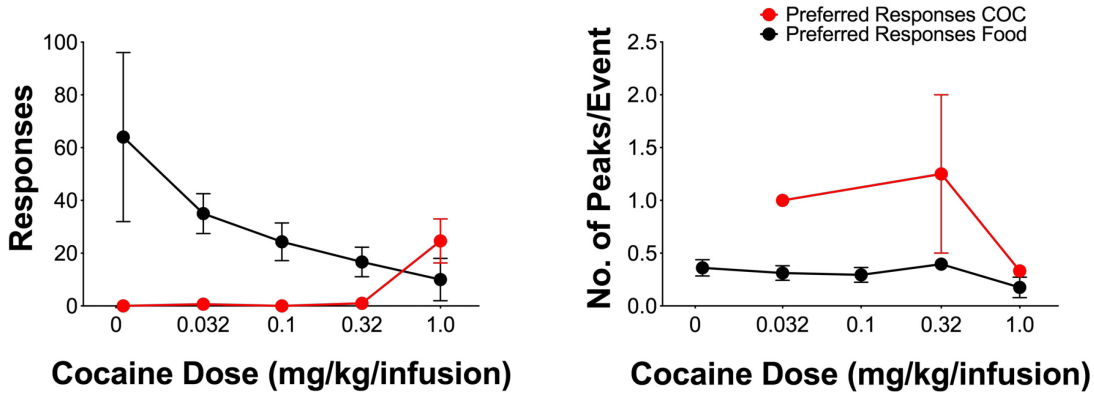


Figure 4.20 Number of Oxygen Peaks That Occurred to Preferred Choice Responses Per The Number of Preferred Responses

There were no differences in the number of oxygen peaks that occurred to preferred choice responses per the number of events that occurred in the **(A)** 1-pellet (right), **(B)** 4-pellet (right), and **(C)** 4-pellet + FR (right) conditions. LME, $p > 0.05$. Data represented as mean ± SEM.

