Anticipatory and hedonic reward response in Major Depression: Underlying molecular correlates and relationship with treatment outcome

by

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Dedication

This work is dedicated to the participants whose generosity of time and effort made this research possible.

To my corneal donor and his family, Eversight Michigan, and the Lions Clubs of Michigan, for restoring my sight and enabling me to do the work I love.

To the City of Detroit and its people: for your hospitality, and inspiring resiliency in the face of Le Nain Rouge's villainy.

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Thank you to my committee for their thoughtful feedback and insights: Dr. Jonides, Dr. Polk, Dr. Reuter-Lorenz, and in particular Dr. Thompson for his remarkable support when I needed it most.

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Elyse Aurbach has been an inspiration and source of strength. I first knew her as a conscientious student and thoughtful scientist, and now as a kind, ambitious, resourceful partner. She has supported me in countless ways, for which I am grateful beyond words, and I am so excited to continue building our future together.

It is by the grace of my parents' hard work and sacrifices that I have had the opportunities that led me here. This milestone is a testament to my family's love and support over my lifetime.

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Abstract

Reward encompasses multiple psychological processes, including motivation to earn a reward and the hedonic enjoyment of a reward. Animal and human research supports roles for the dopamine and mu-opioid systems in facilitating motivation and hedonics, respectively. Loss of interest (apathy) and pleasure (anhedonia) are core symptoms of Major Depressive Disorder, and patients often present with impairments in one or both of these reward processes. Patient studies suggest that the cortico-striatal network shows disordered responses to reward processing and particularly implicate altered dopamine and opioid function. However, gaps in understanding remain: 1) individual differences in the relationship between reward response and neurotransmitter function in depression and 2) the value of reward response as a predictor of antidepressant treatment response are both important areas for investigation.

This dissertation takes a multimodal neuroimaging approach to investigate the molecular and clinical correlates of disordered reward processing in depression. We utilized two modalities: 1) a well-validated reward paradigm during functional magnetic resonance imaging (fMRI) acquisition to measure neural response to anticipatory and hedonic reward in depressed patients and 2) positron emission tomography (PET) scans to measure dopamine and mu-opioid

receptor binding. Participants subsequently completed a 10-week antidepressant treatment regimen.

We investigated the relationship between striatal fMRI responses to reward and DA 2/3 and mu-opioid receptor binding. We replicated fundamental relationships previously established in the literature: we showed that striatal response to reward anticipation is associated with striatal dopamine release and D2/3 receptor availability, whereas striatal response to reward outcome is associated with mu-opioid receptor availability in the thalamus. Furthermore, anterior cingulate response to reward anticipation mediates a previously-established relationship between nucleus accumbens mu-opioid function and antidepressant treatment response.

These results further elucidate the molecular correlates of reward anticipation and hedonics in major depression and establish the anterior cingulate as a mediator of the relationship between mu-opioid function and recovery from depression.

Chapter One.
Introduction

Reward processing and the underlying biology of its subcomponents

Reward is a fundamental modulator of behavior, acting through reinforcement. While often described as though it were a unitary process, accumulating evidence suggests reward may be more aptly used as an umbrella term encompassing multiple psychological processes. Two such processes are the desire to earn a reward and the pleasure of experiencing a reward, here referred to as motivation and hedonics respectively. Notably, motivated and hedonic behavior can be seen across multiple modalities of reward, including primary rewards such as food (Barbano & Cador, 2005), as well as secondary rewards such as money (Knutson et al., 2000). The psychological processes of motivation and hedonics likely interact, but are experimentally dissociable and may become uncoupled in psychiatric illness. Furthermore, each is associated with neurotransmitter systems and neural circuitry that, similar to the behavioral processes they underlie, are different but overlap neuroanatomically and interact physiologically. Specifically, the dopaminergic system is believed to be critical in facilitating motivated response, and the μ-opioid system in hedonic response.

Dopamine and motivation. The neurotransmitter dopamine has been implicated in both reward behavior and voluntary movement, and acts in part by modulating the sensitivity of postsynaptic medium spiny neurons (MSNs) in the striatum (Figure 1.1) to other types of input such as glutamate.

Dopamine acts primarily on postsynaptic G-protein coupled dopamine receptors, which fall into two families: D1-like (D1 and D5 subtypes) and D2-like (D2-D4 subtypes), and the effect of dopamine binding depends on the receptor-type to which it binds. Binding of dopamine to D1-like receptors creates "up-states", that is, increased responsiveness of the MSN to sustained release of glutamate (Surmeier et al., 2007). These receptors are stimulated by phasic firing, in which a rapid burst of action potentials leads to a rise in the release of dopamine at the target synapse (Grace & Bunney, 1984). Conversely, binding of dopamine to D2-like receptors creates "down-states", in which MSN responsiveness is reduced (Hernández-López et al., 2000). D2-like receptors are stimulated by tonic dopamine release, driven by the basal, steady-state firing of dopamine neurons.

The synthesis of dopamine begins with the conversion of tyrosine to L-3,4-dihydroxyphenylalanine (L-dopa) by tyrosine hydroxylase. L-dopa is subsequently converted to dopamine via decarboxylation, and is then stored in vesicles and released into the synapse in response to an action potential. Dopamine undergoes rapid reuptake into the presynaptic terminal by the dopamine transporter (DAT), where it may be repackaged in vesicles for future release or degraded by monoamine oxidase (MAO) into homovanillic acid (HVA). HVA can subsequently be measured in cerebrospinal fluid as an indirect measure of dopamine system function (Blennow et al., 1993).

Dopamine neurons in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNpc) give rise to three major dopaminergic pathways (Figure 1.2; orange, yellow, and purple projections). The mesolimbic pathway connects the VTA with the ventral striatum (including the nucleus accumbens; NAc) and amygdala, and is closely associated with motivational aspects of reward. The nigrostriatal pathway connects the SNpc with dorsal striatum (caudate and putamen) and is mostly strongly associated with motor control. The mesocortical pathway connects the VTA with cortical regions such as the dorsomedial prefrontal cortex, orbitofrontal cortex, and anterior cingulate cortex, and may play a role in aspects of executive function, such as attentional and inhibitory control.

Previous literature implicates the mesolimbic dopamine system in the desire for reward. Augmentation of DA transmission in rats via electrical stimulation (Berridge & Valenstein, 1991), DAT knockdown (Peciña et al, 2003) and drug sensitization (Tindell et al, 2005; Wyvell & Berridge, 2000) induces increased feeding motivation, but with no increase in demonstrated hedonic enjoyment. Likewise, disruption of DA transmission reduces motivation, while hedonic facial responses remain intact (Berridge & Robinson, 1998; Peciña et al, 1997) as does preference for sucrose solutions and highly palatable foods. This link between motivation and dopamine has been demonstrated in humans as well, with DA levels correlating more highly with subjective levels of craving than pleasure (Leyton et al., 2002; Volkow et al., 2002). DA antagonists do not reduce subjective ratings of pleasure for a drug but do diminish craving (Leyton, 2002). Circuitry underlying motivation appears to comprise a larger cortico-striatal-thalamic-cortical loop (Haber, 2011), working in parallel with anatomically similar loops underlying a variety of cognitive processes (Figure 1.3). Special focus has been given to

mesolimbic pathway, although accumulating optogenetics evidence supports a role for the nigrostriatal pathway as well (Ilango et al., 2014; Rossi et al., 2013).

Opioids and hedonics. The endogenous opioid system is perhaps most well-known for its role in analgesia, but is further implicated in mood and hedonic reward.

Endogenous opioid ligands are classically known to bind to three G-protein coupled receptors: mu- (MOR), delta- (DOR), and kappa- (KOR) opioid receptors. Unlike dopamine receptors, opioid receptors are expressed diffusely in the brain, and can be found throughout the cortex, limbic system and thalamus. Animal models have suggested different roles for each receptor-type in mediating behavior, with MOR essential in the response to natural and drug rewards (Matthes et al., 1996; Papaleo et al., 2007), DOR in emotional regulation and drug-reinforcement (Filliol et al., 2000; Roberts et al., 2000), and KOR activity eliciting aversive reactions, potentially maintaining hedonic homeostasis through the opposition of MOR effects (Spanagel et al., 1992). Three major precursor proteins are cleaved to produce endongenous opioid ligands: proopiomelanocortin (POMC) into β-endorphins, proenkephalin (Penk) into enkephalins, and prodynorphin (Pdyn) into dynorphins. Although the receptor binding profiles of endogenous opioid ligands are complex, generally MOR show high affinity for β-endorphins and enkephalins, DOR for enkephalins, and KOR for dynorphins.

The μ-opioid system seems to play a critical role in the evaluation of the pleasant hedonic properties of a stimulus that make it desirable. MOR are densely expressed in the nucleus accumbens, amygdala and medial thalamus. In MOR knockout mice, the reinforcing properties of opiate (Contarino et al., 2002) and non-opiate (Berrendero et al., 2002; Ghozland et al., 2002; Roberts et al., 2000) drugs were abolished. Human studies have similarly found that a MOR

antagonist decreased the perceived pleasantness of food, without changing the rated appetite (Yeomans & Gray, 1997).

Relative to motivation, reward hedonics appear to be mediated by a more restricted limbic circuit. Within striatal structures, such as the nucleus accumbens and ventral pallidum, are small hedonic "hotspots" (Peciña & Berridge, 2005; Smith & Berridge, 2005), containing a high density of opioid receptors. Opioid stimulation of these regions elicits positive hedonic facial reactions to a sweet solution, and potently induces feeding. These hedonic hotspots are found within structures also implicated in motivation, which illustrates an overlap in the circuitry underlying these different reward processes, and suggests the striatum as a potential site of interaction between them.

Neuroimaging insight into the circuitry of reward processing. The use of functional neuroimaging studies of reward in humans has largely replicated the general findings of the animal literature. In the context of motivation, striatal BOLD responses to potential cocaine reward correlate positively with craving (Breiter et al., 1997). The striatum also demonstrates increased response to conditioned cues predicting primary (O'Doherty et al., 2002) and secondary (Knutson et al., 2000) rewards. Similarly, fMRI studies demonstrate significant response of the striatum to rewarding or positive outcomes, including for concrete outcomes such as money (Delgado et al., 2000) as well as more abstract hedonic rewards such as viewing an attractive face (Smith et al., 2010).

While fMRI studies investigating reward processing have used a variety of paradigms, of particular interest is the Monetary Incentive Delay (MID) task (Knutson et al, 2000), which allows for investigation of the neural circuitries underlying motivation and hedonics within the

same task design. In the task, participants have the opportunity to win and lose money based on their performance on a reaction-time challenge. Responses to cues indicating potential gain serve as measures of reward anticipation and motivation, while responses to feedback indicating actual gain serve as measures of reward hedonics.

Interim summary. Reward processing is comprised of multiple subcomponents, including motivation and hedonics. Motivation has been associated with the dopaminergic system, in particular the mesolimbic pathway connecting the VTA and ventral striatum. Hedonic response has been associated with the endogenous opioid system, in particular mu-opioid receptors, and is believed to be facilitated by "hedonic hotspots", areas of dense MOR expression including the ventral striatum. Human neuroimaging experiments provide additional support for the striatum as a critical region for mediating both motivational and hedonic reward, with the Monetary Incentive Delay task serving as a well-validated paradigm for eliciting the neural circuitry underlying these processes.

Even as the field continues to develop its understanding of the psychological processes comprising reward, and the neurobiological systems that facilitate them, accumulating evidence implicates disruptions in reward processing in a variety of psychiatric conditions, including addiction, schizophrenia, and major depression.

Major depression and disrupted reward processing

Major depression is a prevalent and debilitating illness, and represents a significant social and economic burden on society. Characterized by depressed mood and a loss of pleasure, major depression affects an estimated 16.2% of adults in the United States at some point in their

lifetimes (Kessler et al., 2003). The annual cost of MDD in the US is estimated at $83.1 billion, with nearly two thirds of this cost a result of functional disability (Greenberg et al., 1899).

With persistent depressed mood and loss of interest or pleasure as its core symptoms, major depression frequently includes other distressing symptoms such as fatigue, diminished ability to think or concentrate, and extreme alterations in sleep, appetite, or psychomotor behavior (American Psychiatric Association, 2013) (Figure 1.4). Patients present with different constellations of these symptoms, resulting in a variety of clinical manifestations that all fall under the diagnosis of major depression. For example, one patient may feel extreme sadness and worthlessness, frequently cry without understanding why, constantly fidget, overeat, and suffer from insomnia. Another patient may feel bereft of emotion or pleasure, an inability to concentrate, fatigue, a lack of appetite and slowed movement. While both symptoms profiles are undoubtedly burdensome on the affected individuals, they reflect different (and in some ways opposing) conditions that likely result from different etiologies. This presents a challenge to the investigation of the biology of major depression, as samples are frequently heterogeneous and contain patients with different symptoms and etiologies, which may blur important individual differences driving their unique profiles.

One way to address the challenge of heterogeneity in mental health research samples is reflected in the National Institute of Mental Health's (NIMH) research domain criteria (RDoC) approach of examining functional domains that are disrupted in psychiatric illness, which may be more closely linked to alterations in underlying biology (i.e. abnormalities at the circuit, cellular, or molecular level) than the diagnoses themselves (Insel & Cuthbert, 2009). With this in mind, it may be beneficial to focus on a disrupted functional process underlying a core symptom of the illness.

While much work has investigated the biology of disturbed emotion regulation in major depression (see Rive et al., 2013 for a review of the neuroimaging literature), the present work joins the body of literature investigating the biology of disrupted reward processing. Individuals with MDD frequently present with reduced motivation (apathy) and pleasure (anhedonia). In experiments with MDD patients, this disruption in motivation emerges behaviorally as reduced subjective positivity to anticipated rewards (McFarland & Klein, 2009) and reduced willingness to expend effort for rewards (Cléry-Melin et al., 2011; Treadway et al, 2009). Converging lines of evidence support a role for dopaminergic dysfunction in the pathophysiology of depression, including reduced concentrations of the DA metabolite homovanillic acid (Lambert et al., 2000), and euphoric effects of DA receptor agonists (Tremblay et al, 2005) particularly in severely depressed patients (Tremblay et al, 2002). The pro-depressive effect of catecholamine depletion in susceptible individuals (Hasler et al., 2009) further suggests a role of dopamine in mood dysregulation. These data support the role of DA in motivation and a link between DA dysfunction and depression.

While experiments using self-report measures of pleasure generally fail to demonstrate impaired behavioral hedonic response in depression (Dichter et al., 2010), biological research does support a role for alterations of hedonic neural circuitry and the μ-opioid system in MDD pathology. Evidence indicates that endogenous opioid system tone and activation is dysregulated in depression (Hsu et al., 2015; Kennedy, 2006), and opioid agonists elicit significant and rapid mood elevation in depressed patients (Ehrich et al., 2015). Furthermore, in non-depressed participants, individual differences in μ-opioid function have been linked to affective responses (Liberzon et al., 2002; Zubieta et al, 2003).

The human neuroimaging literature of reward processing in depression has produced heterogenous results, however a few consistent findings have emerged in meta-analyses. First, striatal response to both reward anticipation and outcome is reduced in MDD patients relative to controls. Second, medial prefrontal cortex (mPFC) and anterior cingulate cortex (ACC) response to reward anticipation is increased relative to controls (Zhang et al., 2015; Zhang et al., 2013)

Interim summary. The above literature identifies, in patients with major depression, alterations in behavior, neural circuitry, and neurotransmitter systems associated with different aspects of reward processing; most notably, reduced motivation, disrupted dopamine and opioid neurotransmission, and altered patterns of cortico-striatal response. Substantial research has been performed to investigate the role of neurotransmitters in reward, the neural circuitry underlying different aspects of reward, and disruptions of these in MDD. However, the relationships between motivation/DA and hedonics/μ-opioids are still not well-understood in depressed patients. Given the above evidence that these reward processes and their associated neurotransmitter systems are disrupted in MDD, additional investigation is warranted into their respective contributions to the pathophysiology of depression (Figure 1.5).

Molecular and clinical correlates of reward response

Previous research investigating the relationship between dopamine receptor activity and reward-related BOLD response supports the importance of cortico-striatal interactions in reward. In cocaine abusers, DA D2 receptor availability in the ventral striatum negatively correlates with mPFC BOLD response to reward outcome (Asensio et al., 2010). Reward-elicited dopamine release in the ventral striatum is positively correlated with ventral striatal BOLD reward response

for both anticipation (Schott et al., 2008) and outcome (Weiland et al., 2016), and mPFC BOLD reward response to anticipation (Weiland et al., 2014).

Experiments investigating the link between BOLD reward response and clinical measures of disrupted reward behavior have thus far focused on schizophrenia, and demonstrate that ventral striatal response to reward anticipation correlates negatively with clinical measures of apathy (Kirschner et al., 2016; Simon et al., 2010) and anhedonia. Interestingly, the negative relationship between reward response and anhedonia was not observed in patients with major depression (Arrondo et al., 2015).

Interim summary. The literature supports a link between ventral striatum dopamine function and reward responses in both the ventral striatum and mPFC, placing further emphasis on cortico-striatal interactions in reward function. In patients with schizophrenia, reduced ventral striatal response to reward is associated with greater apathy and anhedonia. The nature of these relationships in individuals with depression remains unclear, although some evidence suggests the relationship may differ across diagnoses.

Biomarkers for antidepressant treatment response

The search for effective antidepressant treatment is challenging for many affected individuals. Following treatment with an initial SSRI antidepressant, an average of 4 weeks are needed to attain response and at least 6 weeks to attain remission, although remission can take 12 weeks or longer, if achieved at all (Trivedi et al., 2006). Most patients fail to enter remission with the first antidepressant prescribed, and subsequently experience a period of serial trial-and-error with different combinations of medications (Leuchter et al., 2009). It typically requires 1

year to identify a successful treatment (Keitner et al., 1992; Rush, 2007). Ineffective treatment carries a financial cost, in addition to side effects and the continued burden of the disease, making it difficult to maintain further compliance with treatment. Twenty-six percent of patients who fail to improve with the first treatment stop taking medication, frequently within the first 2 weeks (Warden et al., 2007), and up to 42% of patients discontinue medication within the first 30 days (Olfson et al., 2006). Given the rates of treatment failure, it is important to develop an understanding of which individuals are likely to respond to antidepressant treatment, and which are not.

To address this issue, a growing body of research has focused on identifying predictors of antidepressant treatment response, primarily using neuroimaging techniques such as positron emission tomography (PET; Mayberg et al., 1997; Milak et al., 2009), electroencephalography (EEG; (Arns et al., 2015; Korb et al., 2009)), and functional magnetic resonance imaging (fMRI; Chen et al., 2007; Langenecker et al., 2007). Some of these studies examined the brain at rest, and others while engaged in cognitive tasks such as face processing and response inhibition. And yet the function of reward circuitry, believed to be a component of the pathophysiology of major depression, remains understudied as a predictor of antidepressant treatment response (Phillips et al., 2015).

Dopamine and μ-opioid systems in antidepressant treatment. Given the robust evidence that dopamine and μ-opioid systems are disrupted in major depression, it is unsurprising that recovery from depression with treatment may affect these systems, and that modulation of these systems may impact depressive symptoms. Studies investigating the impact of SSRI depressants on dopamine receptor binding have found mixed results: Montgomery et al. (2007) found

reduced DA D2/3 receptor availability in the dorsal striatum of patients with MDD taking SSRI antidepressants, compared to healthy controls. Hirvonen et al. (2011) did not find a change in striatal DA D2/3 receptor availability following four months of SSRI treatment, but did note increased DA D2/3 receptor availability in the thalamus. A meta-analysis of randomized, double-blind trials found norepinephrine/dopamine reuptake inhibitor buproprion to be equally effective as SSRIs (Thase et al., 2005), and it may be particularly effective in ameliorating anhedonia (Tomarken et al., 2004), which is frequently inadequately addressed by SSRIs. Similarly, recent trials of μ-opioid partial agonist buprenorphine have demonstrated reduced suicidal ideation in response to an ultra-low dose (Yovell et al., 2015), and reduction in depression scores in response to a low-dose in conjunction with a μ-opioid antagonist (Ehrich et al., 2015; Fava et al., 2016).

Interim summary. Difficulty in finding effective treatment, and the financial and emotional stress it engenders, represents an additional burden on patients with MDD. A rapidly growing literature seeks to alleviate this burden by identifying pre-treatment predictors of treatment response. While disrupted reward processing is a core symptom of major depression, and modulation of its associated neurotransmitter systems can elicit antidepressant effects, neural reward response and DA D2/3 and MOR activity remain understudied as potential predictors or treatment response.

Current Directions

Elucidating the underlying neurobiology of major depression, as well as the factors that influence response to available treatments, is critical to reducing the substantial personal and

societal burdens inflicted by the disease. The current literature has established two key principles which motivate the present research. First, anticipatory and hedonic reward behaviors are disrupted in depression, as are the neurotransmitter systems (DA/μ-opioid) and neural circuitry thought to underlie those behaviors. Second, modulation of dopamine and μ-opioid systems can elicit antidepressant effects. Pre-treatment measures of these systems, together with reward response, represent promising but understudied candidates for biomarkers of antidepressant treatment response. Therefore, the present research seeks to clarify the relationship between disrupted neural reward response in depression and three measures with which it is putatively associated: dopamine/μ-opioid receptor activity, clinical scales of reward dysfunction, and antidepressant treatment response.

The experiments in the following chapters use two neuroimaging techniques: functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), collected on the same sample of participants with major depression. FMRI uses a magnetic field to measure changes in the concentration of oxygenated blood that occur in response to brain activity. As previous literature has established impaired neural reward response in major depression, this technique provides us with a measure of the neural response to anticipatory and hedonic reward by having participants perform a Monetary Incentive Delay task during fMRI acquisition. PET uses radiotracers to observe metabolic activity; more specifically, it can be used to investigate the availability of certain receptor types as well as the change in availability in response to a challenge. A decrease in receptor availability for the radiotracer in response to a challenge would be interpreted as activation of the endogenous system; that is, increased release of the endogenous ligand. Given the putative alterations in dopamine and μ-opioid systems in major

depression, this technique provides us with a measure of DA D2/3 and MOR availability, as well as dopamine and opioid peptide release in response to a challenge.

First, we investigate how anticipatory and hedonic reward response are associated with individual differences in DA D2/3 and MOR availability, as well as clinical measures of apathy and anhedonia, in patients with major depression. This work builds on previous literature examining the relationship between dopamine neurotransmission and reward response, as well as previous work linking reward response and clinical scales of reward.

Second, we investigate how anticipatory and hedonic reward responses predict subsequent antidepressant treatment response, as well as how this relationship can be modelled in the context of dopamine and μ-opioid receptor binding. This work adds to previous findings of neuroimaging predictors of treatment response in depression.

Overall, the present research seeks to build on the field's current knowledge of disrupted neural response to anticipatory and hedonic reward in major depression by exploring relationships with dopamine and μ-opioid receptor activity, clinical reward disruption, and antidepressant treatment response.