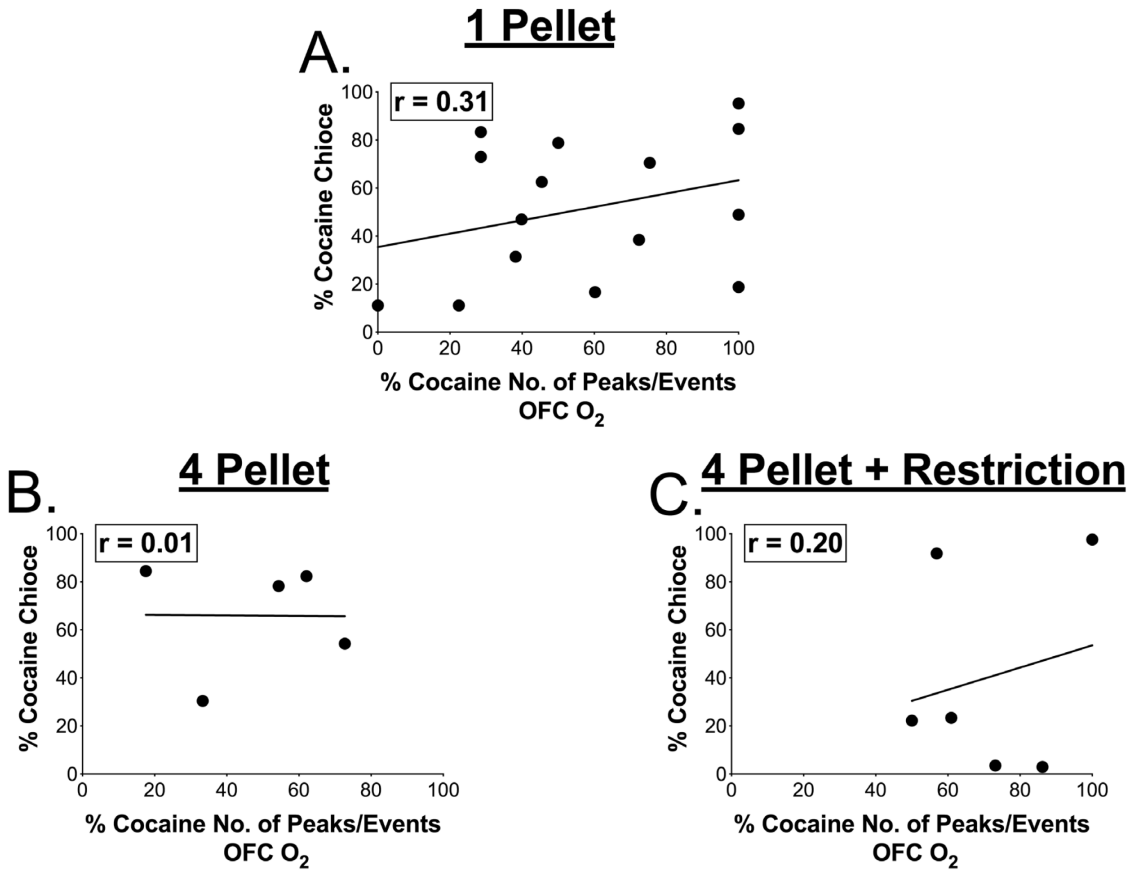


Figure 4.21 Correlation Between Number of Preferred Choice Responses & The Number of Oxygen Peaks That Occurred Per Preferred Choice Responses

The percentage of oxygen peaks that occurred per preferred COC choices relative to all preferred choices was not correlated with COC preference for the (**A**) 1-pellet, (**B**) 4-pellet, or (**C**) 4-pellet + FR conditions. Linear regression, $p > 0.05$.

CHAPTER 5

GENERAL DISCUSSION

The purpose of these studies was to better understand the neurobehavioral mechanisms of cocaine-use disorder. Considering that aberrant glutamate neurotransmission has been associated with cocaine abuse and relapse (see Kalivas, 2009 for review) the first of these studies explored glutamate neurotransmission in freely-moving rats that performed in a cocaine/food multiple schedule. As previously mentioned, most non-clinical experiments exploring the effects of cocaine on the glutamate system use behavioral paradigms that do not expose the same animal to drug and non-drug reinforcers making cocaine-specific neural changes hard to determine (Cunningham et al., 2015; Huff & LaLumiere, 2015; Saddoris et al., 2016). Considering no person takes drugs in isolation and is exposed to a myriad of reinforcers, here we used multiple schedules of reinforcement (Weissenborn et al., 1995; Weissenborn et al., 1996; Carelli et al., 2000; Stairs et al., 2010) in an attempt to isolate cocaine-specific glutamatergic effects. By coupling glutamate biosensors with the multiple schedule behavioral paradigm, this first experiment was able to isolate cocaine and food reward-related glutamatergic changes in the PrL and NAcC.

Previous work has shown that glutamate neurotransmission in the NAcC and the PrL is involved in drug seeking and relapse (McFarland et al., 2003; Kalivas et al., 2005) and that cocaine self-administration increases glutamate levels above baseline in rats chronically exposed to cocaine (Miguens et al., 2007). Thus, it was not surprising that an increase in glutamate release was observed from cocaine delivery compared to food delivery. However, a significant increase in the number of glutamate peaks from food events compared to cocaine events was also observed. This result was surprising considering that the probability of glutamate release is shown to increase during cocaine reinstatement and self-administration (Moran et al., 2005; Madayag et al., 2007;

Miguens et al., 2007; Kalivas 2009 for review). However, as mentioned, most studies looking at the effects of cocaine on glutamate use separate animals for experimental and control conditions. Thus, the increase in the number of glutamate events to food related behavior compared to cocaine related behavior observed here could be due to the fact that all animals in this study were exposed to both reinforcers. Conversely, this increase in the number of glutamate peaks to food events could be due to the fact that all rats had a greater history with the food reinforcer. The fact that differential reinforcer histories can cause different neural adaptations (Nestler, 2001; Kalivas & O'Brien, 2008) lends credence to this idea.