Figures

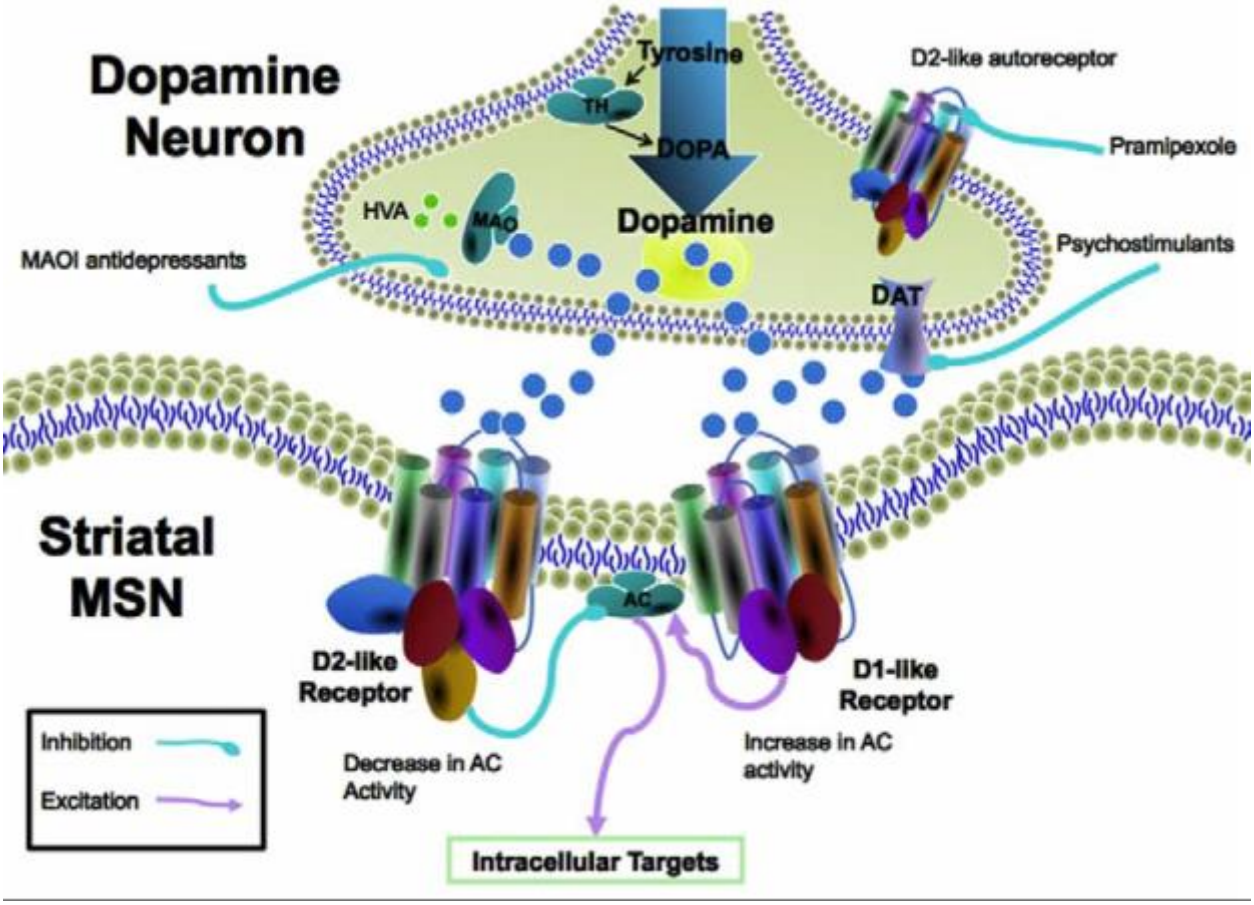


Figure 1.1. Dopaminergic neurotransmission at a striatal synapse. Tyrosine is converted through a multistep process into dopamine. Dopaminergic neurons projecting to the striatum synapse onto medium spiny neurons (MSNs) and may bind to a D1-like receptor (increasing MSN responsiveness to glutamate) or a D2-like receptor (decreasing MSN responsiveness to glutamate). Dopamine in the synapse undergoes reuptake by a dopamine transporter and is repackaged for future release or converted to homovanillic acid. Adapted from Treadway and Zald (2011)

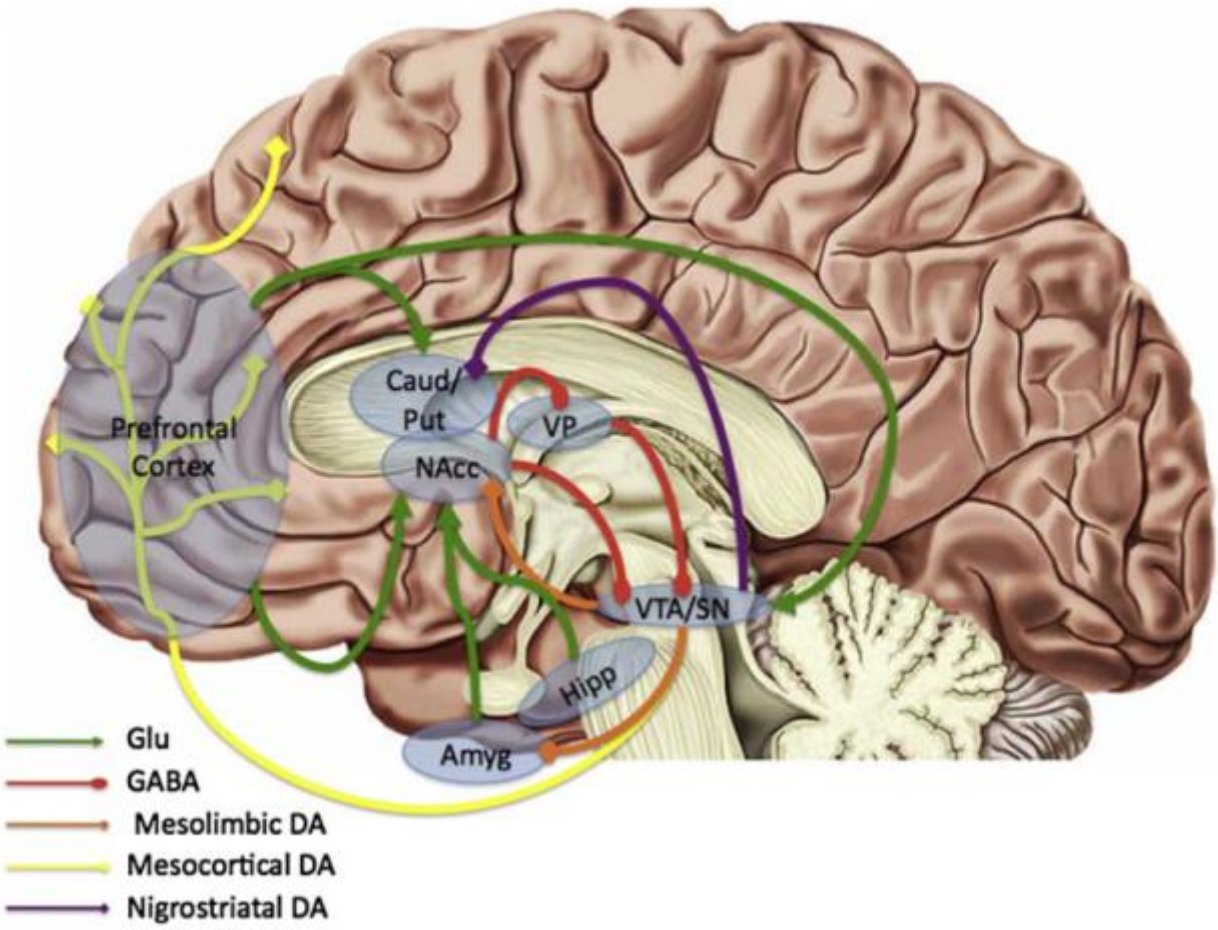


Figure 1.2. Major dopaminergic pathways. The mesolimbic dopamine pathway (orange) is thought to play a critical role in reward processing. The nigrostriatal pathway is involved in motor control, although accumulating evidence suggests it also has a role in reward. Reprinted from Treadway and Zald (2011)

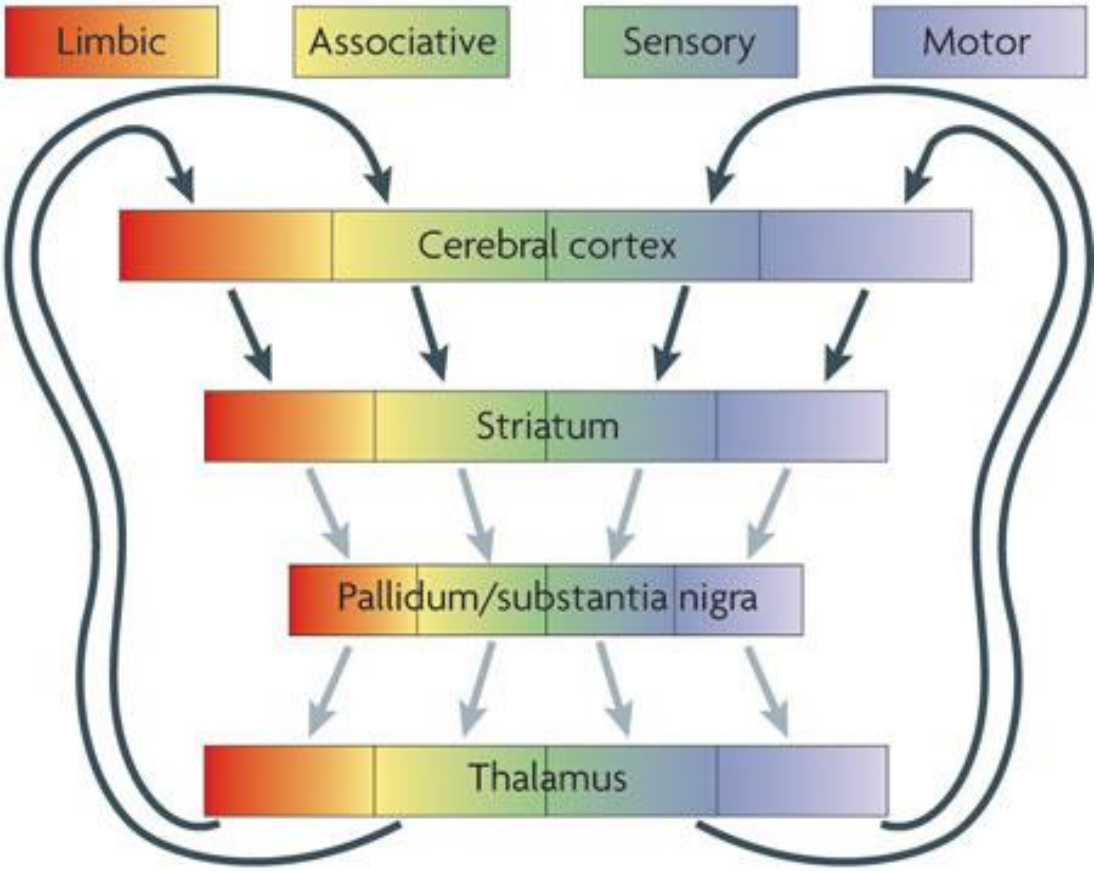


Figure 1.3. Cortico-striatal-thalamic-cortical loops underlying motivation and other cognitive processes. Adapted from Redgrave et al (2010).

Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either: (1) Depressed mood or (2) Loss of interest or pleasure Note: Do not include symptoms that are clearly due to a general medical condition, or mood-incongruent delusions or hallucinations.
(1) Depressed mood most of the day, nearly every day, as indicated by either subjective report (eg, feels sad or empty) or observation made by others (eg, appears tearful). Note: In children and adolescents, can be irritable mood
(2) Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others)
(3) Significant weight loss when not dieting or weight gain (eg, a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day. Note: In children, consider failure to make expected weight gains
(4) Insomnia or hypersomnia nearly every day
(5) Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)
(6) Fatigue or loss of energy nearly every day
(7) Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely s elf-reproach or guilt about being sick)
(8) Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)
(9) Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

Figure 1.4. DSM-V criteria for diagnosis of Major Depressive Disorder. Anhedonia is one of two core symptoms of depression. Reprinted from Kennedy (2008).

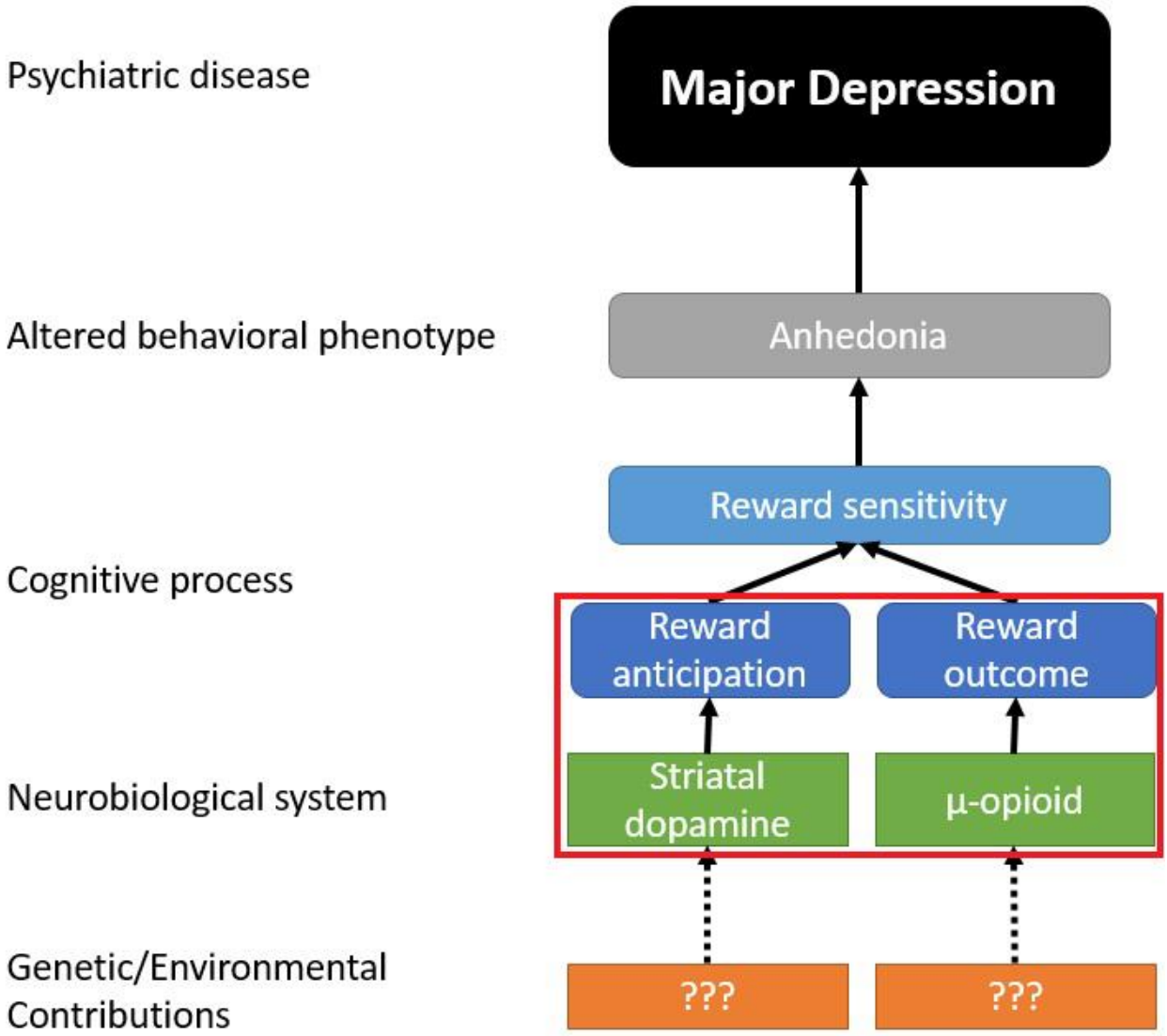


Figure 1.5. RDoC approach to investigating disrupted reward in major depression. The relationships between DA/reward anticipation and μ-opioid/reward outcome (red box) in patients with major depression are not well understood. This gap motivates the research presented in Chapter 2.

Works Cited

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders* (Fifth Edition). American Psychiatric Association. Retrieved from http://psychiatryonline.org/doi/book/10.1176/appi.books.9780890425596

Arns, M., Etkin, A., Hegerl, U., Williams, L. M., DeBattista, C., Palmer, D. M., … Gordon, E. (2015). Frontal and rostral anterior cingulate (rACC) theta EEG in depression: Implications for treatment outcome? *European Neuropsychopharmacology*, *25*(8), 1190–1200. https://doi.org/10.1016/j.euroneuro.2015.03.007

Arrondo, G., Segarra, N., Metastasio, A., Ziauddeen, H., Spencer, J., Reinders, N. R., … Murray, G. K. (2015). Reduction in ventral striatal activity when anticipating a reward in depression and schizophrenia: a replicated cross-diagnostic finding. *Psychology for Clinical Settings*, 1280. https://doi.org/10.3389/fpsyg.2015.01280

Asensio, S., Romero, M. J., Romero, F. J., Wong, C., Alia-Klein, N., Tomasi, D., … Goldstein, R. Z. (2010). Striatal dopamine D2 receptor availability predicts the thalamic and medial prefrontal responses to reward in cocaine abusers three years later. *Synapse*, *64*(5), 397–402. https://doi.org/10.1002/syn.20741

Barbano, M. F., & Cador, M. (2005). Various aspects of feeding behavior can be partially dissociated in the rat by the incentive properties of food and the physiological state. *Behavioral Neuroscience*, *119*(5), 1244–1253. https://doi.org/10.1037/0735-7044.119.5.1244

Berrendero, F., Kieffer, B. L., & Maldonado, R. (2002). Attenuation of Nicotine-Induced Antinociception, Rewarding Effects, and Dependence in μ-Opioid Receptor Knock-Out Mice. *The Journal of Neuroscience*, *22*(24), 10935–10940.

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Research Reviews*, *28*(3), 309–369. https://doi.org/10.1016/S0165-0173(98)00019-8

Berridge, K. C., & Valenstein, E. S. (1991). What psychological process mediates feeding evoked by electrical stimulation of the lateral hypothalamus? *Behavioral Neuroscience*, *105*(1), 3–14. https://doi.org/10.1037/0735-7044.105.1.3

Blennow, K., Wallin, A., Gottfries, C. G., Månsson, J.-E., & Svennerholm, L. (1993). Concentration gradients for monoamine metabolites in lumbar cerebrospinal fluid. *Journal of Neural Transmission - Parkinson's Disease and Dementia Section*, *5*(1), 5–15. https://doi.org/10.1007/BF02260910

Breiter, H. C., Gollub, R. L., Weisskoff, R. M., Kennedy, D. N., Makris, N., Berke, J. D., … Hyman, S. E. (1997). Acute Effects of Cocaine on Human Brain Activity and Emotion. *Neuron*, *19*(3), 591–611. https://doi.org/10.1016/S0896-6273(00)80374-8

Chen, C.-H., Ridler, K., Suckling, J., Williams, S., Fu, C. H. Y., Merlo-Pich, E., & Bullmore, E. (2007). Brain Imaging Correlates of Depressive Symptom Severity and Predictors of Symptom Improvement After Antidepressant Treatment. *Biological Psychiatry*, *62*(5), 407–414. https://doi.org/10.1016/j.biopsych.2006.09.018

Cléry-Melin, M.-L., Schmidt, L., Lafargue, G., Baup, N., Fossati, P., & Pessiglione, M. (2011). Why Don't You Try Harder? An Investigation of Effort Production in Major Depression. *PLOS ONE*, *6*(8), e23178. https://doi.org/10.1371/journal.pone.0023178

Contarino, A., Picetti, R., Matthes, H. W., Koob, G. F., Kieffer, B. L., & Gold, L. H. (2002). Lack of reward and locomotor stimulation induced by heroin in μ-opioid receptor-deficient mice. *European Journal of Pharmacology*, *446*(1–3), 103–109. https://doi.org/10.1016/S0014-2999(02)01812-5

Delgado, M. R., Nystrom, L. E., Fissell, C., Noll, D. C., & Fiez, J. A. (2000). Tracking the Hemodynamic Responses to Reward and Punishment in the Striatum. *Journal of Neurophysiology*, *84*(6), 3072–3077.

Dichter, G. S., Smoski, M. J., Kampov-Polevoy, A. B., Gallop, R., & Garbutt, J. C. (2010). Unipolar depression does not moderate responses to the Sweet Taste Test. *Depression and Anxiety*, *27*(9), 859–863. https://doi.org/10.1002/da.20690

Ehrich, E., Turncliff, R., Du, Y., Leigh-Pemberton, R., Fernandez, E., Jones, R., & Fava, M. (2015). Evaluation of Opioid Modulation in Major Depressive Disorder. *Neuropsychopharmacology*, *40*(6), 1448–1455. https://doi.org/10.1038/npp.2014.330

Fava, M., Memisoglu, A., Thase, M. E., Bodkin, J. A., Trivedi, M. H., de Somer, M., … Ehrich, E. (2016). Opioid Modulation With Buprenorphine/Samidorphan as Adjunctive Treatment for Inadequate Response to Antidepressants: A Randomized Double-Blind Placebo-Controlled Trial. *American Journal of Psychiatry*, *173*(5), 499–508. https://doi.org/10.1176/appi.ajp.2015.15070921

Filliol, D., Ghozland, S., Chluba, J., Martin, M., Matthes, H. W. D., Simonin, F., … Kieffer, B. L. (2000). Mice deficient for δ- and μ-opioid receptors exhibit opposing alterations of emotional responses. *Nature Genetics*, *25*(2), 195–200. https://doi.org/10.1038/76061

Ghozland, S., Matthes, H. W. D., Simonin, F., Filliol, D., Kieffer, B. L., & Maldonado, R. (2002). Motivational Effects of Cannabinoids Are Mediated by μ-Opioid and κ-Opioid Receptors. *The Journal of Neuroscience*, *22*(3), 1146–1154.

Grace, A. A., & Bunney, B. S. (1984). The control of firing pattern in nigral dopamine neurons: burst firing. *Journal of Neuroscience*, *4*(11), 2877–2890.

Greenberg, P. E., Kessler, R. C., Birnbaum, H. G., Leong, S. A., Lowe, S. W., & Corey-Lisle, P. K. (1899). The Economic Burden of Depression in the United States: How Did It Change Between 1990 and 2000? *The Journal of Clinical Psychiatry*, *64*(12), 1465–1475.

Haber, S. N. (2011). Neuroanatomy of Reward: A View from the Ventral Striatum. In J. A. Gottfried (Ed.), *Neurobiology of Sensation and Reward*. Boca Raton (FL): CRC Press/Taylor & Francis. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK92777/

Hasler, G., Luckenbaugh, D. A., Snow, J., Meyers, N., Waldeck, T., Geraci, M., … Drevets, W. C. (2009). Reward Processing After Catecholamine Depletion in Unmedicated, Remitted Subjects with Major Depressive Disorder. *Biological Psychiatry*, *66*(3), 201–205. https://doi.org/10.1016/j.biopsych.2009.02.029

Hernández-López, S., Tkatch, T., Perez-Garci, E., Galarraga, E., Bargas, J., Hamm, H., & Surmeier, D. J. (2000). D2 Dopamine Receptors in Striatal Medium Spiny Neurons Reduce L-Type Ca2+ Currents and Excitability via a Novel PLCβ1–IP3–Calcineurin-Signaling Cascade. *Journal of Neuroscience*, *20*(24), 8987–8995.

Hirvonen, J., Hietala, J., Kajander, J., Markkula, J., Rasi-Hakala, H., Salminen, J. K., … Karlsson, H. (2011). Effects of antidepressant drug treatment and psychotherapy on striatal and thalamic dopamine D2/3 receptors in major depressive disorder studied with [11C]raclopride PET. *Journal of Psychopharmacology*, *25*(10), 1329–1336. https://doi.org/10.1177/0269881110376691

Hsu, D. T., Sanford, B. J., Meyers, K. K., Love, T. M., Hazlett, K. E., Walker, S. J., … Zubieta, J.-K. (2015). It still hurts: altered endogenous opioid activity in the brain during social rejection and acceptance in major depressive disorder. *Molecular Psychiatry*, *20*(2), 193–200. https://doi.org/10.1038/mp.2014.185

Ilango, A., Kesner, A. J., Keller, K. L., Stuber, G. D., Bonci, A., & Ikemoto, S. (2014). Similar Roles of Substantia Nigra and Ventral Tegmental Dopamine Neurons in Reward and Aversion. *Journal of Neuroscience*, *34*(3), 817–822. https://doi.org/10.1523/JNEUROSCI.1703-13.2014

Insel, T. R., & Cuthbert, B. N. (2009). Endophenotypes: Bridging Genomic Complexity and Disorder Heterogeneity. *Biological Psychiatry*, *66*(11), 988–989. https://doi.org/10.1016/j.biopsych.2009.10.008

Keitner GI, Ryan CE, Miller IW, & Norman WH. (1992). Recovery and major depression: factors associated with twelve-month outcome. *American Journal of Psychiatry*, *149*(1), 93–99. https://doi.org/10.1176/ajp.149.1.93

Kennedy, S. H. (2008). Core symptoms of major depressive disorder: relevance to diagnosis and treatment. *Dialogues in Clinical Neuroscience*, *10*(3), 271.

Kennedy SE, Koeppe RA, Young EA, & Zubieta J. (2006). Dysregulation of endogenous opioid emotion regulation circuitry in major depression in women. *Archives of General Psychiatry*, *63*(11), 1199–1208. https://doi.org/10.1001/archpsyc.63.11.1199

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K. R., … Wang, P. S. (2003). The Epidemiology of Major Depressive Disorder: Results From the National Comorbidity Survey Replication (NCS-R). *JAMA*, *289*(23), 3095. https://doi.org/10.1001/jama.289.23.3095

Kirschner, M., Hager, O. M., Bischof, M., Hartmann, M. N., Kluge, A., Seifritz, E., … Kaiser, S. (2016). Ventral striatal hypoactivation is associated with apathy but not diminished expression in patients with schizophrenia. *Journal of Psychiatry & Neuroscience*, *41*(2), 152–161. https://doi.org/10.1503/jpn.140383

Knutson, B., Westdorp, A., Kaiser, E., & Hommer, D. (2000). FMRI Visualization of Brain Activity during a Monetary Incentive Delay Task. *NeuroImage*, *12*(1), 20–27. https://doi.org/10.1006/nimg.2000.0593

Korb, A. S., Hunter, A. M., Cook, I. A., & Leuchter, A. F. (2009). Rostral anterior cingulate cortex theta current density and response to antidepressants and placebo in major depression. *Clinical Neurophysiology*, *120*(7), 1313–1319. https://doi.org/10.1016/j.clinph.2009.05.008

Lambert G, Johansson M, Ågren H, & Friberg P. (2000). Reduced brain norepinephrine and dopamine release in treatment-refractory depressive illness: Evidence in support of the catecholamine hypothesis of mood disorders. *Archives of General Psychiatry*, *57*(8), 787–793. https://doi.org/10.1001/archpsyc.57.8.787

Langenecker, S. A., Kennedy, S. E., Guidotti, L. M., Briceno, E. M., Own, L. S., Hooven, T., … Zubieta, J.-K. (2007). Frontal and Limbic Activation During Inhibitory Control Predicts Treatment Response in Major Depressive Disorder. *Biological Psychiatry*, *62*(11), 1272–1280. https://doi.org/10.1016/j.biopsych.2007.02.019

Leuchter, A. F., Cook, I. A., Marangell, L. B., Gilmer, W. S., Burgoyne, K. S., Howland, R. H., … Greenwald, S. (2009). Comparative effectiveness of biomarkers and clinical indicators for predicting outcomes of SSRI treatment in Major Depressive Disorder: Results of the BRITE-MD study. *Psychiatry Research*, *169*(2), 124–131. https://doi.org/10.1016/j.psychres.2009.06.004

Leyton, M., Boileau, I., Benkelfat, C., Diksic, M., Baker, G., & Dagher, A. (2002). Amphetamine-Induced Increases in Extracellular Dopamine, Drug Wanting, and Novelty Seeking: A PET/[11C]Raclopride Study in Healthy Men. *Neuropsychopharmacology*, *27*(6), 1027–1035. https://doi.org/10.1016/S0893-133X(02)00366-4

Liberzon, I., Zubieta, J. K., Fig, L. M., Phan, K. L., Koeppe, R. A., & Taylor, S. F. (2002). μ-Opioid receptors and limbic responses to aversive emotional stimuli. *Proceedings of the National Academy of Sciences*, *99*(10), 7084–7089. https://doi.org/10.1073/pnas.102174799

Matthes, H. W. D., Maldonado, R., Simonin, F., Valverde, O., Slowe, S., Kitchen, I., … Kieffer, B. L. (1996). Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the μ-opioid-receptor gene. *Nature*, *383*(6603), 819–823. https://doi.org/10.1038/383819a0

Mayberg, H. S., Brannan, S. K., Mahurin, R. K., Jerabek, P. A., Brickman, J. S., Tekell, J. L., … Fox, P. T. (1997). Cingulate function in depression: a potential predictor of treatment response. *Neuroreport*, *8*(4), 1057–1061.

McFarland, B. R., & Klein, D. N. (2009). Emotional reactivity in depression: diminished responsiveness to anticipated reward but not to anticipated punishment or to nonreward or avoidance. *Depression and Anxiety*, *26*(2), 117–122. https://doi.org/10.1002/da.20513

Milak, M. S., Parsey, R. V., Lee, L., Oquendo, M. A., Olvet, D. M., Eipper, F., … Mann, J. J. (2009). Pretreatment regional brain glucose uptake in the midbrain on PET may predict remission from a major depressive episode after three months of treatment. *Psychiatry Research: Neuroimaging*, *173*(1), 63–70. https://doi.org/10.1016/j.pscychresns.2008.09.004

Montgomery, A. J., Stokes, P., Kitamura, Y., & Grasby, P. M. (2007). Extrastriatal D2 and striatal D2 receptors in depressive illness: Pilot PET studies using [11C]FLB 457 and [11C]raclopride. *Journal of Affective Disorders*, *101*(1–3), 113–122. https://doi.org/10.1016/j.jad.2006.11.010

O'Doherty, J. P., Deichmann, R., Critchley, H. D., & Dolan, R. J. (2002). Neural Responses during Anticipation of a Primary Taste Reward. *Neuron*, *33*(5), 815–826. https://doi.org/10.1016/S0896-6273(02)00603-7

Olfson, M., Marcus, S. C., Tedeschi, M., & Wan, G. J. (2006). Continuity of Antidepressant Treatment for Adults With Depression in the United States. *American Journal of Psychiatry*, *163*(1), 101–108. https://doi.org/10.1176/appi.ajp.163.1.101

Papaleo, F., Kieffer, B. L., Tabarin, A., & Contarino, A. (2007). Decreased motivation to eat in μ-opioid receptor-deficient mice. *European Journal of Neuroscience*, *25*(11), 3398–3405. https://doi.org/10.1111/j.1460-9568.2007.05595.x

Peciña, S., & Berridge, K. C. (2005). Hedonic Hot Spot in Nucleus Accumbens Shell: Where Do μ-Opioids Cause Increased Hedonic Impact of Sweetness? *The Journal of Neuroscience*, *25*(50), 11777–11786. https://doi.org/10.1523/JNEUROSCI.2329-05.2005

Peciña, S., Berridge, K. C., & Parker, L. A. (1997). Pimozide Does Not Shift Palatability: Separation of Anhedonia from Sensorimotor Suppression by Taste Reactivity. *Pharmacology Biochemistry and Behavior*, *58*(3), 801–811. https://doi.org/10.1016/S0091-3057(97)00044-0

Peciña, S., Cagniard, B., Berridge, K. C., Aldridge, J. W., & Zhuang, X. (2003). Hyperdopaminergic Mutant Mice Have Higher "Wanting" But Not "Liking" for Sweet Rewards. *The Journal of Neuroscience*, *23*(28), 9395–9402.

Phillips, M. L., Chase, H. W., Sheline, Y. I., Etkin, A., Almeida, J. R. C., Deckersbach, T., & Trivedi, M. H. (2015). Identifying Predictors, Moderators, and Mediators of Antidepressant Response in Major Depressive Disorder: Neuroimaging Approaches. *American Journal of Psychiatry*, *172*(2), 124–138. https://doi.org/10.1176/appi.ajp.2014.14010076

Redgrave, P., Rodriguez, M., Smith, Y., Rodriguez-Oroz, M. C., Lehericy, S., Bergman, H., … Obeso, J. A. (2010). Goal-directed and habitual control in the basal ganglia: implications for Parkinson's disease. *Nature Reviews Neuroscience*, *11*(11), 760–772. https://doi.org/10.1038/nrn2915

Rive, M. M., van Rooijen, G., Veltman, D. J., .Phillips, M. L., Schene, A. H., & Ruhé, H. G. (2013). Neural correlates of dysfunctional emotion regulation in major depressive disorder. A systematic review of neuroimaging studies. *Neuroscience & Biobehavioral Reviews*, *37*(10, Part 2), 2529–2553. https://doi.org/10.1016/j.neubiorev.2013.07.018

Roberts, A. J., McDonald, J. S., Heyser, C. J., Kieffer, B. L., Matthes, H. W. D., Koob, G. F., & Gold, L. H. (2000). μ-Opioid Receptor Knockout Mice Do Not Self-Administer Alcohol ,. *Journal of Pharmacology and Experimental Therapeutics*, *293*(3), 1002–1008.

Rossi, M. A., Sukharnikova, T., Hayrapetyan, V. Y., Yang, L., & Yin, H. H. (2013). Operant Self-Stimulation of Dopamine Neurons in the Substantia Nigra. *PLOS ONE*, *8*(6), e65799. https://doi.org/10.1371/journal.pone.0065799

Rush, A. J. (2007). Limitations in Efficacy of Antidepressant Monotherapy. *The Journal of Clinical Psychiatry*, *68*(suppl 10), 8–10.

Schott, B. H., Minuzzi, L., Krebs, R. M., Elmenhorst, D., Lang, M., Winz, O. H., … Bauer, A. (2008). Mesolimbic Functional Magnetic Resonance Imaging Activations during Reward Anticipation Correlate with Reward-Related Ventral Striatal Dopamine Release. *The Journal of Neuroscience*, *28*(52), 14311–14319. https://doi.org/10.1523/JNEUROSCI.2058-08.2008

Simon, J. J., Biller, A., Walther, S., Roesch-Ely, D., Stippich, C., Weisbrod, M., & Kaiser, S. (2010). Neural correlates of reward processing in schizophrenia — Relationship to apathy and depression. *Schizophrenia Research*, *118*(1–3), 154–161. https://doi.org/10.1016/j.schres.2009.11.007

Smith, D. V., Hayden, B. Y., Truong, T.-K., Song, A. W., Platt, M. L., & Huettel, S. A. (2010). Distinct Value Signals in Anterior and Posterior Ventromedial Prefrontal Cortex. *Journal of Neuroscience*, *30*(7), 2490–2495. https://doi.org/10.1523/JNEUROSCI.3319-09.2010

Smith, K. S., & Berridge, K. C. (2005). The Ventral Pallidum and Hedonic Reward: Neurochemical Maps of Sucrose "Liking" and Food Intake. *The Journal of Neuroscience*, *25*(38), 8637–8649. https://doi.org/10.1523/JNEUROSCI.1902-05.2005

Spanagel, R., Herz, A., & Shippenberg, T. S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(6), 2046–2050.

Surmeier, D. J., Ding, J., Day, M., Wang, Z., & Shen, W. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends in Neurosciences*, *30*(5), 228–235. https://doi.org/10.1016/j.tins.2007.03.008

Thase, M. E., Haight, B. R., Richard, N., Rockett, C. B., Mitton, M., Modell, J. G., … Wang, Y. (2005). Remission Rates Following Antidepressant Therapy With Bupropion or Selective Serotonin Reuptake Inhibitors: A Meta-Analysis of Original Data From 7 Randomized Controlled Trials. *The Journal of Clinical Psychiatry*, *66*(8), 974–981.

Tindell, A. J., Berridge, K. C., Zhang, J., Peciña, S., & Aldridge, J. W. (2005). Ventral pallidal neurons code incentive motivation: amplification by mesolimbic sensitization and amphetamine. *European Journal of Neuroscience*, *22*(10), 2617–2634. https://doi.org/10.1111/j.1460-9568.2005.04411.x

Tomarken, A. J., Dichter, G. S., Freid, C., Addington, S., & Shelton, R. C. (2004). Assessing the effects of bupropion SR on mood dimensions of depression. *Journal of Affective Disorders*, *78*(3), 235–241. https://doi.org/10.1016/S0165-0327(02)00306-3

Treadway, M. T., Buckholtz, J. W., Schwartzman, A. N., Lambert, W. E., & Zald, D. H. (2009). Worth the "EEfRT"? The Effort Expenditure for Rewards Task as an Objective Measure of Motivation and Anhedonia. *PLOS ONE*, *4*(8), e6598. https://doi.org/10.1371/journal.pone.0006598

Tremblay LK, Naranjo CA, Cardenas L, Herrmann N, & Busto UE. (2002). Probing brain reward system function in major depressive disorder: Altered response to dextroamphetamine. *Archives of General Psychiatry*, *59*(5), 409–416. https://doi.org/10.1001/archpsyc.59.5.409

Tremblay LK, Naranjo CA, Graham SJ, & et al. (2005). Functional neuroanatomical substrates of altered reward processing in major depressive disorder revealed by a dopaminergic probe. *Archives of General Psychiatry*, *62*(11), 1228–1236. https://doi.org/10.1001/archpsyc.62.11.1228

Trivedi, M. H., Rush, A. J., Wisniewski, S. R., Nierenberg, A. A., Warden, D., Ritz, L., … Fava, M. (2006). Evaluation of Outcomes With Citalopram for Depression Using Measurement-Based Care in STAR*D: Implications for Clinical Practice. *American Journal of Psychiatry*, *163*(1), 28–40. https://doi.org/10.1176/appi.ajp.163.1.28

Volkow, N. D., Wang, G.-J., Fowler, J. S., Logan, J., Jayne, M., Franceschi, D., … Pappas, N. (2002). "Nonhedonic" food motivation in humans involves dopamine in the dorsal striatum and methylphenidate amplifies this effect. *Synapse*, *44*(3), 175–180. https://doi.org/10.1002/syn.10075

Warden, D., Rush, A. J., Trivedi, M. H., Fava, M., & Wisniewski, S. R. (2007). The STAR*D Project results: a comprehensive review of findings. *Current Psychiatry Reports*, *9*(6), 449–459.

Weiland, B. J., Heitzeg, M. M., Zald, D., Cummiford, C., Love, T., Zucker, R. A., & Zubieta, J.-K. (2014). Relationship between impulsivity, prefrontal anticipatory activation, and striatal dopamine release during rewarded task performance. *Psychiatry Research: Neuroimaging*, *223*(3), 244–252. https://doi.org/10.1016/j.pscychresns.2014.05.015

Weiland, B. J., Zucker, R. A., Zubieta, J.-K., & Heitzeg, M. M. (2016). Striatal dopaminergic reward response relates to age of first drunkenness and feedback response in at-risk youth. *Addiction Biology*, n/a-n/a. https://doi.org/10.1111/adb.12341

Wyvell, C. L., & Berridge, K. C. (2000). Intra-Accumbens Amphetamine Increases the Conditioned Incentive Salience of Sucrose Reward: Enhancement of Reward "Wanting" without Enhanced "Liking" or Response Reinforcement. *The Journal of Neuroscience*, *20*(21), 8122–8130.

Yeomans, M. R., & Gray, R. W. (1997). Effects of Naltrexone on Food Intake and Changes in Subjective Appetite During Eating: Evidence for Opioid Involvement in the Appetizer Effect. *Physiology & Behavior*, *62*(1), 15–21. https://doi.org/10.1016/S0031-9384(97)00101-7

Yovell, Y., Bar, G., Mashiah, M., Baruch, Y., Briskman, I., Asherov, J., … Panksepp, J. (2015). Ultra-Low-Dose Buprenorphine as a Time-Limited Treatment for Severe Suicidal Ideation: A Randomized Controlled Trial. *American Journal of Psychiatry*, *173*(5), 491–498. https://doi.org/10.1176/appi.ajp.2015.15040535

Zhang, B., Lin, P., Shi, H., Öngür, D., Auerbach, R. P., Wang, X., … Wang, X. (2015). Mapping anhedonia-specific dysfunction in a transdiagnostic approach: an ALE meta-analysis. *Brain Imaging and Behavior*, 1–20. https://doi.org/10.1007/s11682-015-9457-6

Zhang, W.-N., Chang, S.-H., Guo, L.-Y., Zhang, K.-L., & Wang, J. (2013). The neural correlates of reward-related processing in major depressive disorder: A meta-analysis of functional magnetic resonance imaging studies. *Journal of Affective Disorders*, *151*(2), 531–539. https://doi.org/10.1016/j.jad.2013.06.039

Zubieta J, Ketter TA, Bueller JA, & et al. (2003). Regulation of human affective responses by anterior cingulate and limbic μ-opioid neurotransmission. *Archives of General Psychiatry*, *60*(11), 1145–1153. https://doi.org/10.1001/archpsyc.60.11.1145

Chapter Two.
Anticipatory and Hedonic Reward Responses in Major Depression Reflect Individual Differences in Dopamine and μ-Opioid Systems

Introduction

Individuals with Major Depressive Disorder (MDD) frequently present with a disruption in healthy reward processing. Disrupted reward represents a core symptom of the illness, and has emerged in behavioral (Cléry-Melin et al., 2011; McFarland & Klein, 2009; Treadway et al., 2009) and neuroimaging (Forbes et al., 2009; Pizzagalli et al., 2008; Smoski et al., 2009) studies of reward with depressed patients. Reward processing alterations in MDD encompass two major components: the desire to seek rewards (loss of interest) and the pleasure of experiencing rewards (loss of pleasure). Each component is likely to be associated with distinct – and experimentally dissociable – neurotransmitter systems and neural circuitry. However, the molecular substrates of these alterations are still not well understood.

Motivation and dopamine. Previous literature implicates the mesolimbic dopamine (DA) system in the desire for reward. Augmentation and disruption of DA transmission in rodents (Berridge & Robinson, 1998; Berridge & Valenstein, 1991; Peciña et al., 1997; Peciña, et al., 2003; Tindell et al., 2005; Wyvell & Berridge, 2000) and humans (Leyton et al., 2002; Volkow et al., 2002)

reveals manipulation of motivation without affecting hedonic response. The circuitry underlying motivation appears to comprise a larger cortico-striatal-thalamic-cortical network (Haber, 2011), and special focus has been given to mesolimbic pathway, connecting DA neurons in the ventral tegmental area (VTA) with the nucleus accumbens (NAc). Converging lines of evidence support a role for DA dysfunction in the pathophysiology of depression, including altered striatal DA binding (Cannon et al, 2008), and reduced concentrations of a DA metabolite (Lambert et al, 2000). Furthermore, augmented (Tremblay et al, 2005) and disrupted (Hasler et al., 2009) DA neurotransmission in depressed individuals can exert anti- and pro-depressant effects, respectively.

Hedonics and μ-opioids. Studies investigating hedonics in both rodents and humans have implicated the μ-opioid system in the evaluation of the pleasant hedonic properties of a stimulus that make it desirable (Berrendero et al., 2002; Contarino et al., 2002; Ghozland et al., 2002; Roberts et al., 2000; Yeomans & Gray, 1997). Relative to motivation, reward hedonics appear to be mediated by a more restricted limbic circuit: the NAc and ventral pallidum contain small hedonic "hotspots" (Peciña & Berridge, 2005; Smith & Berridge, 2005), which have a high density of opioid receptors. Stimulation of the μ-opioid receptor in these regions elicits positive hedonic facial reactions to a sweet solution, and potently induces feeding. In non-depressed participants, individual differences in μ-opioid function have been linked to affective responses (Liberzon et al., 2002; Zubieta et al, 2003). In depression, evidence indicates that endogenous opioid tone is dysregulated (Kennedy et al., 2006), and opioid modulation elicits significant and rapid mood elevation in depressed patients (Ehrich et al., 2015), although their usefulness in treatment is limited by the risk of abuse and addiction.

Clinical and molecular correlates of anticipatory and hedonic reward response. Here, we investigate the molecular correlates of an extensively-used reward processing fMRI paradigm, the Monetary Incentive Delay (MID) task (Knutson et al, 2000) in twenty-nine patients with MDD. We aimed to determine the relationship between fMRI responses during the anticipation of monetary gain, clinical measures of reward responsivity (self-reported measures of apathy and anhedonia), and in vivo correlates of opioid and DA receptor availability and release using Positron Emission Tomography (PET). We hypothesized that (1) lower striatal fMRI responses to anticipation of monetary rewards would correlate with higher apathy scores, increased DA receptor binding, and reduced DA neurotransmission in the striatum. We further hypothesized that (2) lower striatal fMRI responses to monetary rewards would correlate with higher anhedonia scores, increased μ-opioid receptor binding, and reduced opioid neurotransmission in the striatum.

Methods

Participants

Written informed consent was obtained from all participants. Procedures were approved by the University of Michigan Institutional Review Board for Human Subject Use and the Radioactive Drug Research Committee.

Thirty-five right-handed medication-free participants were recruited via advertisement, and diagnosed with DSM-V Major Depressive Disorder (MDD; 23 females, age range 18 – 59 years, mean ± SD: 32.09 ± 12.57) using the Mini International Neuropsychiatric Inventory (MINI) 6.0. Inclusion criteria included diagnosis of MDD using the MINI and Hamilton Rating

Scale for Depression (HRSD; Hamilton, 1960) scores > 12. At screening, participants completed the Apathy Evaluation Scale (AES; Marin, 1996) and Snaith-Hamilton Pleasure Scale (SHAPS; Snaith et al., 1995). Exclusion criteria included suicidal ideation, comorbid medical, neurological or psychiatric conditions, pregnancy, and use of psychotropic agents.

Study Design

Full study design description has been previously described (Peciña et al, 2015). Briefly, patients were randomized to (1) 1-week "active" oral placebo treatment (2 pills per day), with expectations that it represented a fast-acting antidepressant agent, or (2) 1-week "inactive" oral placebo, with disclosure that it was an inactive control (Figure 2.1). After a 3-day washout period without pills, participants were crossed over into the group to which they were not previously assigned. After each placebo week, participants underwent a positron emission tomographic (PET) scanning session. As a challenge to induce endogenous opioid and DA system activation and determine acute placebo effects, the PET session following the 1-week active oral placebo included the administration of an IV active placebo. This consisted of 1mL of 0.9% isotonic saline-introduced IV every 4 minutes over 20 minutes, starting at minute 42 and lasting for 15 seconds each time. Patients were made aware that the study drug was to be administered through a computer-generated human voice recording, followed by a second-by-second count of the infusion timing (15 seconds). No IV placebo followed the inactive placebo condition. Following the 1-week inactive oral placebo, participants underwent a functional magnetic resonance imaging (fMRI) scanning session.