Results from experiment 1 also showed that the number of glutamate peaks that occurred to a given behavioral event was positively correlated to that respective event. This finding is similar to glutamate measures taken from the basolateral amygdala and OFC in other behavioral paradigms (Malvaez et al., 2015, Malvaez et al., 2019). However, while these results are reassuring, they should be interpreted with caution because it is possible that the number of glutamate peaks increased simply because the amount of behavior increased. Thus, it could be that more glutamate peaks were more likely to occur simply because more behavior was emitted. That being said, there is evidence that the NAcC does not participate in the processing of movements (Shultz et al., 1992; Carelli & Deadwyler, 1997). Also, the relationship between the number of glutamate peaks and the number of behavioral responses was not 1:1 suggesting that the number of glutamate peaks occurring to behavioral events may be due to more than just responding. Confidence in this conclusion comes from the fact that the number of peaks that occurred to the start of the food component was greater than the number of peaks that occurred to the start of the cocaine component even though the number of components was equal. Overall, these results suggest that the number of glutamate peaks are likely encoding reinforcer specific information and are not just due to the amount of responding.

In an attempt to control for the problematic nature of the number of peaks analysis discussed above the number of glutamate peaks that occurred to a

specific event were divided by the number of responses that occurred. Standardizing the number of peaks to the number of behavioral responses in theory should control for the disproportionate number of responses between event types, thus creating a measure that is less confounded by the amount of behavior. Interestingly, when the data were expressed as the number of glutamate peaks that occurred per event type, more glutamate peaks were observed per cocaine lever press and per cocaine reinforcer earned. Thus, even though more glutamate peaks were associated with food lever presses overall, there were more glutamate peaks generated per a single cocaine lever press and per a cocaine reinforcer. Thus, when looking at this measure, there is dissociation between behavior and glutamatergic activity further suggesting that the number of glutamate peaks is encoding reinforcer specific information. This result is similar to what has previously been found in the literature (e.g. McFarland et al., 2003; Miguens et al., 2007; Kalivas, 2009 for review) in that chronic exposure to cocaine causes an increase in the probability of synaptic glutamate release.

A potential weakness of experiment 1 was in interpreting the meaning of glutamate frequency data in relation to behavior (i.e. more release events only because more behavior occurred). Even though controlling for this issue was attempted, it is likely that these measures are still partially confounded. Thus, it is difficult to say for certain that there were reinforcer specific effects. Further, even though animals were exposed to both reinforcers they could still only respond for one reinforcer at a time. Also, the reinforcer histories were not controlled. These latter two factors make it difficult to say anything about brain representations of value in relation to these two reinforcers or the actions taken to earn them.

Experiment 2 addressed some of the issues in experiment 1. First, a novel choice procedure was used that controlled for the positive feedback function between choices and reinforcement as well as reinforcer history (Beckmann et al., 2019). Also, choice procedures more easily allow for the assessment of the relative value of cocaine and food compared to multiple schedule procedures due to the fact that reinforcers are concurrently available. Also, considering a number

of behavioral responses were held constant between cocaine and food events in this choice procedure, this allowed for a more straightforward interpretation of brain measures between cocaine and food events. Further, considering that reinforcer history was held constant, this allowed for behavioral and brain measures of preference to not be confounded by reinforcer intake (Chow, 2018; Beckmann et al., 2019).

It may seem disjunctive that in experiment 2 oxygen was measured instead of glutamate and that these measurements were taken from a completely different brain region (OFC) than in experiment 1. However, this was decided due to the fact that the interest of experiment 2 was in exploring drug-related decision-making and value. Thus, there was more precedence for taking measures from the OFC compared to the PrL (Kable & Glimcher, 2009). Note that there was equal precedent for taking oxygen measures from the NAcC (Salamone et al., 2007) and in retrospect this should have been done first for reasons of comparison. However, at the time the experiment was designed, evidence from our lab (unpublished) showed that OFC lesions changed choice behavior in our experimental paradigm in distinctive ways. Due to that finding, as well as others (Schoenbaum et al., 2006; Padoa-Schioppa & Conen, 2017), measures were taken from the OFC first. Further, there is a paucity of data exploring glutamate dynamic in decision-making. Thus, it made more sense to explore overall brain activity (here measured by oxygen dynamics).