Monetary Incentive Delay Task

Participants performed a modified version of the MID task (Knutson et al., 2000) during fMRI acquisition, to measure changes in Blood-Oxygen-Level Dependent (BOLD) signal in response to reward anticipation and receipt. Each participant completed three runs, of thirty trials each, in which subjects made a button-response to a simple visual target during a brief response window (Figure 2.2).

At the beginning of each trial, participants saw a cue for 500ms, indicating the one of five trial conditions: potential large reward (+$5), potential small reward (+$0.20), potential large punishment (-$5), potential small punishment (-$0.20), or no money at risk (neutral). Eighteen trials of each condition were presented in pseudo-randomized order. An anticipation period of 1500-5500ms followed the cue. The target image then appeared briefly, cuing the participant to make a button response. The participant won money or avoided losing money if the button was pressed within the response window (~250 ms). The response window was dynamically varied over the course of the task, in response to participant reaction time. Target presentation was followed by a variable delay (2-6 s) and then feedback regarding the outcome of the trial (2 s). An inter-trial interval of 2-6 s separated each trial.

Participants' responses to the MID task were recorded in E-Prime 2.0. Behavioral responses were subsequently inspected to verify the correct response button was used. Trials in which an incorrect response button was used were discarded from analysis.

MRI acquisition and image processing

MRI data were acquired on a Philips Ingenia 3.0-Tesla scanner (Philips Medical Systems; Best, Netherlands). Two-hundred eleven whole-brain functional images were acquired in each

of three runs using a T2*-weighted echo-planar gradient-echo pulse sequence (39 slices acquired sequentially in axial orientation co-planar with AC-PC line, slice thickness = 3.5mm, slice gap = 0mm, echo time = 28ms, repetition time = 2000ms, flip angle = 90°, acquisition matrix = 64mm x 64mm). Stimuli were presented electronically with E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA) using the ESys patient display monitor (Invivo, Orlando, FL).

A high resolution structural image was obtained for anatomic normalization using a T1-weighted, magnetization-prepared rapid gradient echo (MPRAGE) sequence (220 sagittal slices, slice thickness =1 mm, echo time = 4.6ms, repetition time = 9.8ms, flip angle=8°, acquisition matrix = 240mm x 240mm).

Preprocessing was performed on the functional data using FSL (5.0.2.2, http://www.fmrib.ox.ac.uk) and SPM8 (r4667, Wellcome Department of Cognitive Neurology, University College London, UK; www.fil.ion.ucl.ac.uk). Volumes underwent slice-timing and motion correction. Runs containing between-scan translation of more than 3.5mm were excluded, and participants with two or more excluded runs were excluded from group analysis. Data for six participants were rejected for excessive head motion during the fMRI scan. Functional scans were normalized to the MNI152 template, resampled to 2x2x2mm voxels, and underwent spatial smoothing using an isotropic Gaussian kernel of 8.0 mm full-width at half-maximum. Following all exclusions, fMRI data for twenty-nine participants were included in subsequent group analyses.

For each participant, intrasubject effects were modeled in SPM8 using an event-related design including both anticipatory and outcome conditions. For anticipatory conditions, the onsets of the cues for each of the five conditions were modeled as separate regressors. For outcome conditions, onsets of the feedback period for each of the three potential outcomes

(successful monetary outcome, encompassing both monetary reward and avoidance of monetary punishment; unsuccessful monetary outcome, encompassing monetary punishment and failure to earn monetary reward; neutral outcome, where no money was at risk) were modeled as separate regressors. In each model, six motion parameters were included as regressors of no interest. A high pass filter (cutoff 128s) was applied, as well as AR(1) auto-regression correction.

To investigate BOLD response to reward anticipation, a contrast was created for Anticipation of Monetary Gain > Neutral Anticipation (Anticipation of Monetary Gain collapsed across reward magnitude to encompass all trials in which participations could gain money). Anticipation of Monetary Loss > Neutral Anticipation showed similar results to the Monetary Gain contrast. The Monetary Gain contrast was used in subsequent analyses to address our hypotheses regarding reward function in MDD.

To investigate BOLD response to reward outcome, a contrast was created for Successful Monetary Outcome > Unsuccessful Monetary Outcome. A contrast was also created for Unsuccessful Monetary Outcome > Successful Monetary Outcome, in which no significant results were observed.

The number of observations of a given condition varied across the three runs. To account for this, contrast weights were calculated to reflect inverse variance. We determined the contrast weight for a given condition in a given run as the number of observations of the condition in that run, divided by the sum of the square roots of the number of observations of the condition in each run. For example, the contrast weight for Condition A in run 1 is equal to the number of observations of Condition A in run 1 divided by the sum of the square roots of the number of observations of Condition A in run 1, run 2, and run 3.

PET acquisition and image processing

Four 90-minute PET scans were acquired (HR_scanner; Siemens, Knoxville, Tennessee) in 3-dimensional mode (reconstructed full-width/half-maximum resolution, approximately 5.5 mm in plane and 5.0 mm axially), with the septa retracted and scatter correction, as previously described (Peciña et al, 2015; Scott et al., 2007). Of the twenty-nine participants with quality fMRI data, all twenty-nine underwent PET scans for MOR; a subset of seventeen underwent scans for DA D2/3.

Briefly, participants were positioned in the PET scanner gantry, and 2 intravenous (antecubital) lines were placed. A light forehead restraint was used to eliminate intrascan head movement. Radiotracer administrations were separated by at least 2 hours to allow for radiotracer decay. Carbon 11 (^{11}C)–labeled carfentanil was synthesized at high specific activity (>2000 Ci/mmol [the conversion factor for 1Ci is $3.7x10^{10}$ Bq]) by the reaction of [^{11}C]methyl iodide and a normethyl precursor as previously described (Jewett, 2001). [^{11}C]raclopride was synthesized at high specific activity (>2000 Ci/mmol) by the reaction of *O*-desmethyl raclopride with [^{11}C]methyl triflate. Ten to 15 mCi was administered in each of the imaging procedures, with a mean (SD) mass of carfentanil injected of 0.028 (0.013) μg/kg per scan and of raclopride of 0.20 (0.15) μg/kg per scan. These levels ensured that the compounds were administered in tracer quantities, that is, subpharmacological doses occupying less than 1% of the available receptors. Fifty percent of the radiotracer doses were administered as an initial bolus and the remaining 50% by continuous infusion for the remainder of the study. This procedure compensates for the metabolism and distribution of the radiotracer, leading to constant plasma concentrations over time and more rapid equilibration between kinetic compartments. For each

scan, 21 sets of images (frames) were acquired over a 90-minute period with an increasing duration (four 30-second frames, three 1-minute frames, two 2.5-minute frames, eight 5-minute frames, and four 10-minute frames). Images were reconstructed using iterative algorithms (brain mode; Fourier rebinning algorithm with ordered-subsets expectation maximization, 4 iterations, and 16 subsets; no smoothing) into a 128x128-pixel matrix in a 28.8-cm-diameter field of view. Attenuation correction was performed through a 6-minute transmission scan (Ge^{68} source) obtained before the PET study and with iterative reconstruction of the blank/transmission data, followed by segmentation of the attenuation image. Small head motions during PET were corrected by an automated computer algorithm for each subject before analysis, and the images were co-registered with the same software. Time points were then decay corrected during reconstruction of the PET data. Image data were then transformed on a voxel-by-voxel basis into 2 sets of parametric maps, a tracer transport measure (K_1 ratio) and a receptor-related measure (distribution volume ratio [DVR] at equilibrium), using data from 5- to 40-minute post-tracer administration. To avoid the need for arterial blood sampling, these measures were calculated by means of a modified Logan graphical analysis (Logan et al., 1996) using the following reference regions: the occipital cortex (an area with low expression of μ-opioid receptors) for [^{11}C]carfentanil scans and the cerebellum (an area with negligible DA D2/3 receptors) for [^{11}C]raclopride scans. The slope of the Logan plot is equal to the receptor concentration divided by its affinity for the radiotracer ($f_2B_{max}/K_d + 1$ for this receptor site) and has been referred to as the DVR; f_2B_{max}/K_d (or DVR−1) is the "receptor related" measure (also termed BP) or receptor availability in vivo. B_{max} is the receptor concentration and K_d, the receptor-ligand dissociation constant. The term f_2 refers to the concentration of free radiotracer in the extracellular fluid and is considered to represent a constant and very small value. Reductions in the in vivo availability

of receptors, the BP_{ND} measure, after an acute challenge (i.e., placebo administration) are thought to reflect processes, such as competition between radiotracer and endogenous ligand, associated with neurotransmitter release (Narendran & Martinez, 2008).

Clinical scales

The AES-S is an 18-item self-report instrument measuring motivational impairment. Participants respond to statements regarding motivation on a four-point scale, indicating whether each statement is "not at all true", "slightly true", "somewhat true", or "very true". Responses to each item are scored 4 to 1 respectively (with three items reverse-scored), such that total scores may range from 18 to 72, with higher scores indicating greater apathy (impairment in motivation).

The SHAPS is a 14-item self-report instrument measuring hedonic experience. Participants respond to statements regarding experienced pleasure on a four-point scale, indicating whether they "strongly disagree", "disagree", "agree", or "strongly agree" with each statement. Responses of "strongly disagree" and "disagree" are scored 1, while responses of "agree" and "strongly agree" are scored 0, such that total scores may range from 0 to 14, with higher scores indicating greater anhedonia (impairment in hedonic function).

Statistical analysis

For overall flow of analyses in Chapter 2, see Figure 2.4. At the second level, main effects of task were investigated using fMRI contrasts of interest (Anticipation of Monetary Gain > Neutral Anticipation; Successful Monetary Outcome > Unsuccessful Monetary Outcome). We chose to look at Anticipation of Monetary Gain, rather than Monetary Loss, as our interest is in

anticipation of reward rather than avoidance of punishment. That said, BOLD response to Anticipation of Gain and Loss did not significantly differ in main effect regions. We similarly chose to investigate Successful Monetary Outcome > Unsuccessful Monetary Outcome to look at positive-valence specific response to reward hedonics. Unsuccessful Monetary Outcome > Successful Monetary Outcome did not produce any significant results.

The resulting voxelwise maps were thresholded at $p < 0.001$ uncorrected and with cluster extent threshold to correct for multiple comparisons at $p < 0.05$, based on Monte Carlo simulation using 3dClustSim (10000 iterations, NN1, 1-sided; version AFNI_16.2.01, precompiled binary linux_xorg7_64: July 8 2016; Cox, 1996). For each contrast of interest (anticipation and outcome), a mask was generated comprised of significant Main Effect of Task clusters, restricted to the striatum using a mask of DA D2/3 binding (see Appendix 2.1).

Functional MRI contrast values were extracted separately from each of these masks via the MarsBaR toolbox (Brett et al, 2002), and regressed against maps of DA D2/3 and μ-opioid receptor availability, and challenge-induced changes in receptor availability. The resulting voxelwise maps were thresholded at $p < 0.001$ uncorrected. Cluster extent threshold to correct for multiple comparisons at $p < 0.05$ was calculated using 3dClustSim as described above. Results were masked to the striatum using a mask of raclopride binding. As a secondary analysis, correlations with maps of μ-opioid receptor availability were re-examined masked to regions of carfentanil binding.

Additional statistical analyses were performed using the statistical software SPSS 22 (IBM Corp, Armonk, NY). Post-hoc, DA D2/3 and μ-opioid receptor availability values were extracted from significant clusters resulting from correlation analyses as binary cluster masks in MarsBaR. These extracted values were correlated with fMRI contrast variables in SPSS.

Extracted fMRI contrast values for anticipation of monetary gain and successful monetary outcome were correlated with Apathy Evaluation Scale and Snaith-Hamilton Pleasure Scale scores. For SPSS correlations, in cases where variables were non-normally distributed (as determined by Shapiro-Wilk test of normality in SPSS, $p < 0.05$), Spearman's rank correlation coefficient (ρ) was tested. For all others, Pearson's correlation coefficient (r) was used.

Results

Clinical characteristics of sample

Participants' scores on the Apathy Evaluation Scale ranged from 25-66 (mean ± SD = 46.00 ± 9.11). Scores on the Snaith-Hamilton Pleasure Scale ranged from 0-12 (mean ± SD = 5.42 ± 3.51).

Behavioral results

Median reaction time of successful responses across all trial types was 217.7ms (± 56.0 ms). Mean accuracy across all trial types was 54.7% (± 7.8%). For additional behavioral results, see Appendix 2.2.

Imaging results

Relationship between striatal DA D2/3 and μ-opioid BP_{ND} and striatal anticipation of monetary gain during the MID

We first examined the relationship between the anticipation of monetary gain in the striatum during the MID and binding measures of DA D2/3 (n = 17) and μ-opioid (n = 29)

receptors. Averaged BOLD signal from striatal main effects of task during the anticipation of monetary gain was regressed against both DA D2/3 and μ-opioid binding maps.

Increased striatal BOLD response during anticipation of monetary gain correlated positively with DA D2/3 receptor BP_{ND} in the bilateral putamen (Right putamen: 30, -10, 6; z = 4.86, k = 136; left putamen: -26, -14, 6; z = 4.08, k = 49), and right caudate (16, 12, 16; z = 4.25, k = 138). Increased striatal BOLD response during anticipation of monetary gain correlated negatively with DA D2/3 receptor BP_{ND} in the right globus pallidus (26, -6, -6; z = 3.97; k = 115). (Figure 2.5)

No effects were observed between striatal BOLD response during the anticipation of monetary gain and striatal μ-opioid receptor binding potential for either positive or negative contrasts.

Relationship between DA D2/3 and μ-opioid BP_{ND} and striatal processing of successful monetary outcomes during the MID

We next examined the relationship between the processing of successful monetary outcomes during the MID and measures of DA D2/3 (n = 17) and μ-opioid (n = 29) receptor availability. Averaged BOLD signal from a mask incorporating the main effects of task within the striatum during the processing of successful monetary outcomes (see Methods), was regressed against both DA D2/3 and μ-opioid binding maps.

No significant correlations were observed between BOLD response in the striatum during successful monetary outcomes and striatal DA D2/3 receptor availability for either positive or negative contrasts.

No significant correlations were observed between BOLD response in the striatum during successful monetary outcomes and striatal μ-opioid receptor availability for either positive or

negative contrasts. Using a larger mask reflecting areas of μ-opioid receptor binding (see Appendix 2.3), increased BOLD response to successful monetary outcome correlated negatively with μ-opioid receptor availability in the bilateral medial thalamus (peak at 2, -12, 4; $z = 3.36$; $k = 65$) (Figure 2.6). No significant correlations were observed for the opposite contrast.

Relationship between challenge-induced changes in DA D2/3 and μ-opioid BP_{ND} and striatal anticipation of monetary gain during the MID

We then examined the relationship between challenge-induced changes in DA D2/3 ($n = 17$) and μ-opioid ($n = 29$) BP_{ND} and the processing of anticipatory response to monetary gain during the MID, using the same mask described above for the anticipation of monetary gain. Increased BOLD response to anticipation of monetary gain correlated negatively with challenge-induced changes in DA D2/3 BPND in the bilateral putamen (Figure 2.7; left putamen: -26, -2, 0; $z = 3.90$; $k = 50$; right putamen: 26, 4, 2; $z = 4.09$; $k = 35$). No significant correlations were observed for the opposite contrast.

No significant correlations were observed between BOLD responses during anticipation of monetary gain and challenge-induced changes in μ-opioid BP_{ND}, for either positive or negative contrasts.

Relationship between challenge-induced changes in DA D2/3 and μ-opioid BP_{ND} and striatal processing of successful monetary outcomes during the MID

Finally, we examined the relationship between challenge-induced changes in DA D2/3 ($n = 17$) and μ-opioid ($n = 29$) BP_{ND} and the processing of successful monetary outcomes during the MID, using the mask described above for the processing of monetary outcomes.

No significant correlations were observed between BOLD responses during successful monetary outcome and challenge-induced changes in DA D2/3 BP_{ND} or μ-opioid BP_{ND}, for either positive or negative contrasts.

Relationship between anticipation-associated DA D2/3 BP_{ND} and clinical reward scales

Building on the above analyses, we investigated the relationship between anticipation-associated striatal DA D2/3 BP_{ND}, and clinical measures of reward function: apathy, measured by the AES, and anhedonia measures by the SHAPS. Increased DA D2/3 BP_{ND} in the putamen and caudate was associated with greater anhedonia scores (r = .584, p = 0.014), and was associated at a trend level with greater apathy scores (r = .448, p = 0.071). No significant correlation was observed between DA D2/3 BP_{ND} in the globus pallidus and apathy or anhedonia.

Relationship between outcome-associated MOR BP_{ND} and clinical reward scales

We also investigated the relationship between outcome-associated thalamic MOR BP_{ND}, and clinical measures of reward function. No significant correlation was observed between μ-opioid BP_{ND} in the thalamus and apathy or anhedonia.

(See Table 2.1 for summary of significant imaging results.)

Discussion

In the present study, we investigated the relationship between striatal response during anticipation and receipt of a reward, neurotransmitter binding and neurotransmission, and

clinical measures of disrupted reward in patients with Major Depression. The above results demonstrate significant relationships between DA D2/3 receptor availability, fMRI BOLD responses to anticipation of monetary gain, and self-reported measures of apathy and anhedonia. Specifically, we observed a positive correlation between BOLD response to anticipation of monetary gain and DA D2/3 receptor availability in the dorsal striatum, such that lower BOLD response was associated with lower striatal DA D2/3 receptor availability. Given previous findings of both striatal hypoactivation to reward anticipation (Zhang et al, 2013) and elevated striatal DA D2/3 receptor availability (Meyer et al., 2006) in MDD, the direction of this finding was unexpected. That said, investigations into altered DA D2/3 receptor availability in depression have produced mixed results (Savitz & Drevets, 2013), potentially as a result of confounding factors such as smoking status and the presence of psychomotor retardation.

We did not observe the hypothesized correlation between BOLD response to successful monetary outcome and μ-opioid receptor availability in the striatum. However, we did observe a negative correlation between BOLD response to successful monetary outcome and μ-opioid receptor availability in the medial thalamus (a key region of reward circuitry which receives projections from the striatum; Haber & Calzavara, 2009) such that lower BOLD response was associated with greater thalamic μ-opioid receptor availability. These findings suggest that individuals with lower opioid tone in the thalamus demonstrate reduced neural reward response. However, this result does not survive cluster-threshold correction for multiple comparisons, and is at increased risk of reflecting type I error. An alternate analysis, performed by extracting BOLD signal from Main Effect of Task clusters across the whole brain (instead of restricting to the striatum), replicated the negative correlation between BOLD response to successful monetary

outcome and μ-opioid receptor availability in the medial thalamus, and did survive cluster-threshold correction for multiple comparisons (data not shown).

Additionally, we observed a positive correlation between anticipation-associated striatal DA D2/3 receptor availability and scores on clinical scales of disrupted reward, such that greater anhedonia and apathy were associated with greater DA D2/3 receptor availability in the caudate and putamen. This finding is in line with our hypothesis, suggesting lower endogenous tone of DA in individuals with greater clinical reward disruption.

We further observed a significant negative correlation between BOLD reward response and dopamine neurotransmission in the bilateral putamen. Previous studies have demonstrated a positive correlation between NAc dopaminergic release and anticipatory reward activation (Schott et al., 2008; Urban et al., 2011; Weiland et al., 2014), as well as activation to reward outcome (Weiland et al, 2016). One notable consideration that may account for why we observe a different effect in both location (putamen vs nucleus accumbens) and direction (negative correlation with reward response vs positive) compared to the cited work is that previous studies have induced dopamine release using a reward-challenge, which may induce different patterns or magnitude of release than the placebo-challenge in the present study.

In addition to testing our hypothesized relationships, we also observed a significant negative correlation between striatal BOLD response to reward anticipation and striatal BOLD response to successful monetary outcome in patients with MDD. This relationship was unexpected but does parallel some previous findings. de Leeuw et al. (2015) found a negative correlation between reward anticipation and outcome response in the ventral striatum of unaffected siblings of patients with schizophrenia. Other studies have published findings in which BOLD response to reward anticipation and reward outcome are significantly correlated

with a third variable in opposite directions, but did not directly correlate the two BOLD measures (Forbes et al., 2010; Furukawa et al., 2014). At least one study has directly tested this relationship and found no significant effect (Hoogendam et al., 2013). We likewise found no significant relationship between anticipation and outcome response in a separate sample of control subjects ($n = 13$, $p > 0.9$, data not shown). The negative relationship in patients with MDD could reflect variation among participants in how much they transfer incentive salience from the reward outcome to the reward cue. Participants who fail to transfer this incentive salience may demonstrate reduced response to the cue (i.e., anticipation) while still demonstrating a response to reward outcome. Additional investigation into the relationship between striatal responses to reward anticipation and reward outcome are warranted, in both healthy control subjects and clinical samples.

The above findings should be interpreted with the following consideration in mind: anticipation of reward in the MID coincides with motor preparation for a button response (as noted in Treadway & Zald, 2011), such that striatal BOLD response during this condition likely reflects both processes, and must be interpreted with caution. The role of striatal dopamine in both processes further strains our ability to interpret the above relationship between BOLD response and DA D2/3 binding.

These results should be replicated with the inclusion of a healthy control group, which would allow direct comparison of neurotransmitter binding/reward response relationships between depressed and control samples. Such a comparison would help to clarify the context of the above results, and allow for stronger statements about the molecular basis of reward disruption in major depression. Furthermore, investigating the relationship between neural

response to reward and neurotransmission elicited by a reward-challenge in individuals with MDD would help place this work in context of previous studies.

In summary, our results suggest that neural response to reward and clinical measures of reward response in depressed individuals are modulated by DA D2/3 and μ-opioid receptor availability in key regions of reward circuitry.

Tables, Figures, and Legends

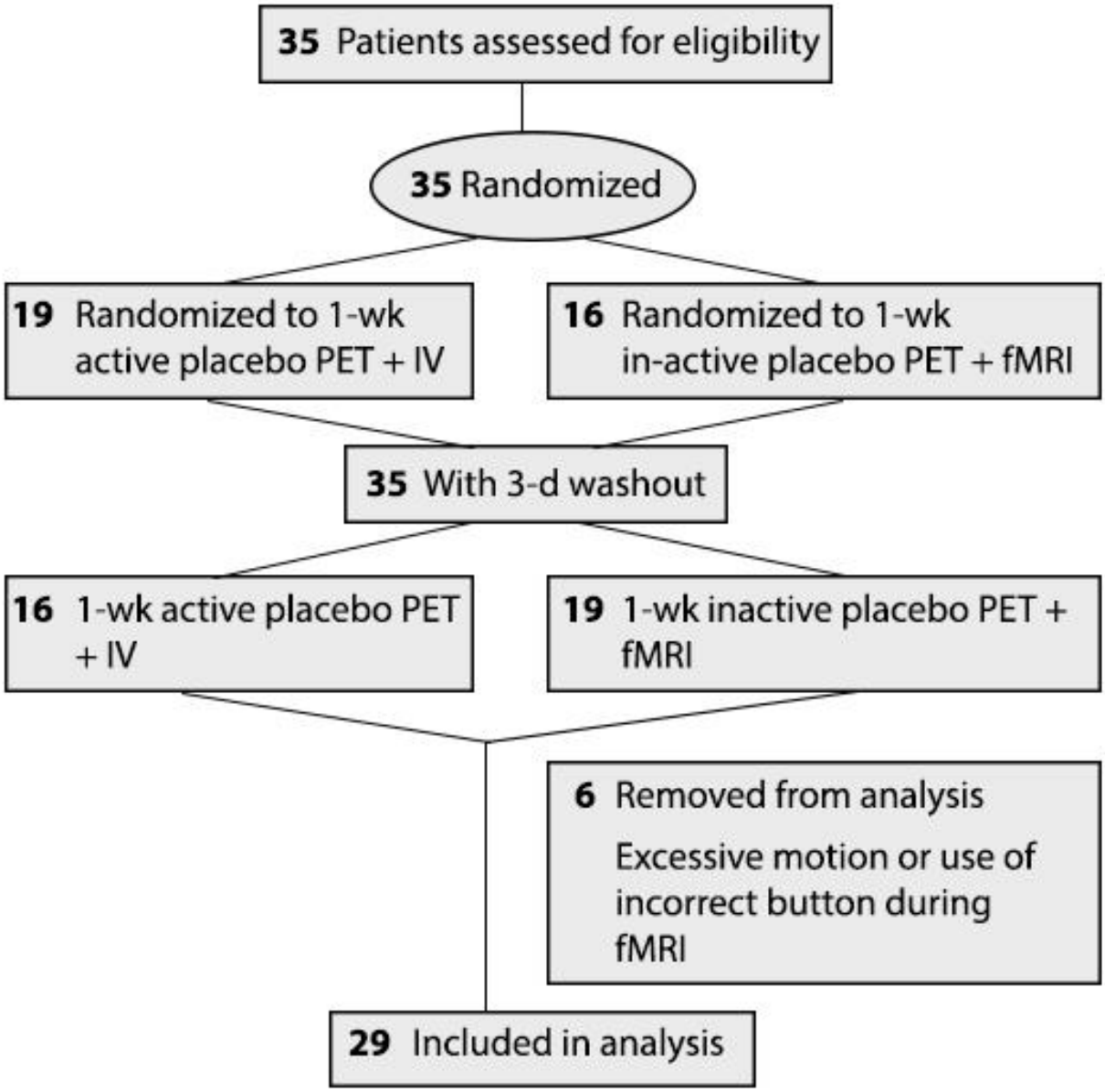


Figure 2.1. **Study design.** Participants were randomized into a 1-week active or inactive oral placebo treatment. Following a three-day washout period, participants crossed over into the other group. Following each treatment period, participants underwent a PET scanning session. Following the active oral placebo treatment, participants received a placebo IV during the PET scan. Following the inactive oral placebo treatment, participants additionally underwent an fMRI scanning session.

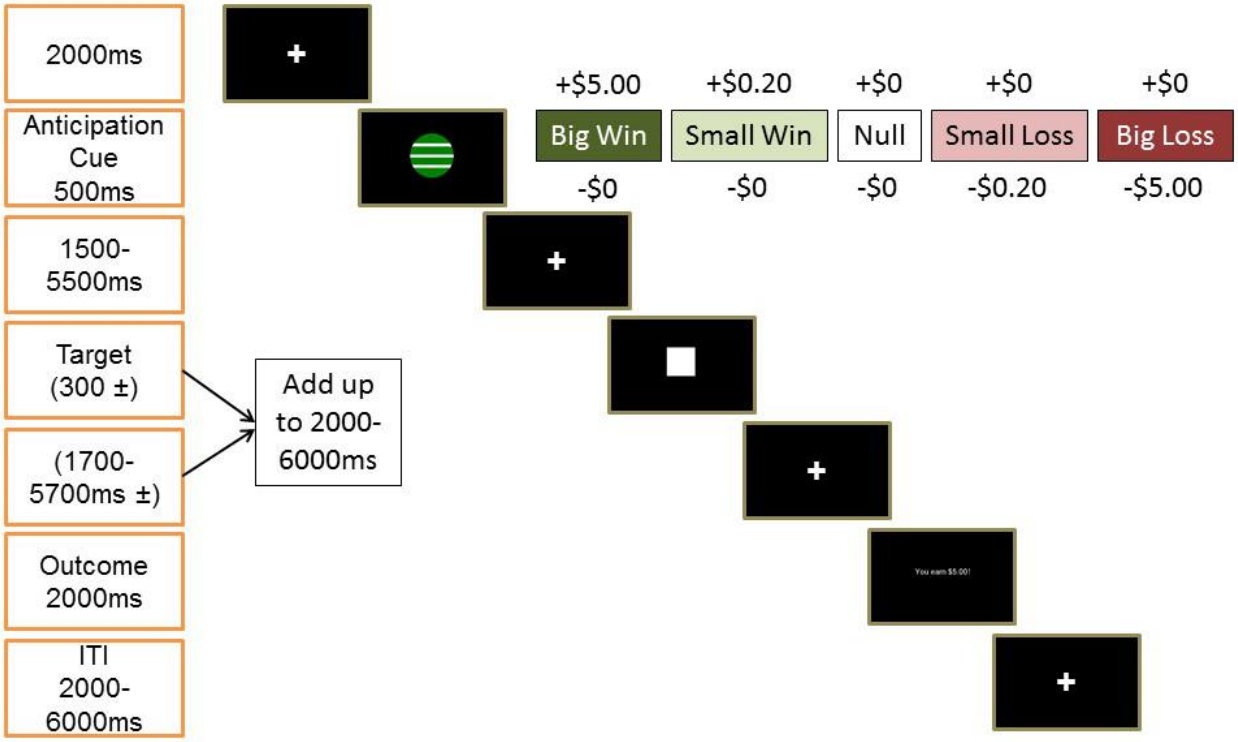


Figure 2.2. **Monetary Incentive Delay task design.** The MID is a reaction-time task probing reward function. Participants receive a cue indicating condition-type (potential win, potential loss, or null). Following a fixation cross, a target appears. The participant makes a button-press as quickly as possible in the response to the target, and subsequently receives feedback describing the outcome of the trial (successful or unsuccessful).

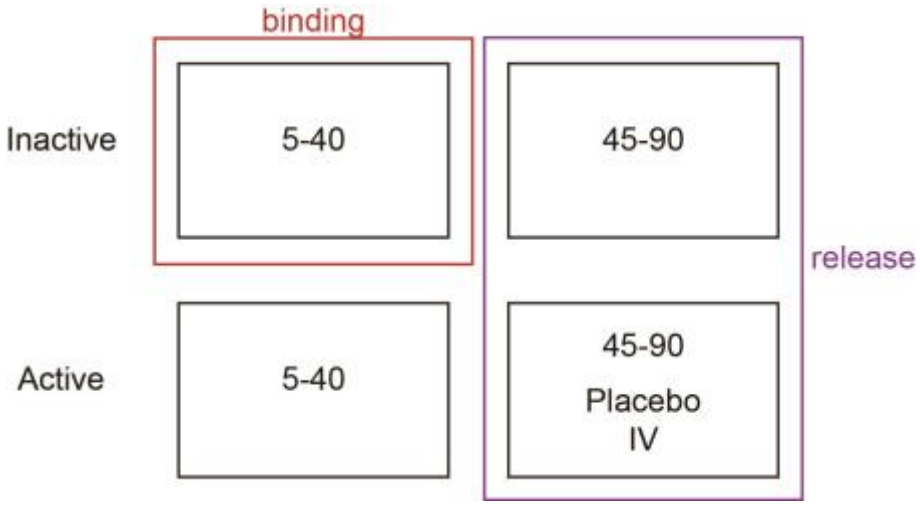


Figure 2.3. **PET measures of binding and release.** In both the inactive and active placebo conditions, and for both [^{11}C]raclopride and [^{11}C]carfentanil, participants underwent two periods of PET scanning. During the 45 minute – 90 minute session in the active condition, investigators introduced a placebo-challenge, meant to elicit dopamine and endogenous opioid release. Measures of receptor availability, or radiotracer "binding", were taken from the baseline 5 minute – 40 minute scan from the inactive condition. Measures of "release", or the change in binding in response to a challenge, were calculated by subtracting the image of the 45 minute – 90 minute scan during active condition (which included placebo IV) from the image of the 45 minute – 90 minute scan during inactive condition.

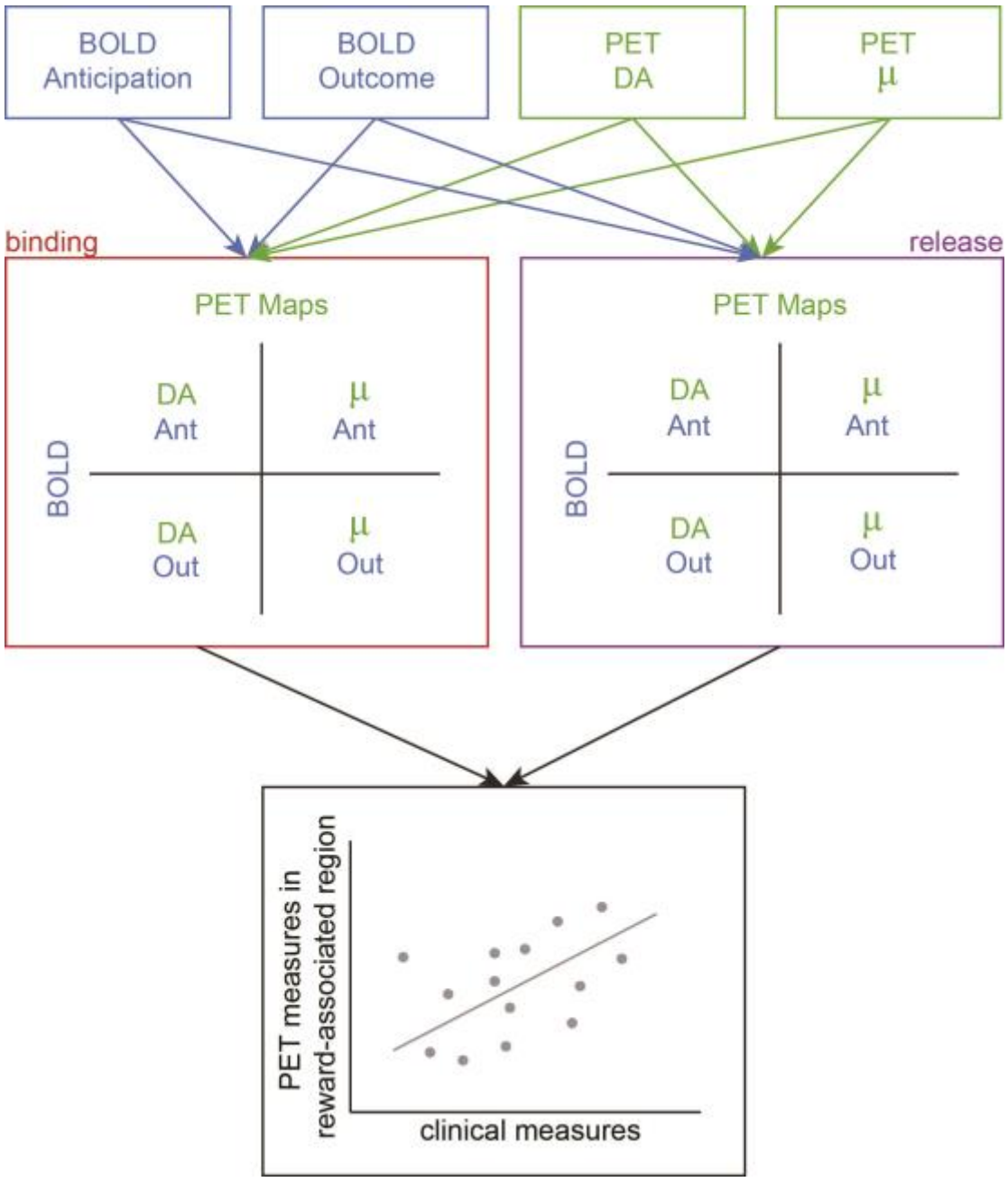


Figure 2.4. **Work flow of analyses for Chapter 2.** We collected striatal BOLD response to reward anticipation and hedonics, as well as dopamine and opioid binding and release. Extracted striatal anticipation and hedonic signals from the fMRI analyses were used as covariates in a voxelwise regression with PET binding and release maps. In significant clusters from those analyses, PET signal was extracted and correlated with clinical measures of reward disruption (AES and SHAPS).

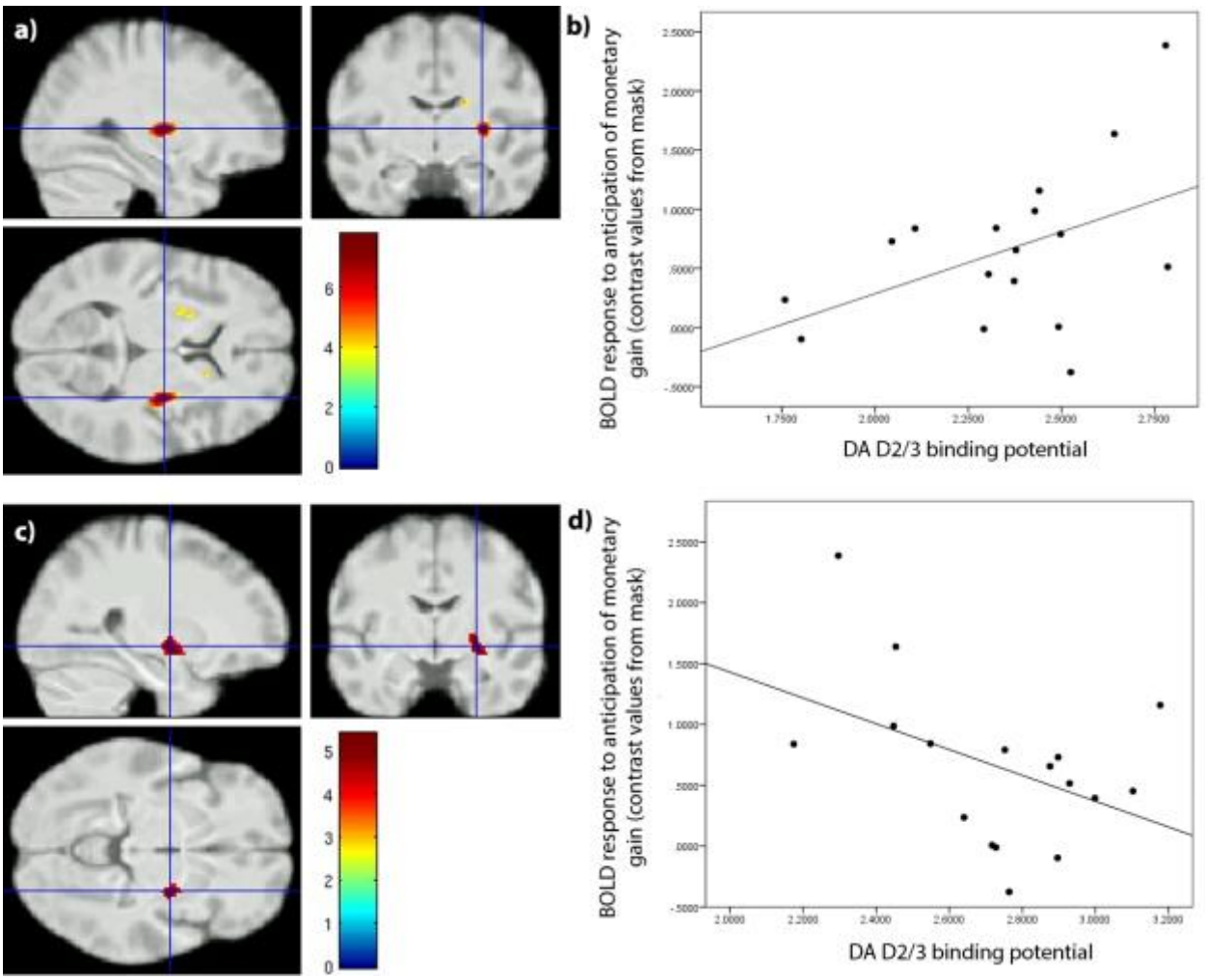


Figure 2.5. **Correlation between anticipatory responses to monetary reward and D2/D3 receptor availability.** a) BOLD response to anticipation of monetary gain was positively correlated with DA D2/3 receptor availability in bilateral putamen (Right putamen: 30, -10, 6; z = 4.86, k = 136; left putamen: -24, 0, 10; z = 4.08, k = 49), and right caudate (16, 12, 16; z = 4.25, k = 138) (n = 17). Map is cluster-wise FWE-corrected at cluster-defining threshold p < 0.001. Color bar reflects T-values. b) Graph of post-hoc correlation between BOLD response to anticipation of monetary gain and DA D2/3 receptor availability averaged across significant clusters (ρ = .355). c) BOLD response to anticipation of monetary gain was negatively correlated with DA D2/3 receptor availability in right globus pallidus (26 -6 -6; z = 3.97; k = 115). Map is cluster-wise FWE-corrected at cluster-defining threshold p < 0.001. Color bar reflects T-values. d) Graph of post-hoc correlation between BOLD response to anticipation of monetary gain and DA D2/3 receptor availability in right globus pallidus cluster (ρ = -.360).

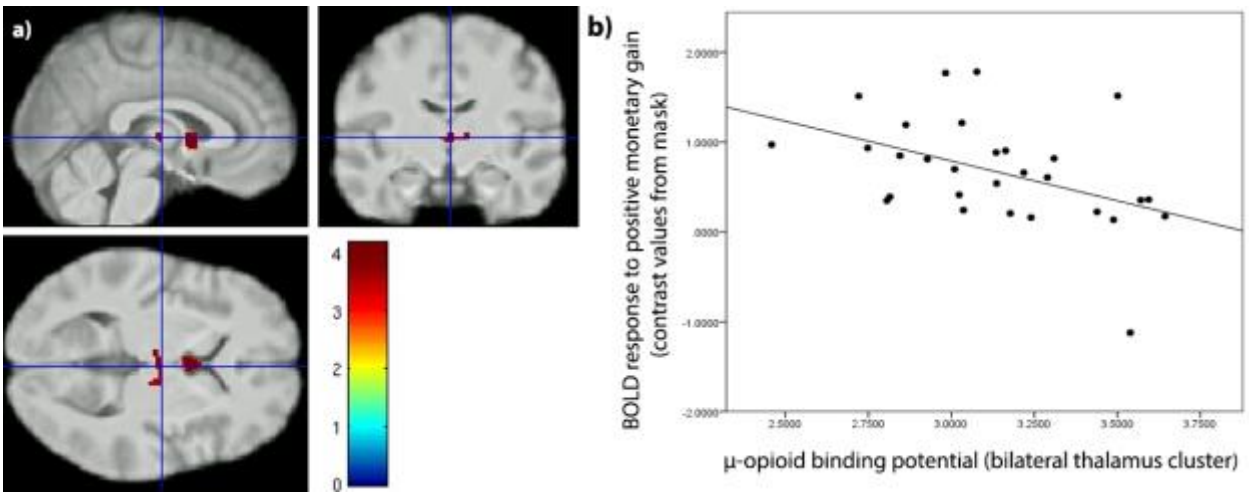


Figure 2.6. **Correlation between response to successful monetary outcome and μ-opioid receptor availability**. a) BOLD response to successful monetary outcome is negatively correlated with μ-opioid receptor availability in bilateral medial thalamus (Left thalamus: -8, -16, 6; z = 4.37; right thalamus: 12 -26 4, z = 4.10; k = 588; n = 29). Map is thresholded at $p < 0.001$ uncorrected. Color bar reflects T-values. b) Graph of post-hoc correlation between BOLD response to successful monetary outcome and μ-opioid receptor availability in the bilateral thalamus clusters (r = -.447).

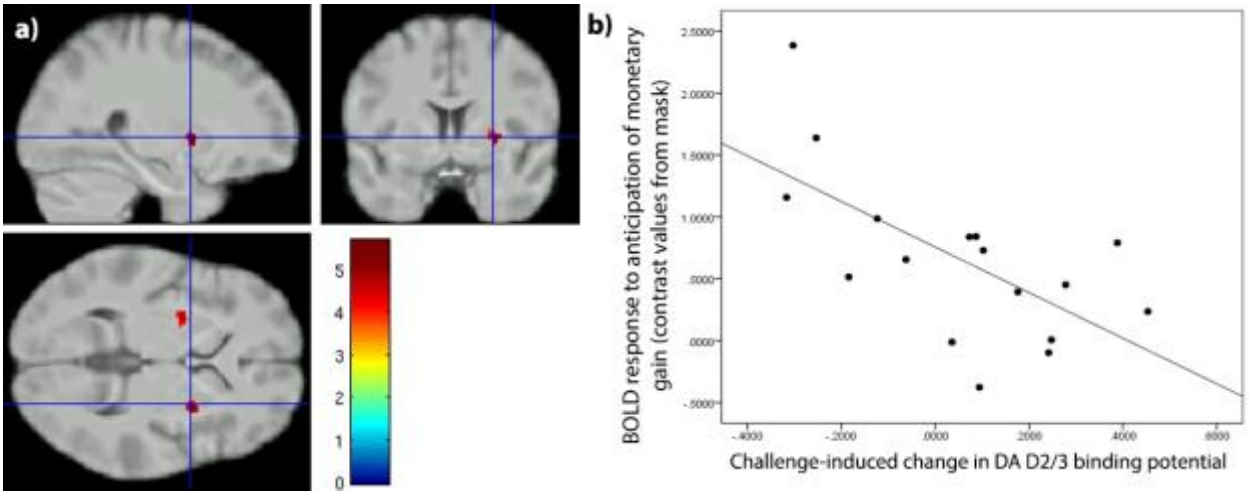


Figure 2.7. **Correlation between anticipation of monetary gain and challenge-induced change in DA D2/3 receptor availability**. a) BOLD response to anticipation of monetary gain is negatively correlated with challenge-induced change in DA D2/3 receptor availability in bilateral putamen (Left putamen: -26, -2, 0; z = 3.90; k = 50; right putamen: 26, 4, 2, z = 4.09; k = 35; n = 17). Map is cluster-wise FWE-corrected at cluster-defining threshold $p < 0.001$. Color bar reflects T-values. b) Graph of post-hoc correlation between BOLD response to successful monetary outcome and challenge-induced change in DA D2/3 receptor availability in the bilateral putamen (ρ = -.613).

Region[a]	*H*[b]	*MNI*[c]	*Cluster size*[d]	*Z*[e]	ρ[f]
A. Anticipation of monetary gain					
DA D2/3 BP_{ND}: Positive correlation					
Putamen	R	30 -10 6	**1088**	4.86	.355
	L	-26 -14 6	**1104**	4.08	
Caudate	R	16 12 16	**392**	4.25	
DA D2/3 BP_{ND}: Negative correlation					
Globus pallidus	R	26 -6 -6	**920**	3.97	-.360
DA D2/3 ΔBP_{ND}: Negative correlation					
Putamen	R	26 4 2	**280**	4.09	-.613
	L	-26 -2 0	**400**	3.90	
Region[a]	*H*[b]	*MNI*[c]	*Cluster size*[d]	*Z*[e]	r[g]
B. Successful monetary outcome					
MOR BP_{ND}: Negative correlation					
Thalamus	L	2 -12 4	520	3.36	-.447

[a] Anatomical region subjectively labeled following consultation of AAL, ICBM, and TD atlases

[b] H: hemisphere

[c] Montreal Neurological Institute (MNI) coordinates of peak voxel (mm)

[d] Cluster size in mm^3; bolded values indicates cluster size surpasses threshold to survive multiple comparison correction

[e] Two-sided voxel-level Z score at peak voxel

[f] Post-hoc: Spearman's rank correlation coefficient, using average signal across clusters

[g] Post-hoc: Pearson correlation coefficient

Table 2.1. **Summary of BOLD contrast correlations with PET binding potential.** A. BOLD response to anticipation of monetary gain was correlated with DA D2/3 binding potential and challenge-induced changes in DA D2/3 binding potential. B. BOLD response to successful monetary outcome was correlated with MOR binding potential. NOTE: One peak is listed per cluster.