Although not significant, it was surprising that drug preference increased when 4 food pellets could be earned compared to the 1-pellet condition as previous research suggests the opposite (Nader & Woolverton, 1991). However, this was likely because rats became sated on food pellets and thus the relative subjective value of cocaine was increased. Expectedly, drug preference decreased when 4 pellets could be earned and rats were food restricted, a finding that is supported by previous work (Beckmann et al., 2019). Overall, these data are in line with other published reports showing that environmental manipulations, including changing reinforcer magnitude, can affect drug-related

decision-making (Carroll & Lac, 1993; Nader & Woolverton, 1991; Schierenberg et al., 2012; Hutsell et al., 2015; Chow, 2018; Beckmann et al., 2019).

There were also generally a greater number of oxygen peaks seen to forced cocaine responses compared to forced food responses even though the number of responses was approximately equal (however, this was not significant). Considering, this effect was seen even when the number of responses was held constant lends support to this being a cocaine specific effect. This result is consistent with previous literature that showed that OFC activity during cocaine sampling was greater than the activity observed during food sampling (Guillem & Ahmed, 2017). Considering reinforcer delivery was not included in the forced choice analysis, the increase in the number of oxygen peaks observed could be due to an increase in cue reactivity to the cocaine lever oppose to the food lever. In fact evidence suggests that BOLD activity in the OFC has been associated with craving (Risinger et al., 2005). However, it is worth noting that after the first block cocaine can be earned and can thus potentially affect brain signaling to other behavior. Considering acute cocaine administration has been shown to increase BOLD activity in the OFC (Kufahl et al., 2005) it is possible that the changes in the number of oxygen peaks observed (during and after the second block) were due to the pharmacological properties of cocaine. However, the oxygen responses here were in a general U-shaped pattern similar to those found to be associated with 'chosen value' by Padoa-Schioppa & Assad (2006). Considering this general trend, and the fact that oxygen signaling to forced responses looked markedly different than when the reinforcer was earned, it suggests that the oxygen signals found in experiment 2 to forced responses may represents some relative encoding of value. However, note that in this study the preference measure is independent of forced responses. Thus, oxygen changes in forced trials should not directly relate to choice preference making these findings hard to compare to Padoa-Schioppa & Assad (2006).

Differences were also observed when the reinforcer was earned. Namely, the number of oxygen peaks was increased when cocaine was earned compared to when food was earned. Again, this is similar to results showing that neuronal

activity increased in the OFC to actions associated with a cocaine reinforcer compared to a food reinforcer (Guillem & Ahmed, 2017). Further, the increased activity to cocaine reinforcer delivery observed here is also similar to studies showing increased OFC activity to drug-related cues and to acute cocaine administration (Kufahl et al., 2005; Risinger et al., 2005; for review Schoenbaum et al., 2006; for review Dom et al., 2007). Considering the number of reinforcers per block is held constant this again suggests dissociation between cocaine and food events. However, considering that drug was being delivered when the increase in oxygen peaks were observed it is likely this increase in oxygen has more to do with the direct effects of cocaine oppose to changes in value per se.

Unlike most choice studies, here the major measure of choice preference was responses on the cocaine or food lever when reinforcement was not available. No differences were observed in the number of oxygen peaks to preferred choice responses. However, there were significant correlations between the number of oxygen peaks observed to cocaine and food preferred responses and cocaine preference. Specifically, the percent of oxygen peaks observed to preferred cocaine responses was positively correlated with cocaine preference. These correlations seem to be driven by a general positive correlation with the number of oxygen peaks that occurred to cocaine preferred responses and cocaine preference and a general negative correlation between the number of oxygen peaks that occurred to food preferred responses and cocaine preference. Again, these results are similar to others found in the literature. For example, Guillem and Ahmed (2017) found that neuronal activity in the OFC was positively correlated with cocaine preference. However, Chow (2018) found no correlation between the percent cocaine cFos+ cells in the OFC and cocaine preference. Considering the procedure used in experiment 2 was the same used in Chow (2018) it is unclear why these results differ. However, it could be due to the fact that the oxygen measures shown here were collected in real-time and are a bit more dynamic than cFos measures. Conversely, it could be that the cFos measure is a more accurate representation of neural activity than oxygen measures. Nevertheless, this study, Chow (2018), and Beckman et

al. (2019) all support the idea that preference is a relative measure determined by different reinforcement dimensions (e.g. frequency, magnitude) and not drug intake (Iglauer & Woods, 1974; Anderson et al., 2002).