Works Cited

Berrendero, F., Kieffer, B. L., & Maldonado, R. (2002). Attenuation of Nicotine-Induced Antinociception, Rewarding Effects, and Dependence in μ-Opioid Receptor Knock-Out Mice. *The Journal of Neuroscience*, *22*(24), 10935–10940.

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Research Reviews*, *28*(3), 309–369. https://doi.org/10.1016/S0165-0173(98)00019-8

Berridge, K. C., & Valenstein, E. S. (1991). What psychological process mediates feeding evoked by electrical stimulation of the lateral hypothalamus? *Behavioral Neuroscience*, *105*(1), 3–14. https://doi.org/10.1037/0735-7044.105.1.3

Cannon, D. M., Klaver, J. M., Peck, S. A., Rallis-Voak, D., Erickson, K., & Drevets, W. C. (2008). Dopamine Type-1 Receptor Binding in Major Depressive Disorder Assessed Using Positron Emission Tomography and [11C]NNC-112. *Neuropsychopharmacology*, *34*(5), 1277–1287. https://doi.org/10.1038/npp.2008.194

Cléry-Melin, M.-L., Schmidt, L., Lafargue, G., Baup, N., Fossati, P., & Pessiglione, M. (2011). Why Don't You Try Harder? An Investigation of Effort Production in Major Depression. *PLOS ONE*, *6*(8), e23178. https://doi.org/10.1371/journal.pone.0023178

Contarino, A., Picetti, R., Matthes, H. W., Koob, G. F., Kieffer, B. L., & Gold, L. H. (2002). Lack of reward and locomotor stimulation induced by heroin in μ-opioid receptor-deficient mice. *European Journal of Pharmacology*, *446*(1–3), 103–109. https://doi.org/10.1016/S0014-2999(02)01812-5

Cox, R. W. (1996). AFNI: Software for Analysis and Visualization of Functional Magnetic Resonance Neuroimages. *Computers and Biomedical Research*, *29*(3), 162–173. https://doi.org/10.1006/cbmr.1996.0014

Ehrich, E., Turncliff, R., Du, Y., Leigh-Pemberton, R., Fernandez, E., Jones, R., & Fava, M. (2015). Evaluation of Opioid Modulation in Major Depressive Disorder. *Neuropsychopharmacology*, *40*(6), 1448–1455. https://doi.org/10.1038/npp.2014.330

Forbes, E. E., Hariri, A. R., Martin, S. L., Silk, J. S., Moyles, D. L., Fisher, P. M., … Dahl, R. E. (2009). Altered Striatal Activation Predicting Real-World Positive Affect in Adolescent Major Depressive Disorder. *American Journal of Psychiatry*, *166*(1), 64–73. https://doi.org/10.1176/appi.ajp.2008.07081336

Forbes, E. E., Ryan, N. D., Phillips, M. L., Manuck, S. B., Worthman, C. M., Moyles, D. L., … Dahl, R. E. (2010). Healthy Adolescents' Neural Response to Reward: Associations with Puberty, Positive Affect, and Depressive Symptoms. *Journal of the American Academy of Child and Adolescent Psychiatry*, *49*(2), 162–172e5.

Furukawa, E., Bado, P., Tripp, G., Mattos, P., Wickens, J. R., Bramati, I. E., … Moll, J. (2014). Abnormal Striatal BOLD Responses to Reward Anticipation and Reward Delivery in ADHD. *PLOS ONE*, *9*(2), e89129. https://doi.org/10.1371/journal.pone.0089129

Ghozland, S., Matthes, H. W. D., Simonin, F., Filliol, D., Kieffer, B. L., & Maldonado, R. (2002). Motivational Effects of Cannabinoids Are Mediated by μ-Opioid and κ-Opioid Receptors. *The Journal of Neuroscience*, *22*(3), 1146–1154.

Haber, S. N. (2011). Neuroanatomy of Reward: A View from the Ventral Striatum. In J. A. Gottfried (Ed.), *Neurobiology of Sensation and Reward*. Boca Raton (FL): CRC Press/Taylor & Francis. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK92777/

Haber, S. N., & Calzavara, R. (2009). The cortico-basal ganglia integrative network: The role of the thalamus. *Brain Research Bulletin*, *78*(2–3), 69–74. https://doi.org/10.1016/j.brainresbull.2008.09.013

Hamilton, M. (1960). A RATING SCALE FOR DEPRESSION. *Journal of Neurology, Neurosurgery, and Psychiatry*, *23*(1), 56.

Hasler, G., Luckenbaugh, D. A., Snow, J., Meyers, N., Waldeck, T., Geraci, M., … Drevets, W. C. (2009). Reward Processing After Catecholamine Depletion in Unmedicated, Remitted Subjects with Major Depressive Disorder. *Biological Psychiatry*, *66*(3), 201–205. https://doi.org/10.1016/j.biopsych.2009.02.029

Hoogendam, J. M., Kahn, R. S., Hillegers, M. H. J., van Buuren, M., & Vink, M. (2013). Different developmental trajectories for anticipation and receipt of reward during adolescence. *Developmental Cognitive Neuroscience*, *6*, 113–124. https://doi.org/10.1016/j.dcn.2013.08.004

Jewett, D. M. (2001). A simple synthesis of [11C]carfentanil using an extraction disk instead of HPLC. *Nuclear Medicine and Biology*, *28*(6), 733–734. https://doi.org/10.1016/S0969-8051(01)00226-8

Kennedy SE, Koeppe RA, Young EA, & Zubieta J. (2006). Dysregulation of endogenous opioid emotion regulation circuitry in major depression in women. *Archives of General Psychiatry*, *63*(11), 1199–1208. https://doi.org/10.1001/archpsyc.63.11.1199

Knutson, B., Westdorp, A., Kaiser, E., & Hommer, D. (2000). FMRI Visualization of Brain Activity during a Monetary Incentive Delay Task. *NeuroImage*, *12*(1), 20–27. https://doi.org/10.1006/nimg.2000.0593

Lambert G, Johansson M, Ågren H, & Friberg P. (2000). Reduced brain norepinephrine and dopamine release in treatment-refractory depressive illness: Evidence in support of the catecholamine hypothesis of mood disorders. *Archives of General Psychiatry*, *57*(8), 787–793. https://doi.org/10.1001/archpsyc.57.8.787

Leeuw, M. de, Kahn, R. S., & Vink, M. (2015). Fronto-striatal Dysfunction During Reward Processing in Unaffected Siblings of Schizophrenia Patients. *Schizophrenia Bulletin*, *41*(1), 94–103. https://doi.org/10.1093/schbul/sbu153

Leyton, M., Boileau, I., Benkelfat, C., Diksic, M., Baker, G., & Dagher, A. (2002). Amphetamine-Induced Increases in Extracellular Dopamine, Drug Wanting, and Novelty Seeking: A PET/[11C]Raclopride Study in Healthy Men. *Neuropsychopharmacology*, *27*(6), 1027–1035. https://doi.org/10.1016/S0893-133X(02)00366-4

Liberzon, I., Zubieta, J. K., Fig, L. M., Phan, K. L., Koeppe, R. A., & Taylor, S. F. (2002). μ-Opioid receptors and limbic responses to aversive emotional stimuli. *Proceedings of the National Academy of Sciences*, *99*(10), 7084–7089. https://doi.org/10.1073/pnas.102174799

Logan, J., Fowler, J. S., Volkow, N. D., Wang, G.-J., Ding, Y.-S., & Alexoff, D. L. (1996). Distribution Volume Ratios without Blood Sampling from Graphical Analysis of PET Data. *Journal of Cerebral Blood Flow & Metabolism*, *16*(5), 834–840. https://doi.org/10.1097/00004647-199609000-00008

Logothetis, N. K., Pauls, J., Augath, M., Trinath, T., & Oeltermann, A. (2001). Neurophysiological investigation of the basis of the fMRI signal. *Nature*, *412*(6843), 150–157. https://doi.org/10.1038/35084005

Marin, R. (1996). Apathy: Concept, Syndrome, Neural Mechanisms, and Treatment. *Seminars in Clinical Neuropsychiatry*, *1*(4), 304–314. https://doi.org/10.1053/SCNP00100304

McFarland, B. R., & Klein, D. N. (2009). Emotional reactivity in depression: diminished responsiveness to anticipated reward but not to anticipated punishment or to nonreward or avoidance. *Depression and Anxiety*, *26*(2), 117–122. https://doi.org/10.1002/da.20513

Meyer, J. H., McNeely, H. E., Sagrati, S., Boovariwala, A., Martin, K., Verhoeff, N. P. L. G., … Houle, S. (2006). Elevated Putamen D 2 Receptor Binding Potential in Major Depression With Motor Retardation: An [11 C]Raclopride Positron Emission Tomography Study. *American Journal of Psychiatry*, *163*(9), 1594–1602. https://doi.org/10.1176/ajp.2006.163.9.1594

Narendran, R., & Martinez, D. (2008). Cocaine abuse and sensitization of striatal dopamine transmission: a critical review of the preclinical and clinical imaging literature. *Synapse (New York, N.Y.)*, *62*(11), 851–869. https://doi.org/10.1002/syn.20566

Peciña, S., & Berridge, K. C. (2005). Hedonic Hot Spot in Nucleus Accumbens Shell: Where Do μ-Opioids Cause Increased Hedonic Impact of Sweetness? *The Journal of Neuroscience*, *25*(50), 11777–11786. https://doi.org/10.1523/JNEUROSCI.2329-05.2005

Peciña, S., Berridge, K. C., & Parker, L. A. (1997). Pimozide Does Not Shift Palatability: Separation of Anhedonia from Sensorimotor Suppression by Taste Reactivity. *Pharmacology Biochemistry and Behavior*, *58*(3), 801–811. https://doi.org/10.1016/S0091-3057(97)00044-0

Peciña, S., Cagniard, B., Berridge, K. C., Aldridge, J. W., & Zhuang, X. (2003). Hyperdopaminergic Mutant Mice Have Higher "Wanting" But Not "Liking" for Sweet Rewards. *The Journal of Neuroscience*, *23*(28), 9395–9402.

Peciña M, Bohnert AB, Sikora M, & et al. (2015). Association between placebo-activated neural systems and antidepressant responses: Neurochemistry of placebo effects in major depression. *JAMA Psychiatry*, *72*(11), 1087–1094. https://doi.org/10.1001/jamapsychiatry.2015.1335

Pizzagalli, D. A., Iosifescu, D., Hallett, L. A., Ratner, K. G., & Fava, M. (2008). Reduced hedonic capacity in major depressive disorder: Evidence from a probabilistic reward task. *Journal of Psychiatric Research*, *43*(1), 76–87. https://doi.org/10.1016/j.jpsychires.2008.03.001

Roberts, A. J., McDonald, J. S., Heyser, C. J., Kieffer, B. L., Matthes, H. W. D., Koob, G. F., & Gold, L. H. (2000). μ-Opioid Receptor Knockout Mice Do Not Self-Administer Alcohol ,. *Journal of Pharmacology and Experimental Therapeutics*, *293*(3), 1002–1008.

Savitz, J. B., & Drevets, W. C. (2013). Neuroreceptor imaging in depression. *Neurobiology of Disease*, *52*, 49–65. https://doi.org/10.1016/j.nbd.2012.06.001

Schott, B. H., Minuzzi, L., Krebs, R. M., Elmenhorst, D., Lang, M., Winz, O. H., … Bauer, A. (2008). Mesolimbic Functional Magnetic Resonance Imaging Activations during Reward Anticipation Correlate with Reward-Related Ventral Striatal Dopamine Release. *The Journal of Neuroscience*, *28*(52), 14311–14319. https://doi.org/10.1523/JNEUROSCI.2058-08.2008

Scott, D. J., Stohler, C. S., Egnatuk, C. M., Wang, H., Koeppe, R. A., & Zubieta, J.-K. (2007). Individual Differences in Reward Responding Explain Placebo-Induced Expectations and Effects. *Neuron*, *55*(2), 325–336. https://doi.org/10.1016/j.neuron.2007.06.028

Smith, K. S., & Berridge, K. C. (2005). The Ventral Pallidum and Hedonic Reward: Neurochemical Maps of Sucrose "Liking" and Food Intake. *The Journal of Neuroscience*, *25*(38), 8637–8649. https://doi.org/10.1523/JNEUROSCI.1902-05.2005

Smoski, M. J., Felder, J., Bizzell, J., Green, S. R., Ernst, M., Lynch, T. R., & Dichter, G. S. (2009). fMRI of alterations in reward selection, anticipation, and feedback in major depressive disorder. *Journal of Affective Disorders*, *118*(1–3), 69–78. https://doi.org/10.1016/j.jad.2009.01.034

Snaith, R. P., Hamilton, M., Morley, S., Humayan, A., Hargreaves, D., & Trigwell, P. (1995). A scale for the assessment of hedonic tone the Snaith-Hamilton Pleasure Scale. *The British Journal of Psychiatry*, *167*(1), 99–103. https://doi.org/10.1192/bjp.167.1.99

Tindell, A. J., Berridge, K. C., Zhang, J., Peciña, S., & Aldridge, J. W. (2005). Ventral pallidal neurons code incentive motivation: amplification by mesolimbic sensitization and amphetamine. *European Journal of Neuroscience*, *22*(10), 2617–2634. https://doi.org/10.1111/j.1460-9568.2005.04411.x

Treadway, M. T., Buckholtz, J. W., Schwartzman, A. N., Lambert, W. E., & Zald, D. H. (2009). Worth the "EEfRT"? The Effort Expenditure for Rewards Task as an Objective Measure of Motivation and Anhedonia. *PLOS ONE*, *4*(8), e6598. https://doi.org/10.1371/journal.pone.0006598

Treadway, M. T., & Zald, D. H. (2011). Reconsidering anhedonia in depression: Lessons from translational neuroscience. *Neuroscience & Biobehavioral Reviews*, *35*(3), 537–555. https://doi.org/10.1016/j.neubiorev.2010.06.006

Tremblay LK, Naranjo CA, Graham SJ, & et al. (2005). Functional neuroanatomical substrates of altered reward processing in major depressive disorder revealed by a dopaminergic probe. *Archives of General Psychiatry*, *62*(11), 1228–1236. https://doi.org/10.1001/archpsyc.62.11.1228

Urban, N. B. L., Slifstein, M., Meda, S., Xu, X., Ayoub, R., Medina, O., … Abi-Dargham, A. (2011). Imaging human reward processing with positron emission tomography and functional magnetic resonance imaging. *Psychopharmacology*, *221*(1), 67–77. https://doi.org/10.1007/s00213-011-2543-6

Volkow, N. D., Wang, G.-J., Fowler, J. S., Logan, J., Jayne, M., Franceschi, D., … Pappas, N. (2002). "Nonhedonic" food motivation in humans involves dopamine in the dorsal striatum and methylphenidate amplifies this effect. *Synapse*, *44*(3), 175–180. https://doi.org/10.1002/syn.10075

Weiland, B. J., Heitzeg, M. M., Zald, D., Cummiford, C., Love, T., Zucker, R. A., & Zubieta, J.-K. (2014). Relationship between impulsivity, prefrontal anticipatory activation, and striatal dopamine release during rewarded task performance. *Psychiatry Research: Neuroimaging*, *223*(3), 244–252. https://doi.org/10.1016/j.pscychresns.2014.05.015

Weiland, B. J., Zucker, R. A., Zubieta, J.-K., & Heitzeg, M. M. (2016). Striatal dopaminergic reward response relates to age of first drunkenness and feedback response in at-risk youth. *Addiction Biology*, n/a-n/a. https://doi.org/10.1111/adb.12341

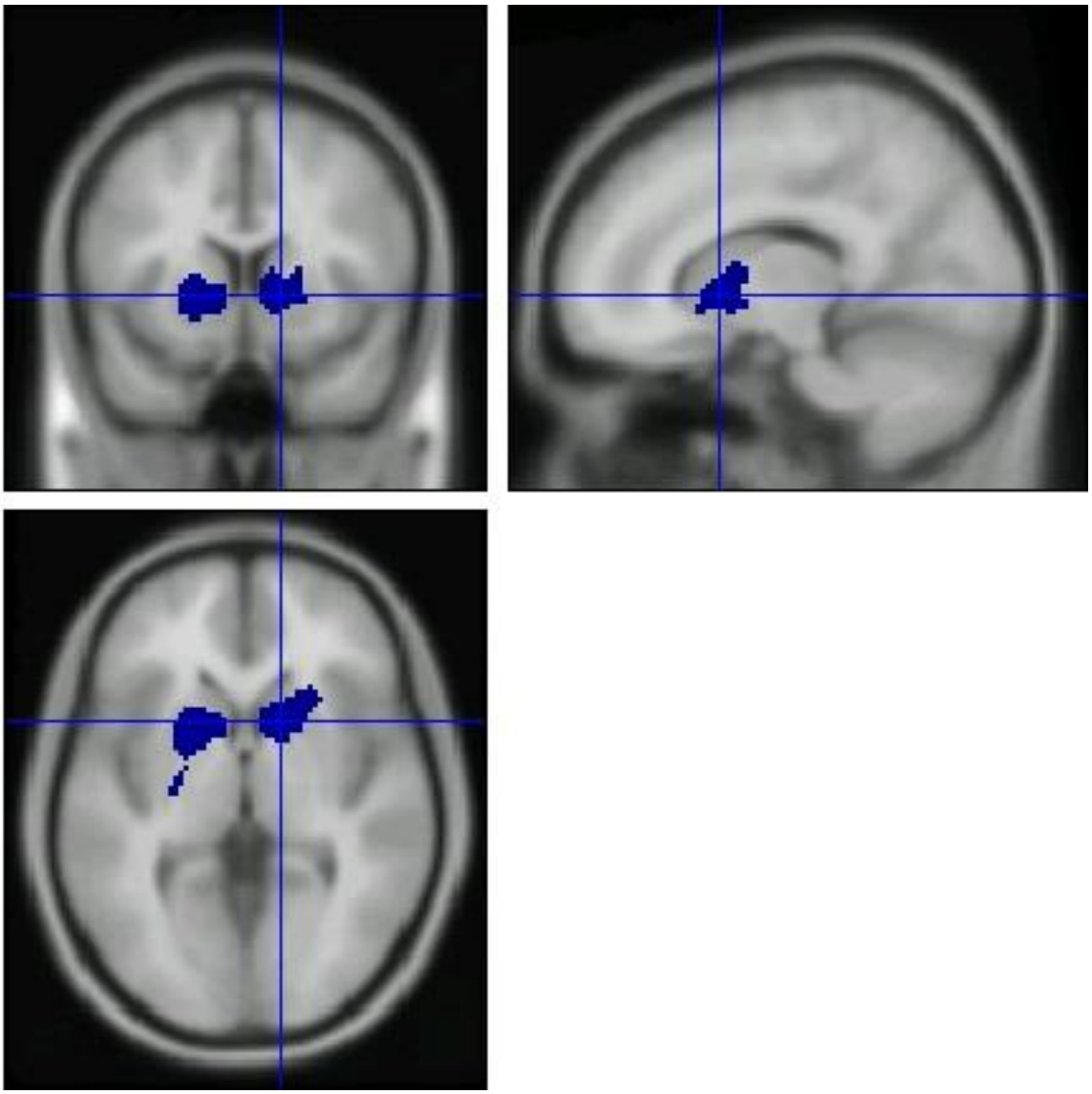
Wyvell, C. L., & Berridge, K. C. (2000). Intra-Accumbens Amphetamine Increases the Conditioned Incentive Salience of Sucrose Reward: Enhancement of Reward "Wanting" without Enhanced "Liking" or Response Reinforcement. *The Journal of Neuroscience*, *20*(21), 8122–8130.

Yeomans, M. R., & Gray, R. W. (1997). Effects of Naltrexone on Food Intake and Changes in Subjective Appetite During Eating: Evidence for Opioid Involvement in the Appetizer Effect. *Physiology & Behavior*, *62*(1), 15–21. https://doi.org/10.1016/S0031-9384(97)00101-7

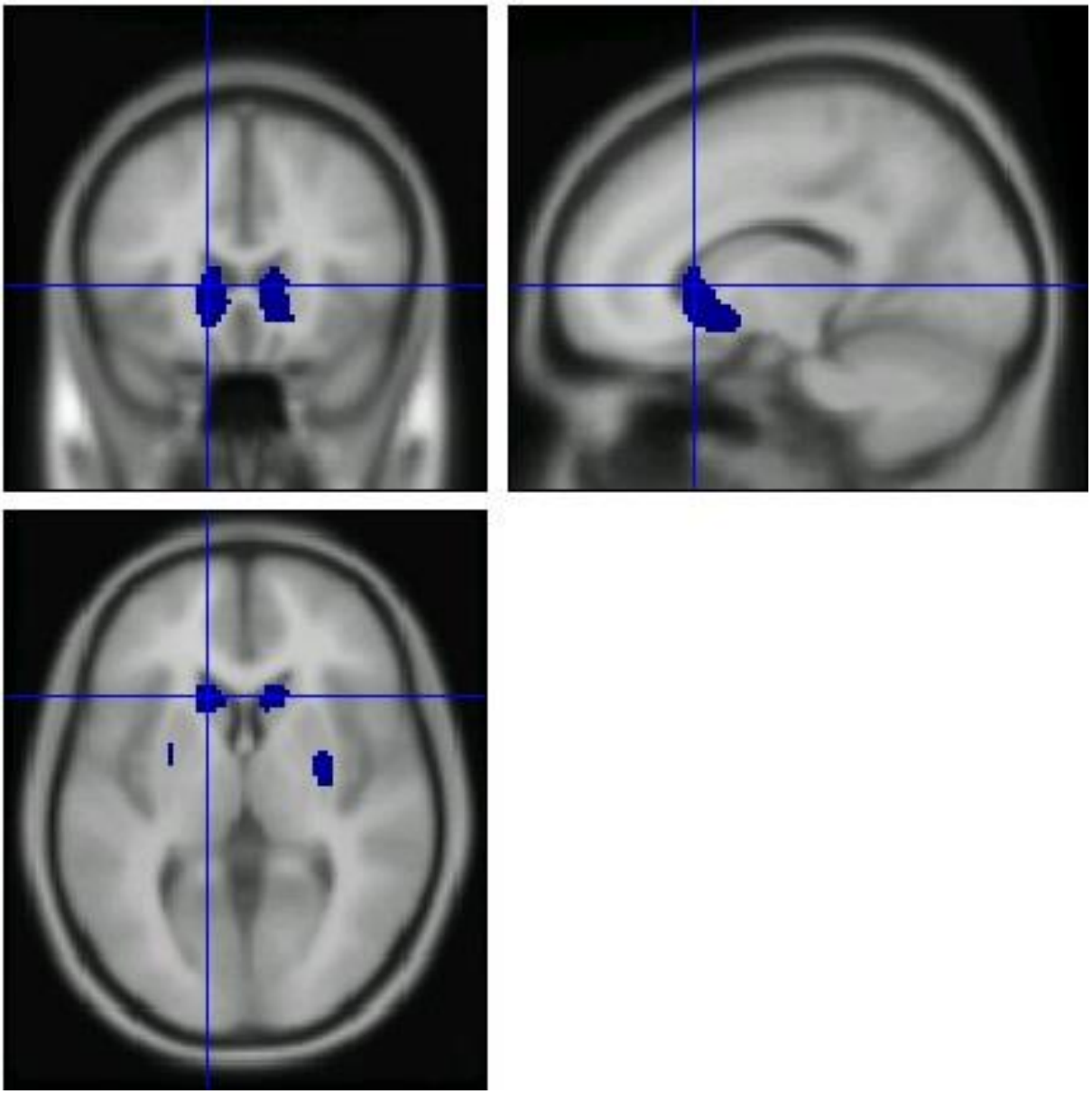
Zhang, W.-N., Chang, S.-H., Guo, L.-Y., Zhang, K.-L., & Wang, J. (2013). The neural correlates of reward-related processing in major depressive disorder: A meta-analysis of functional magnetic resonance imaging studies. *Journal of Affective Disorders*, *151*(2), 531–539. https://doi.org/10.1016/j.jad.2013.06.039

Zubieta J, Ketter TA, Bueller JA, & et al. (2003). Regulation of human affective responses by anterior cingulate and limbic μ-opioid neurotransmission. *Archives of General Psychiatry*, *60*(11), 1145–1153. https://doi.org/10.1001/archpsyc.60.11.1145

Appendix 2.1. Masks for Main Effect of Task.



Appendix Figure 2.8. **Anticipation of Monetary Gain.** A mask was constructed from the significant striatal Main Effect of Task clusters for the Anticipation of Monetary Gain > Neutral Anticipation contrast (cluster-wise FWE-corrected at cluster-defining threshold $p < 0.001$).



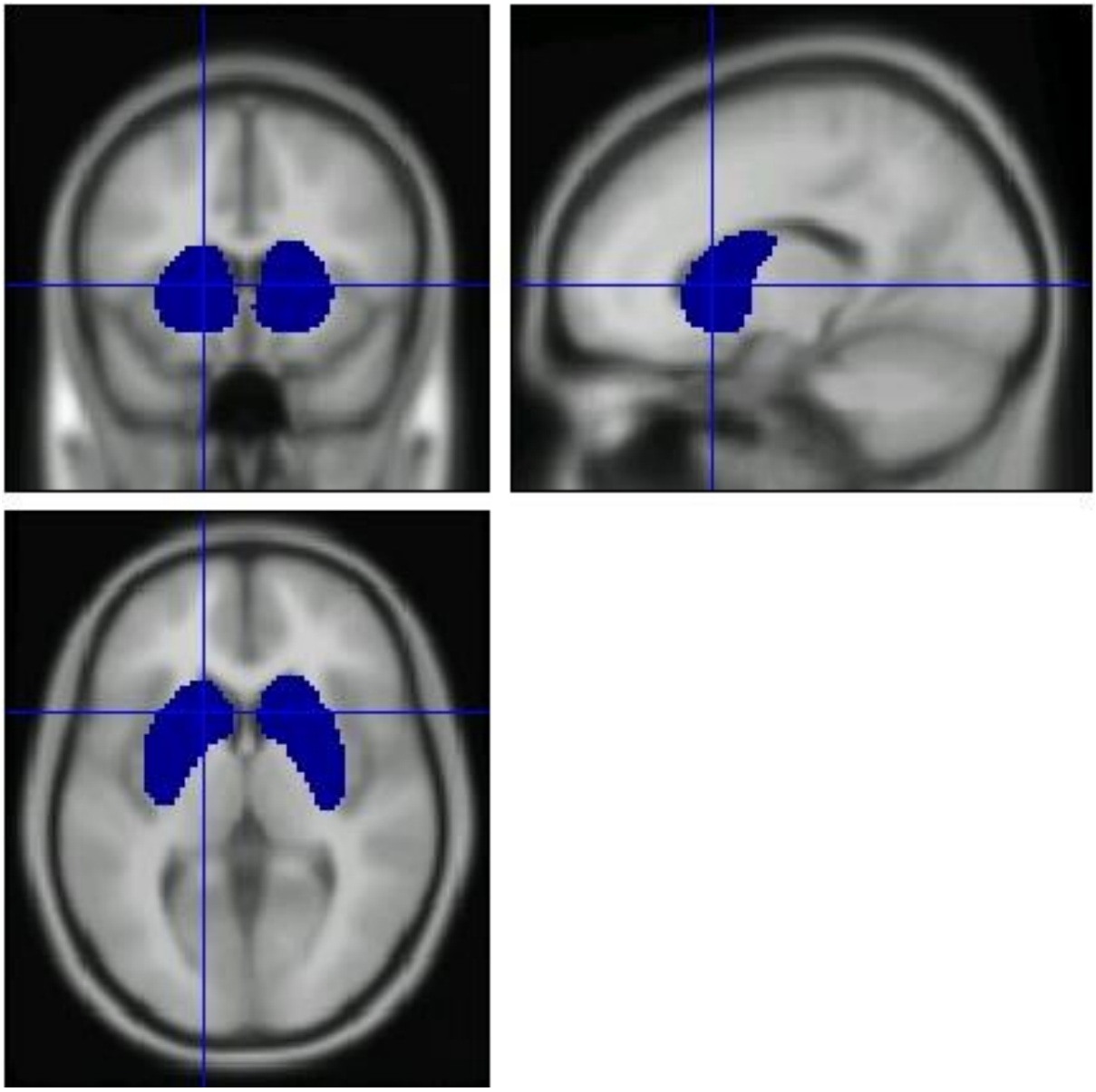
Appendix Figure 2.9. **Successful Monetary Outcome.** A mask was constructed from the significant striatal Main Effect of Task clusters for the Successsful Monetary Outcome > Unsuccessful Monetary Outcome contrast (cluster-wise FWE-corrected at cluster-defining threshold $p < 0.001$).

Appendix 2.2. Behavioral Task Data.

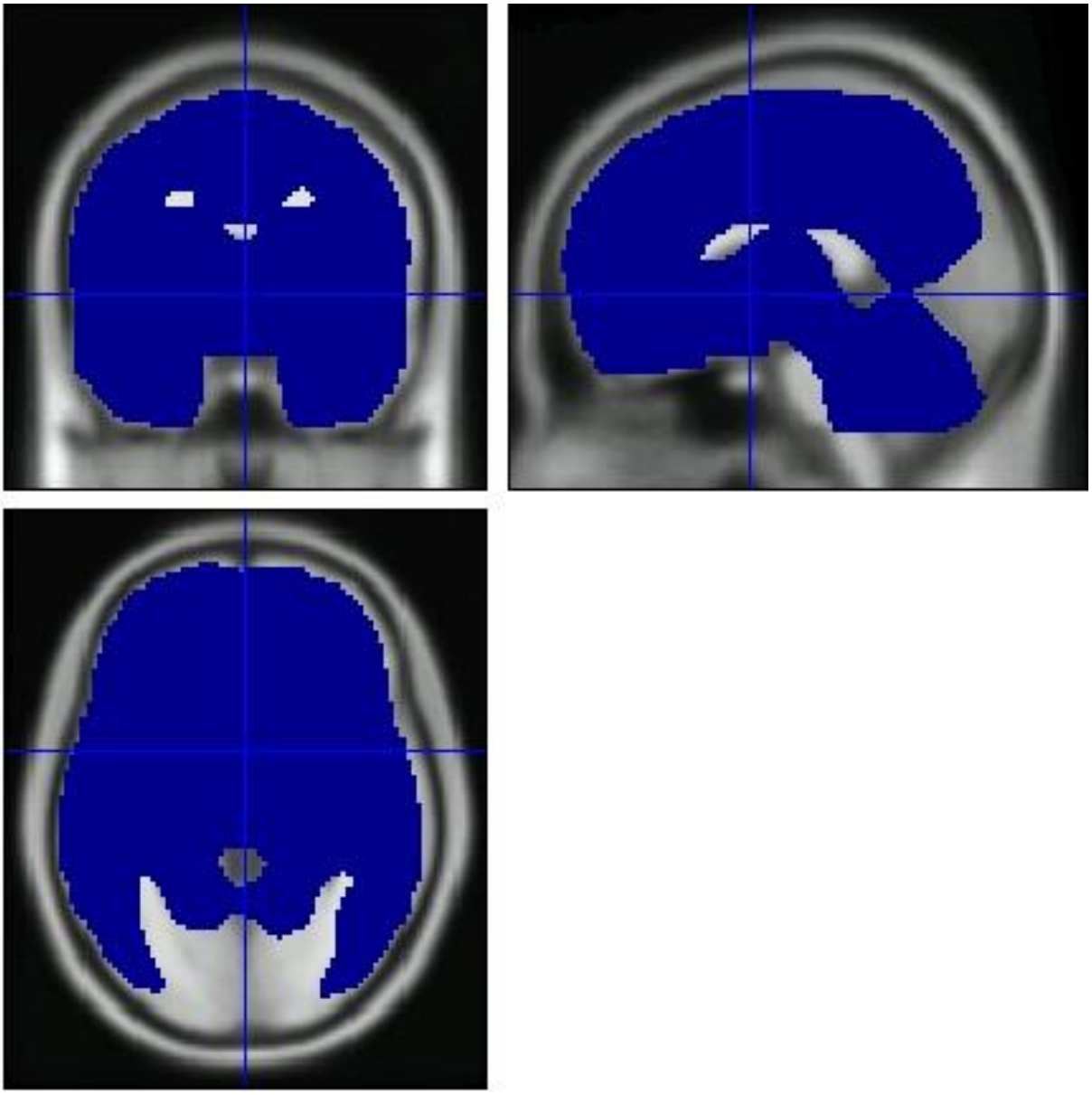
Behavioral measure	All trials	Gain trials	Null trials	Loss trials
Reaction time (ms)	217.7 ± 56.0	214.9 ± 60.9	234.2 ± 46.2	217.7 ± 53.7
Accuracy (% correct)	54.7 ± 7.8	56.7 ± 10.3	44.1% ± 18.4	58.0 ± 11.4

Appendix Table 2.2. **Behavioral Data for Monetary Incentive Delay task.** Median reaction time for successful responses and accuracy were measured during the Monetary Incentive Delay task. Results are presented as mean ± SD both collapsed across trial conditions (All trials), and for each trial condition.

Appendix 2.3. Masks of PET binding.



Appendix Figure 2.10. **Mask indicating regions of DA D2/3 binding.** A mask was constructed from group raclopride binding maps and encompasses the striatum. This mask was used when viewing results to examine only findings in the striatum.



Appendix Figure 2.11. **Mask indicating regions of μ-opioid receptor binding.** A mask was constructed from group carfentanil binding maps and encompasses whole brain, with the exception of occipital lobe and ventricles. While our hypotheses regarding μ-opioid receptor binding focused on the striatum, this mask was used in secondary analyses to investigate the relationship between BOLD reward response and μ-opioid receptor binding outside the striatum.

Chapter Three.
Anterior Cingulate Response to Reward Anticipation Predicts Improvement with Antidepressant Treatment

Introduction

Major depression is a prevalent and debilitating illness, and represents a significant social and economic burden on society. Characterized by depressed mood and a loss of pleasure, major depression affects an estimated 16.2% of adults in the United States at some point in their lifetimes (Kessler et al., 2003). The annual cost of MDD in the US is estimated at $83.1 billion, with nearly two thirds of this cost a result of functional disability (Greenberg et al., 2003). Much of this cost can be attributed to the long period of time it takes patients to recover from the illness in addition to the limited response to available treatments (Leuchter et al., 2009).

The search for an effective treatment is challenging for many affected individuals. It typically requires 1 year to identify a successful treatment (Keitner et al., 1992; Rush, 2007). Ineffective treatment carries a financial cost, in addition to side effects and the continued burden of the disease, making it difficult to maintain further compliance with treatment.

To address this issue, a growing body of research has focused on identifying predictors of antidepressant treatment response, primarily using functional neuroimaging techniques (Mayberg

et al., 1997; Arns et al., 2015; Langenecker et al., 2007; Chen et al., 2007). And yet the function of reward circuitry, believed to be a component of the pathophysiology of major depression, as a predictor of antidepressant treatment response remains understudied (Phillips et al., 2015).

Previous studies of incentive processing in individuals with major depression have found dysregulation in the underlying circuitry (Knutson et al., 2008; Pizzagalli et al., 2009). Associated neurotransmitter systems may also be affected: dopamine plays a critical role in experiencing the rewarding effects of incentives, and is proposed to be involved in disturbed incentive response in major depression (Nestler & Carlezon, 2006). However, while the literature of neurochemical dysregulation in depression has focused primarily on dopamine and its fellow monoamine neurotransmitters serotonin and norepinephrine, the opioid system represents an underexplored avenue for understanding the etiology and treatment of major depression. Endogenous μ-opioid tone is dysregulated in major depression (Kennedy et al., 2006), and treatment with μ-opioid agonists has been associated with significant mood improvement (Karp et al., 2014). Of particular interest are findings associating antidepressant treatment response with a μ-opioid receptor variant (Garriock et al., 2010) and pre-treatment μ-opioid tone in the nucleus accumbens (Peciña et al., 2015). The role of dopamine and opioid function in both incentive processing and major depression suggest that a multimodal approach, capturing both the function of reward circuitry and its associated neurotransmission, is needed to most effectively characterize incentive processing as a predictor of antidepressant treatment response.

To address this question, we measured BOLD response to two reward processes (incentive anticipation and successful monetary outcome), and dopamine and μ-opioid function via PET, prior to a ten-week antidepressant trial. We hypothesized that one or both of the following relationships would emerge: 1) striatal response to reward anticipation would correlate

with subsequent antidepressant treatment response, as well as striatal DA D2/3 receptor availability, and 2) striatal response to successful reward outcome would correlate with subsequent antidepressant treatment response, as well as striatal μ-opioid receptor availability.

Methods

Participants

Written informed consent was obtained from all participants. Procedures were approved by the University of Michigan Institutional Review Board for Human Subject Use and the Radioactive Drug Research Committee.

Thirty-five right-handed medication-free participants were recruited via advertisement, and diagnosed with DSM-V Major Depressive Disorder (MDD; 23 females, age range 18 – 59 years, mean ± SD: 32.09 ± 12.57) using the Mini International Neuropsychiatric Inventory (MINI) 6.0. Inclusion criteria included diagnosis of MDD using the MINI and Hamilton Rating Scale for Depression (HRSD; Hamilton, 1960) scores > 12. Exclusion criteria included suicidal ideation, comorbid medical, neurological or psychiatric conditions, pregnancy, or use of psychotropic agents.

Sixteen right-handed healthy control (HC) participants with no history of psychiatric illness (8 females, age range 20 – 47 years, mean ± SD: 30.25 ± 8.11) were recruited concurrently as part of a separate study, and screened using the MINI.

Study design

All participants performed a Monetary Incentive Delay task while functional magnetic resonance imaging (fMRI) data was collected. MDD participants also underwent a positron

emission tomographic (PET) scanning session. Following PET and fMRI scans, MDD participants began a 10-week open-label trial with the selective serotonin reuptake inhibitor citalopram (20-40 mg daily). Depressive symptoms were evaluated at weeks 0, 2, 4, 8 and 10 using the Quick Inventory of Depressive Symptomatology (QIDS-SR16) self-report measure (Rush et al, 2003). Twenty-two of the 35 MDD participants (62.9%) completed the 10-week trial, with partial follow-up data available for all 35 participants.

Monetary Incentive Delay Task

Participants performed a modified version of the MID task (Knutson et al., 2000) during fMRI acquisition, to measure changes in Blood-Oxygen-Level Dependent (BOLD) signal in response to reward anticipation and receipt. Each participant completed three runs, of thirty trials each, in which subjects made a button-response to a simple visual target during a brief response window.

At the beginning of each trial, participants saw a cue for 500ms, indicating the one of five trial conditions: potential large reward (+$5), potential small reward (+$0.20), potential large punishment (-$5), potential small punishment (-$0.20), or no money at risk (neutral). Eighteen trials of each condition were presented in pseudo-randomized order. An anticipation period of 1500-5500ms followed the cue. The target image then appeared briefly, cuing the participant to make a button response. The participant won money or avoided losing money if the button was pressed within the response window (~250 ms). The response window was dynamically varied over the course of the task, in response to participant reaction time. Target presentation was followed by a variable delay (2-6 s) and then feedback regarding the outcome of the trial (2 s). An inter-trial interval of 2-6 s separated each trial.

Participants' responses to the MID task were recorded in E-Prime 2.0. Behavioral responses were subsequently inspected to verify the correct response button was used. Trials in which an incorrect response button was used were discarded from analysis.

MRI acquisition and image processing.

MRI data were acquired on a Philips Ingenia 3.0-Tesla scanner (Philips Medical Systems; Best, Netherlands). Two-hundred eleven whole-brain functional images were acquired in each of three runs using a T2*-weighted echo-planar gradient-echo pulse sequence (39 slices acquired sequentially in axial orientation co-planar with AC-PC line, slice thickness = 3.5mm, slice gap = 0mm, echo time = 28ms, repetition time = 2000ms, flip angle = 90°, acquisition matrix = 64mm x 64mm). Stimuli were presented electronically with E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA) using the ESys patient display monitor (Invivo, Orlando, FL).

A high resolution structural image was obtained for anatomic normalization using a T1-weighted, magnetization-prepared rapid gradient echo (MPRAGE) sequence (220 sagittal slices, slice thickness =1 mm, echo time = 4.6ms, repetition time = 9.8ms, flip angle=8°, acquisition matrix = 240mm x 240mm).

Preprocessing was performed on the functional data using FSL (5.0.2.2, http://www.fmrib.ox.ac.uk) and SPM8 (r4667, Wellcome Department of Cognitive Neurology, University College London, UK; www.fil.ion.ucl.ac.uk). Volumes underwent slice-timing and motion correction. Runs containing between-scan translation of more than 3.5mm were excluded, and participants with two or more excluded runs were excluded from group analysis. Data for six MDD participants were rejected for excessive head motion during the fMRI scan. Functional scans were normalized to the MNI152 template, resampled to 2x2x2mm voxels, and

underwent spatial smoothing using an isotropic Gaussian kernel of 8.0 mm full-width at half-maximum.

For each participant, intrasubject effects were modeled in SPM8 using an event-related design including both anticipatory and outcome conditions. For anticipatory conditions, the onsets of the cues for each of the five conditions were modeled as separate regressors. For outcome conditions, onsets of the feedback period for each of the three potential outcomes (successful monetary outcome, encompassing both monetary reward and avoidance of monetary punishment; unsuccessful monetary outcome, encompassing monetary punishment and failure to earn monetary reward; neutral outcome, where no money was at risk) were modeled as separate regressors. In each model, six motion parameters were included as regressors of no interest. A high pass filter (cutoff 128s) was applied, as well as AR(1) auto-regression correction.

To investigate BOLD response to incentive anticipation, a contrast was created for Incentive Anticipation > Neutral Anticipation (Anticipation of Incentive collapsed across reward magnitude and valance to encompass all trials in which participations could gain or lose money). Anticipation of Monetary Loss and Anticipation of Monetary Gain did not differ significantly in main effect of task regions.

To investigate BOLD response to reward outcome, a contrast was created for Successful Monetary Outcome > Unsuccessful Monetary Outcome. A contrast was also created for Unsuccessful Monetary Outcome > Successful Monetary Outcome, in which no significant results were observed.

The number of observations of a given condition varied across the three runs. To account for this, contrast weights were calculated to reflect inverse variance. We determined the contrast weight for a given condition in a given run as the number of observations of the condition in that

run, divided by the sum of the square roots of the number of observations of the condition in each run. For example, the contrast weight for Condition A in run 1 is equal to the number of observations of Condition A in run 1 divided by the sum of the square roots of the number of observations of Condition A in run 1, run 2, and run 3.

PET acquisition and image processing.

Within the MDD cohort, four 90-minute PET scans were acquired (HR_scanner; Siemens, Knoxville, Tennessee) in 3-dimensional mode (reconstructed full-width/half-maximum resolution, approximately 5.5 mm in plane and 5.0 mm axially), with the septa retracted and scatter correction, as previously described (Peciña et al, 2015; Scott et al., 2007). Of the twenty-nine participants with quality fMRI data, all twenty-nine underwent PET scans for MOR; a subset of seventeen underwent scans for DA D2/3.

Briefly, participants were positioned in the PET scanner gantry, and 2 intravenous (antecubital) lines were placed. A light forehead restraint was used to eliminate intrascan head movement. Radiotracer administrations were separated by at least 2 hours to allow for radiotracer decay. Carbon 11 (^{11}C)–labeled carfentanil was synthesized at high specific activity (>2000 Ci/mmol [the conversion factor for 1Ci is $3.7x10^{10}$ Bq]) by the reaction of [^{11}C]methyl iodide and a normethyl precursor as previously described (Jewett, 2001). [^{11}C]raclopride was synthesized at high specific activity (>2000 Ci/mmol) by the reaction of *O*-desmethyl raclopride with [^{11}C]methyl triflate. Ten to 15 mCi was administered in each of the imaging procedures, with a mean (SD) mass of carfentanil injected of 0.028 (0.013) μg/kg per scan and of raclopride of 0.20 (0.15) μg/kg per scan. These levels ensured that the compounds were administered in tracer quantities, that is, subpharmacological doses occupying less than 1% of the available

receptors. Fifty percent of the radiotracer doses were administered as an initial bolus and the remaining 50% by continuous infusion for the remainder of the study. This procedure compensates for the metabolism and distribution of the radiotracer, leading to constant plasma concentrations over time and more rapid equilibration between kinetic compartments. For each scan, 21 sets of images (frames) were acquired over a 90-minute period with an increasing duration (four 30-second frames, three 1-minute frames, two 2.5-minute frames, eight 5-minute frames, and four 10-minute frames). Images were reconstructed using iterative algorithms (brain mode; Fourier rebinning algorithm with ordered-subsets expectation maximization, 4 iterations, and 16 subsets; no smoothing) into a 128x128-pixel matrix in a 28.8-cm-diameter field of view. Attenuation correction was performed through a 6-minute transmission scan (Ge^{68} source) obtained before the PET study and with iterative reconstruction of the blank/transmission data, followed by segmentation of the attenuation image. Small head motions during PET were corrected by an automated computer algorithm for each subject before analysis, and the images were coregistered with the same software. Time points were then decay corrected during reconstruction of the PET data. Image data were then transformed on a voxel-by-voxel basis into 2 sets of parametric maps, a tracer transport measure (K_1 ratio) and a receptor-related measure (distribution volume ratio [DVR] at equilibrium), using data from 5- to 40-minute posttracer administration. To avoid the need for arterial blood sampling, these measures were calculated by means of a modified Logan graphical analysis (Logan et al., 1996) using the following reference regions: the occipital cortex (an area devoid of μ-opioid receptors) for [^{11}C]carfentanil scans and the cerebellum (an area with negligible DA D2/3 receptors) for [^{11}C]raclopride scans. The slope of the Logan plot is equal to the receptor concentration divided by its affinity for the radiotracer ($f_2B_{max}/K_d + 1$ for this receptor site) and has been referred to as the DVR; f_2B_{max}/K_d (or DVR−1)

is the "receptor related" measure (also termed BP) or receptor availability in vivo. B_{max} is the receptor concentration and K_d, the receptor-ligand dissociation constant. The term f_2 refers to the concentration of free radiotracer in the extracellular fluid and is considered to represent a constant and very small value. Reductions in the in vivo availability of receptors, the BP_{ND} measure, after an acute challenge (i.e., placebo administration) are thought to reflect processes, such as competition between radiotracer and endogenous ligand, associated with neurotransmitter release (Narendran & Martinez, 2008).