Even though experiment 2 controlled for many of the issues observed in experiment 1 it was not without pitfalls. For example, the number of preferred choice responses was still free to vary and was susceptible to being confounded with the amount of behavior that occurred in a similar way as was discussed in experiment 1. However, it is worth noting that, as in experiment 1, there was not a 1:1 relationship between the number of oxygen peaks that occurred to preferred responses and the number of preferred response that occurred suggesting that these results were not completely confounded. Further, there were positive correlations between the number of oxygen peaks that occurred per preferred food and cocaine trials and cocaine preference. Considering the trial measure looked at all oxygen peaks in a trial and not just those related to behavioral events suggest that the oxygen signal to preference is not solely due to the amount of behavior.

These experiments showed a number of interesting results. However, there were several changes that could have been made that may have allowed richer comparisons. For example, brain manipulations could have been made (using pharmacology, DREADDs, etc.) in experiment 1 to assess if the glutamatergic signal could be specifically manipulated and behavior specifically changed. Further, glutamate could have been measured in the PrL and NAcC in experiment 2 so that results could have been more comparable between the two experiments. Conversely, oxygen measures could have been taken from the PrL and NAcC in experiment 2 so that comparisons could be made between the two experiments more easily. In experiment 2, frequency manipulations in the choice procedure (like those made in Chow, 2018 and Beckmann et al., 2019) could have been done to assess how oxygen dynamics in the OFC change to those manipulations in order to make cross-study comparisons more direct. The data could have also been analyzed such that we looked at signaling some time before an event occurred in order to see if pre-event signaling was predictive of

behavior. Considering that BOLD fMRI measures have a slower temporal resolution than electrochemical measures (second vs. millisecond timescale; see Glover, 2011 for review) the oxygen measures here could have been taken at a lower frequency (e.g. 1 Hz) in order to increase translational efficacy. Thus, if the oxygen measures were taken at a slower temporal resolution than the measures here would have been more easily comparable to clinical BOLD fMRI studies. Further, in both studies only signal peaks were analyzed. However, it is possible that decreases (troughs) are also an important aspect of glutamate and oxygen signaling. Considering the temporal properties of the troughs observed in experiment 1's glutamate signals it is more likely the downward signals are due to electrical noise oppose to a sudden increase in glutamate uptake especially because the rate of glutamate uptake (Danbolt, 1998) could likely not account for this decrease. Nevertheless, the physiological relevance of dips in the glutamate signal cannot be assumed to be trivial. Note that dips in oxygen signaling are reported to be related to neuronal oxidative metabolism (Malonek et al., 1997) and thus are likely to be of physiological importance. Thus, in future studies signal peaks and troughs should both be analyzed in order to gain a more robust picture of the processes that occur during reward-related behavior. Nevertheless, the studies herein highlighted results not previously observed.

The findings from experiment 1 and experiment 2 add to the current body of knowledge in a number of ways. First, from a methodological point of view, these experiments show the feasibility of coupling biosensor technology, drug self-administration, and fairly complex behavioral paradigms. At the very least, this lays the groundwork for others to use these methodologies to further explore substance-use disorder. Second, experiment 1 is the only experiment (to the knowledge of the author) comparing glutamate measures from the PrL and NAcC in the same animal exposed to cocaine and food, and experiment 2 is the only experiment that assessed oxygen dynamics in the OFC in a procedure that can separate preference from drug intake.

Specifically, experiment 1 highlighted that cocaine caused an increase above baseline in glutamate compared food; however, the number of glutamate

peaks was greater to food related events. That being said, the number of glutamate peaks per response and reinforcer was greater for cocaine. Thus, this experiment did show reinforcer specific changes in glutamate signaling in both release amplitude and frequency measures. Overall, these data show that differential glutamate signaling does happen between food and cocaine. However, the glutamate system does participate in signaling to both reinforcers in a similar way in both brain regions. Thus, this highlights the impetus to further study these interactions and to be cautious when considering glutamatergic drugs for substance-use disorder. Further, experiment 2 showed that environmental manipulations (namely food magnitude changes) shift cocaine preference. Further, it was found that responses associated with cocaine and earning cocaine cause more activity in the OFC than events related to food. Further, there was some evidence that OFC oxygen (as measured through the number of oxygen peaks that occurred) followed cocaine preference. Thus, overall these data cautiously suggest that the OFC plays a role in the subjective valuations that occur in drug-related decision-making.

Taken together, both studies suggest that the PrL, NAcC, and OFC are related to drug-taking and drug-related decision-making, respectively. Further, these experiments show that environmental manipulations can shape drug-taking behavior, that cocaine can be substituted with a non-drug commodity, and that oxygen dynamics during drug-related behavior are relative in nature. Overall, these experiments lay the groundwork to further study drug-specific neurobehavioral changes and allow for the exploration of behavioral and pharmacological treatments that specifically decrease drug-taking behavior.

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CURRICULUM VITAE

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Education

2010 B.A., Psychology, Transylvania University (Cum Laude)

2013 M.S., Medical Sciences with A Focus in Neuroscience, University of Kentucky

Professional Positions

2007 Mental Health Associate, Eastern State Psychiatric Hospital (Summer)

2010-2011 Research Assistant, Department of Psychiatry, University of Kentucky College of Medicine, Laboratory of Catherine A. Martin, M.D.

2011-2014 Research Assistant, Department of Anatomy & Neurobiology, University of Kentucky College of Medicine, Laboratory of Greg A. Gerhardt, Ph.D. & Paul E.A. Glaser, M.D., Ph.D.

2014- Graduate Research Assistant, Behavioral Neuroscience & Psychopharmacology, Department of Psychology, University of Kentucky College of Arts and Sciences, Laboratory of Joshua S. Beckmann, Ph.D.

2018- Contract Research Scientist, GISMO Therapeutics Inc., Lexington, KY.

Scholastic and Professional Honors

Graduate Student Poster Presentation Award, Bluegrass Chapter for Neuroscience Spring Neuroscience Day, April 2013

Staff Poster Presentation Award, Bluegrass Chapter for Neuroscience Spring Neuroscience Day, April 2014

Graduate Student Poster Presentation Award, Bluegrass Chapter for Neuroscience Spring Neuroscience Day, April 2016

CPDD Travel Award for Early Career Investigators, College on Problems of Drug Dependence, Palm Springs, CA, June 2016.

Publications

Journals

Batten SR & Upchurch MB (2010). Psychoneuroimmunology: an analysis of HIV/AIDS and cancer. URJHS 9: http://www.kon.org/urc/v9/batten.html.

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Batten SR, Hicks KB, Dwoskin LP, Beckmann JS (submitted). Isolating reward changes in diet-induced obese rats: a demand analysis.

Batten SR, Beckmann JS (in preparation). Combining multiple schedules of reinforcement with glutamate biosensors to examine the effects of cocaine

and food on prelimbic and accumbal glutamatergic signaling in freely-moving rats.

Book Chapters

Burmeister JJ, Hascup ER, Hascup KN, Davis V, **Batten SR**, Pomerleau F, Quintero JE, Huettl P, Talauliker PM, Stromberg I, Gerhardt GA (2015). Real-time in vivo neurotransmitter measurements using enzyme-based ceramic microelectrode arrays: what we have learned about glutamate signaling. In: Advances in Real-Time Molecular Neuroscience (Wilson G and Michael A, Eds.). World Scientific.

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Abstracts

Martin CA, Guenthner G, Charnigo R, **Batten SR**, Lile JA, Kelly TH (2011). Psychological symptoms and modafinil effects on smoking cessation. International research symposium for The College on Problems of Drug Dependence in Hollywood, FL.

Davis VA, **Batten SR**, Quintero JE, Pomerleau F, Huettl P, Gerhardt GA (2012). Measuring nitric oxide in the rat corpus cavernosum: can it be done? Appalachian Health Summit Center for Clinical and Translational Science meeting in Lexington, KY.

Davis VA, Stephens ML, **Batten SR**, Price DA, Alcala, R, Pomerleau F, Huettl P, Quintero JE, Slevin JT, Gerhardt GA (2012). Enzyme Based Microelectrode Arrays Offer Novel Insights into Mechanisms of Epilepsy. *In Vivo* Methods, Monitoring Molecules in Neuroscience in London, England.

Batten SR, Matveeva EA, Whiteheart SW, Vanaman TC, Glaser PEA, Slevin JT, Gerhardt GA (2013). Tomosyn Dysregulation Leads to Aberrant Glutamate Release in the Dentate Gyrus of the Hippocampus in a Murine Model of Epileptogenesis. Presented by Batten SR at the Bluegrass Chapter for Neuroscience Spring Neuroscience Day in Lexington, KY.

Davis VA, **Batten SR**, Lourenço CF, Quintero JE, Pomerleau F, Huettl P, Gerhardt GA, Laranjinha J, Barbosa RM. Teasing Apart Nitric Oxide's Role in Erection (2013). Society for Neuroscience in San Diego, CA.

Batten SR, Matveeva EA, Quintero JE, Pomerleau F, Huettl P, Whiteheart SW, Vanaman TC, Glaser PEA, Gerhardt GA, Slevin JT (2013). Tomosyn Dysregulation Leads to Aberrant Glutamate Release in the Dentate Gyrus of the Hippocampus: Implications for Epileptogenesis? Society for Neuroscience in San Diego, CA.

Davis VA, Stephens ML, **Batten SR**, Price DA, Alcala R, McKee HR, Gerhardt GA, Slevin JT (2013). Carpe datum! Enzyme based microelectrode arrays offer novel insights into mechanics of epilepsy. American Epilepsy Society in Washington, D.C.

Batten SR, Matveeva EA, Whiteheart SW, Vanaman TC, Glaser PEA, Slevin JT, Gerhardt GA (2013). Tomosyn Dysregulation Leads to Aberrant Glutamate Release in the Dentate Gyrus of the Hippocampus in a Murine Model of Epileptogenesis. 2nd Annual Epilepsy Symposium University of Kentucky.

Batten SR, Matveeva EA, Alcala R, Whiteheart SW, Vanaman TC, Slevin JT, Gerhardt GA (2014). Examining How Tomosyn Affects Kindling-Sensitivity: Is Aberrant Glutamatergic Neurotransmission in the Hippocampus to Blame? Bluegrass Chapter for Neuroscience Spring Neuroscience Day in Lexington, KY.

Batten SR, Matveeva EA, Alcala R, Whiteheart SW, Vanaman TC, Slevin JT, Gerhardt GA (2014). Reduction of Tomosyn Expressions Leads to a Kindling-Sensitive Phenotype in a Murine Model of Epilepsy. American Academy of Neurology in Philadelphia, PA.

Beckmann JS, **Batten SR,** Quintero JE, Gerhardt GA (2014). Glutamate dynamics in the rat nucleus accumbens core and prelimbic cortex during Pavlovian Conditioned Approach. American College of Neuropsychopharmacology in Phoenix, AZ.

Batten SR, Beckmann JS (2016). Combining multiple schedules of reinforcement with glutamate biosensors to examine the effects of cocaine and food on prelimbic glutamatergic signaling. Bluegrass Chapter for Neuroscience Spring Neuroscience Day in Lexington, KY

Batten SR, Beckmann JS (2016). Evaluating Sucrose and Saccharin Value in Diet Induced Obese Rats. Barnstable Brown Obesity and Diabetes Research Day in Lexington, KY.

Batten SR, Beckmann JS (2016). Combining multiple schedules of reinforcement with glutamate biosensors to examine the effects of cocaine and food on prelimbic and accumbal glutamatergic signaling. College on Problems of Drug Dependence in Palm Springs, CA.

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Wang WX, Vekaria H, Spry M, Cloud A, **Batten SR**, Beckmann JS, Sullivan PG, Springer JE (2017). Effects of TBI on microRNA association with mitochondria and intervention using a novel nanoparticle miRNA delivery strategy. National Neurotrauma Society in Snowbird, UT.

Batten SR, Beckmann JS (2017). Evaluating Sucrose and Saccharin Value in Diet Induced Obese Rats. Society for Neuroscience Meeting in Washington, DC.

Batten SR, Beckmann JS (2018). Exploring the role of orbitofrontal cortex function in drug-related decision-making. Society for Neuroeconomics Meeting in Philadelphia, PA.

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July 16, 2019