Statistical analysis

For work flow of analyses, see Figure 3.1.

At the second level, fMRI contrasts of interest (Incentive Anticipation > Neutral Anticipation; Successful Monetary Outcome > Unsuccessful Monetary Outcome) were regressed in separate analyses against reduction in depressive symptoms with antidepressant treatment (QIDS-SR16 Week 0 – Week 10). The resulting voxelwise maps were thresholded at $p < 0.001$ uncorrected. The cluster-extent threshold to correct for multiple comparisons at $p < 0.05$ was calculated based on Monte Carlo simulation using 3dClustSim (10000 iterations, NN1, 1-sided; version AFNI_16.2.01, precompiled binary linux_xorg7_64: July 8 2016; Cox, 1996).

BOLD signal from significant clusters was extracted via the MarsBaR toolbox (Brett et al, 2002), and regressed against maps of DA D2/3 and μ-opioid receptor availability. The resulting voxelwise maps were thresholded at $p < 0.001$ uncorrected. Again, the cluster-extent threshold to correct for multiple comparisons at $p < 0.05$ was calculated using 3dClustSim.

Additional statistical analyses were performed using the statistical software SPSS 22 (IBM Corp, Armonk, NY). MDD were divided into high- (n=12) and low- (n=10) antidepressant

responder groups using a median split, based on participants' change in QIDS-SR16 scores from week 0 to week 10 of the antidepressant clinical trial. A one-way analysis of variance (ANOVA) was performed on the extracted response to incentive anticipation, between healthy controls, low-antidepressant responder MDD, and high-antidepressant responder MDD. Mediation analysis was performed using a bootstrapping approach with 5000 bootstrap samples, as implemented in the PROCESS SPSS macro (Preacher & Hayes, 2008).

Results

Participant demographics

Healthy control and MDD participant samples did not significantly differ in age or gender. MDD participants reported significantly higher depressive symptoms at screening, as measured by the QIDS-16-SR, $t(49) = 9.80$, $p < 0.001$.

Behavioral results

Healthy control and MDD participants did not significantly differ on accuracy or median reaction time for successful responses either overall, or specifically in gain trials, loss trials, and null trials (data not shown).

Imaging results

Relationship between incentive anticipation and successful monetary outcome during the MID and subsequent antidepressant treatment response

We first examined the relationship between antidepressant treatment response and responses to incentive anticipation and successful monetary outcome during the MID. Decreases

in depressive symptoms after ten weeks of antidepressant treatment, as measured by Quick Inventory of Depressive Symptomatology (QIDS-SR16) self-report measure (Rush et al, 2003) were regressed against anticipation and outcome contrast maps.

Clinical improvement with treatment negatively correlated with BOLD response to anticipatory incentive processing in a mediofrontal cluster encompassing aspects of rostral anterior cingulate cortex (rACC), dorsal anterior cingulate cortex (dACC), and medial prefrontal cortex (mPFC) (-6, 50, 24, $z = 4.22$, $k = 229$; $n = 22$; Fig 3.2a), such that greater BOLD response predicted poorer treatment response (Fig 3.2b). (NOTE: In the interest of brevity, for the remainder of this document the significant cluster reported in this paragraph is referred to as rACC). No significant correlations were observed for the opposite contrast.

Low antidepressant responders ($n = 10$) demonstrated significantly increased rACC BOLD response to incentive anticipation relative to high antidepressant responders ($n = 12$) and healthy controls ($n = 13$) ($F = 11.8$, $p < 0.001$; Fig 3.2c).

No significant correlations were observed between BOLD response to successful monetary outcome and antidepressant treatment response, for either positive or negative contrasts.

Relationship between DA D2/3 and μ-Opioid BP_{ND} and rACC response to incentive anticipation

We then examined the relationship between DA D2/3 ($n = 17$) and μ-opioid ($n = 29$) BP_{ND} and rACC response to incentive anticipation during the MID. Rostral ACC response to incentive anticipation correlated negatively with μ-opioid BP_{ND} in the nucleus accumbens (right NAc: 10, 4, -8, $z = 4.09$, $k = 105$; left NAc: -20, 4, -8, z = 3.82; k = 223; Fig 3.3a), such that

greater rACC BOLD response was associated with reduced μ-opioid receptor availability (Fig 3.3b). No significant correlations were observed for the opposite contrast.

No significant correlations were observed between rACC response to incentive anticipation and DA D2/3 receptor availability, for either positive or negative contrasts.

Mediation analysis

Given that rACC response to incentive anticipation was significantly correlated with μ-opioid binding potential in the nucleus accumbens, and each of those variables independently predicted treatment outcome, we next performed a mediation analysis. Two models were examined: in the first, we tested if rACC response to incentive anticipation mediated the relationship between NAc mu-opioid binding potential and treatment outcome. In the second, we tested if NAc mu-opioid binding potential mediated the relationship between rACC response to incentive anticipation and treatment outcome.

Model 1: Mediation of rACC response to incentive anticipation on NAc MOR BP_{ND} prediction of antidepressant treatment outcome

We observed that rACC response to incentive anticipation is a significant mediator of NAc MOR BP_{ND} effects on antidepressant treatment outcome (Sobel test: $Z = 2.16$, $p = 0.031$). Controlling for mediation effect of the rACC response significantly reduced the association between NAc MOR BP_{ND} and treatment outcome (total effect c: $b = 11.80$, $SE = 3.02$, $p = 0.009$; direct effect c': $b = 5.46$, $SE = 3.58$, $p = .144$) (Figure 3.4a).

Model 2: Mediation of NAc MOR BP_{ND} on rACC response to incentive anticipation prediction of antidepressant treatment outcome

We observed that NAc MOR BP_{ND} is not a significant mediator of rACC response to incentive anticipation effects on antidepressant treatment outcome (Sobel test: $Z = -1.39$, $p = 0.164$). Controlling for mediation effect of the NAc MOR BP_{ND} did not significantly reduce the association between rACC response to incentive anticipation and treatment outcome (total effect c: $b = -3.20$, $SE = 0.67$, $p = 0.001$; direct effect c': $b = -2.30$, $SE = 0.87$, $p = .0164$) (Figure 3.4b).

For full SPSS output of mediation analysis for Model 1, see Appendix 3.1.

Discussion

In the present study, we examined the association between two aspects of reward processing – anticipation and outcome – and antidepressant treatment response, as well as the role of associated neurotransmitters. We found that response to reward anticipation was negatively associated with subsequent antidepressant treatment response, such that greater response predicted reduced improvement. We further found that rACC response to reward anticipation mediated a previously identified relationship between μ-opioid receptor availability in the nucleus accumbens and antidepressant treatment response (Peciña et al., 2015). These findings hold important implications for understanding the neurobiological network underlying disrupted reward processing in Major Depression, and also for improving predictive models of individual treatment response.

We observed a significant negative correlation between BOLD response to incentive anticipation in a mediofrontal region (including aspects of rACC, dACC, and mPFC) and

subsequent improvement with antidepressant treatment, which supports the hypothesis that individual differences in anticipatory reward response, but not reward outcome, predict treatment outcome. This finding joins a significant body of literature implicating the ACC as a critical region in predicting antidepressant response (Arns et al., 2015; see Pizzagalli, 2011 for review). Interestingly, the literature is divided regarding the direction of the relationship. While some studies find a positive relationship between ACC activity and treatment outcome, we join the body of literature demonstrating a negative relationship. Possible explanations for this apparent discrepancy are highlighted by differences in methodology across studies. First, many of the results demonstrating a positive relationship between ACC activity and treatment outcome were done using resting-state activity or metabolism instead of task-related response (Pizzagalli et al, 2001; Mayberg et al, 1997). Second, some previous studies have used treatments other than SSRI antidepressants, such as sleep deprivation or ketamine (Clark et al, 2006; Salvadore et al, 2009), in which the mechanism of action and recovery is likely different. There is clearly a variety of approaches used to investigate the relationship between ACC activity and treatment outcome in depression, and additional investigation is needed to better understand the impact of task, treatment-type, and neuroimaging technique. That said, even as differences in methodology may impact the direction of the relationship, it is interesting that ACC function continues to be implicated in treatment response. In investigating this relationship in the context of healthy controls, we observed that subsequent treatment low-responders demonstrated hyperactive rACC response to incentive anticipation compared to both controls and subsequent treatment high-responders.

We also observed that the treatment outcome-predictive BOLD response in the rACC was negatively correlated with μ-opioid (but not DA D2/3) receptor availability, specifically in

the nucleus accumbens. This finding conflicts with our hypothesis that treatment outcome-predictive BOLD response to reward anticipation would correlate with DA D2/3 binding. The nucleus accumbens is rich in μ-opioid receptors, which are thought to be involved in hedonic reward processing rather than anticipatory reward processing (Peciña et al, 2006), although opioid receptor binding also modulates dopamine neurotransmission (Fields & Margolis, 2015). This provides a potential avenue for μ-opioid receptors to influence anticipatory reward processes via dopaminergic neurons in the striatum.

We replicated a previously published positive correlation between μ-opioid receptor availability in the nucleus accumbens and subsequent improvement with treatment (Peciña et al., 2015) using a subset of the published sample, i.e. participants who both completed the 10-week treatment trial and produced acceptable fMRI data. This finding is in line with previous evidence suggesting a role for the endogenous opioid system in modulating treatment response (Kennedy et al, 2006), and the possibility of modulating opioid receptor activity to elicit recovery from depression (Ehrich et al., 2015).

Finally, we observed a mediation relationship in which rACC response to incentive anticipation mediates the relationship between μ-opioid receptor availability in the NAc and antidepressant treatment outcome. Given that frontal-striatal loops are critical to motivated behavior and are thought to be affected in major depression, we speculate that these individual differences among participants in rACC response and MOR receptor availability may provide a glimpse of the neurobiology underlying capacity for recovery with antidepressants.

However, a limitation of using a functional neuroimaging approach is that the observed relationships with treatment outcome are correlational. While the present findings further characterize the relationship between reward response, antidepressant treatment outcome, and

neurotransmitter binding, additional research is needed to elucidate the mechanism (if such a mechanism exists) that links individual variation in anticipatory incentive response and NAc μ-opioid receptor availability to treatment response.

In this chapter, we have referred to the treatment-predictive reward cluster as rostral anterior cingulate cortex, although it crosses neuroanatomical borders into other regions such as dACC and mPFC. While highly interconnected, these regions also have their own specific patterns of connectivity, and this approach is not meant to suggest these regions are homogenous. However, the approach does (albeit unintentionally) reflect a source of imprecision in the field, which is naming conventions for anatomical locations. In reviewing the literature, the label of rostral anterior cingulate is used to refer to locations from subgenual cingulate all the way up to dorsal ACC and medial PFC. This imprecision groups together findings from neuroanatomically distinct regions and may blur, rather than sharpen, what we might learn from those results.

In summary, we find that rostral anterior cingulate response to incentive anticipation predicts subsequent antidepressant treatment outcome, and mediates the relationship between μ-opioid receptor availability in the nucleus accumbens and treatment outcome. These results, taken in the context of established reward circuitry, suggest a potential cingulo-striatal dysfunction in a subset of patients with Major Depression which diminishes recovery with antidepressant medication. Further investigation into the nature of this mechanism is needed to provide insight into a potential roadblock to recovery.

Tables, Figures and Legends

	Healthy (n=16)	**Depressed (n=35)**	***p*-value**
Age in years (mean ± SD)	30.25 (± 8.11)	32.09 (± 12.57)	$p = .538$ [1]
Sex (male/female)	8/8	12/23	$p = .286$ [2]
QIDS at screening (mean ± SD)	2.93 (± 2.40)	15.72 (± 4.59)	$p < 0.001$[1]

[1] independent-sample t-test, 2-sided
[2] Pearson Chi-square, 2-sided

Table 3.1. **Characteristics of healthy and depressed participants.** Healthy controls and MDD participants did not significantly differ in age or sex. MDD participants had more severe depressive symptoms than healthy controls.

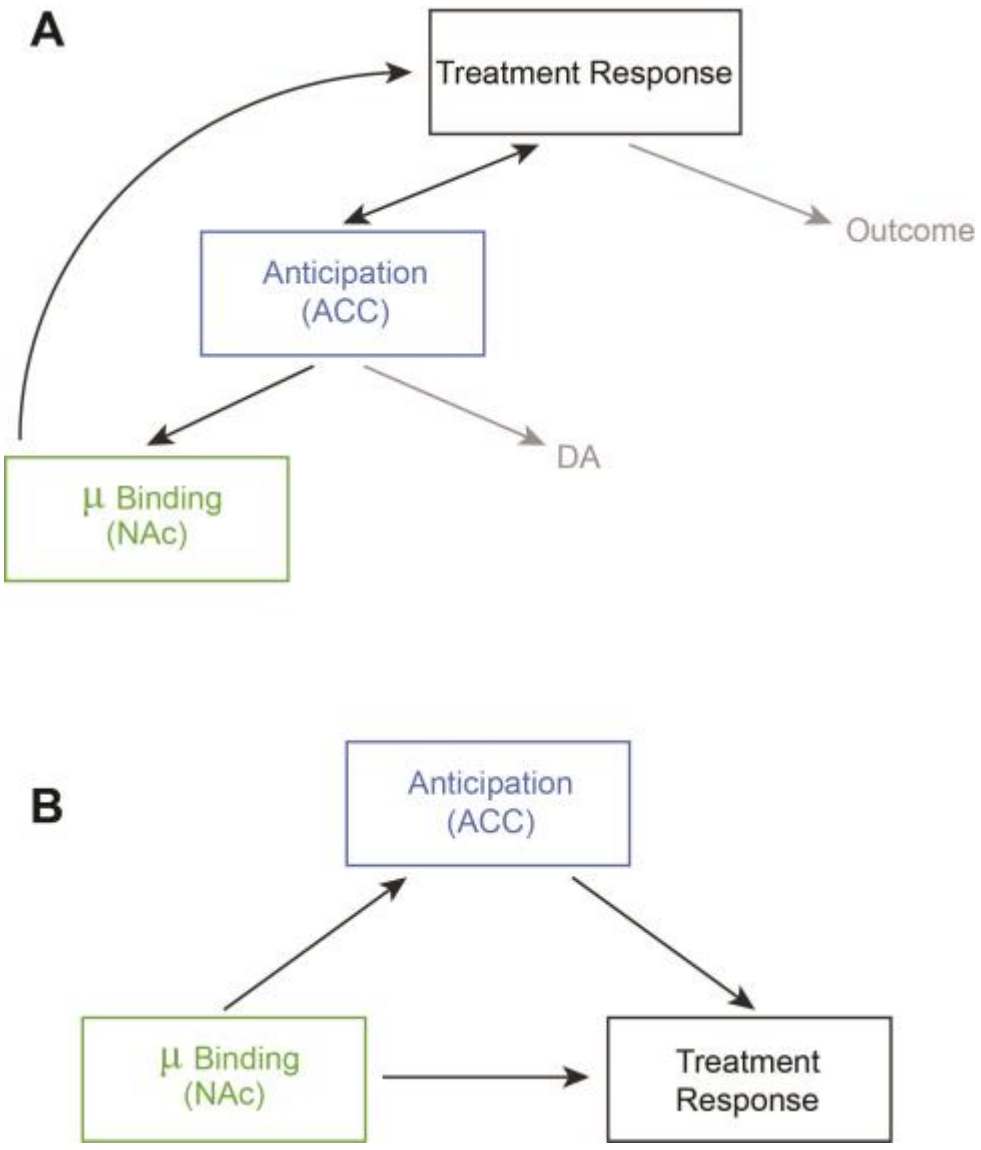


Figure 3.1. **Work flow of analyses for Chapter 3.** (A) Treatment response was used as a covariate in a voxelwise regression against fMRI contrast maps of reward anticipation and reward outcome. Signal was extracted from significant clusters and used as a covariate in a voxelwise regression against mu-opioid and dopamine binding maps. (B) We identified two variables as predictors of treatment response, and ran mediation analyses to investigate the relationship.

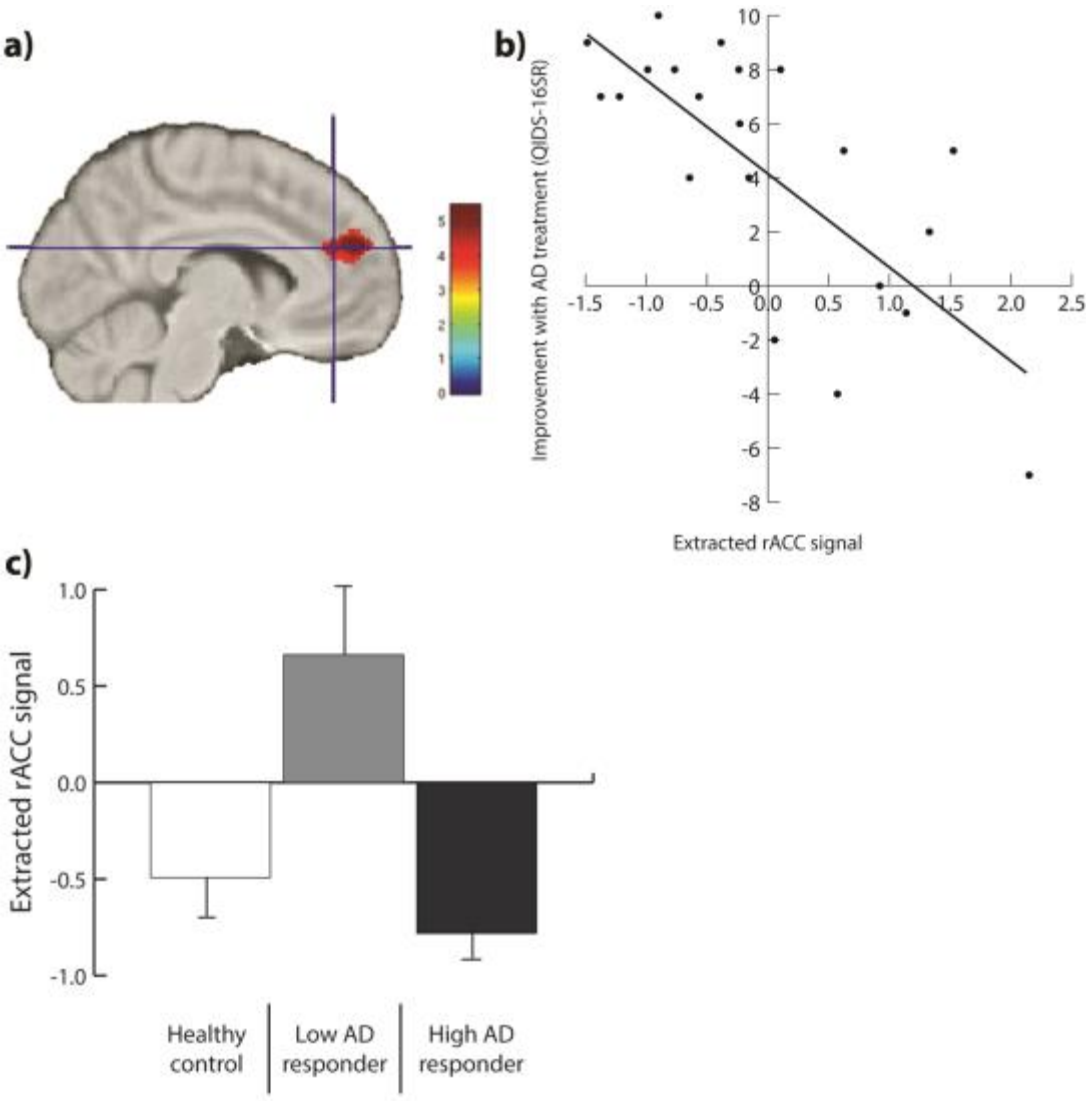


Figure 3.2. **Correlation between incentive anticipation BOLD response and improvement with antidepressant treatment.** (a) Baseline BOLD response to incentive anticipation in the rostral anterior cingulate cortex (rACC) is negatively correlated with improvement in depressive symptoms with antidepressant treatment, as measured by the change in QIDS-SR16 from week 0 to week 10 of treatment. Map is thresholded at $p < 0.001$ uncorrected. Color bar reflects T-values. (b) $r = -.735$ (c) Low antidepressant responders show a significantly hyperactive rACC response to incentive anticipation relative to healthy controls and high-antidepressant responders ($F = 11.8$, $p < 0.001$). Error bars represent SEM.

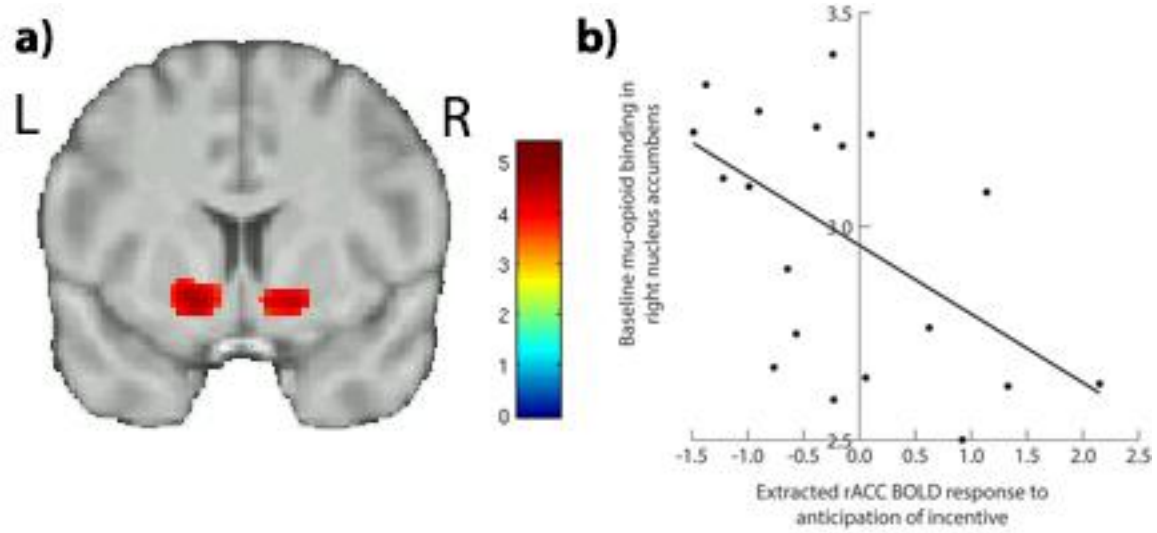


Figure 3.3. **Correlation between μ-opioid binding and rostral ACC BOLD response to incentive anticipation.** (a) Baseline μ-opioid binding in the nucleus accumbens (NAc) is negatively correlated with rACC BOLD response to incentive anticipation. Map is thresholded at $p < 0.001$ uncorrected. Color bar reflects T-values. (b) $r = -.532$.

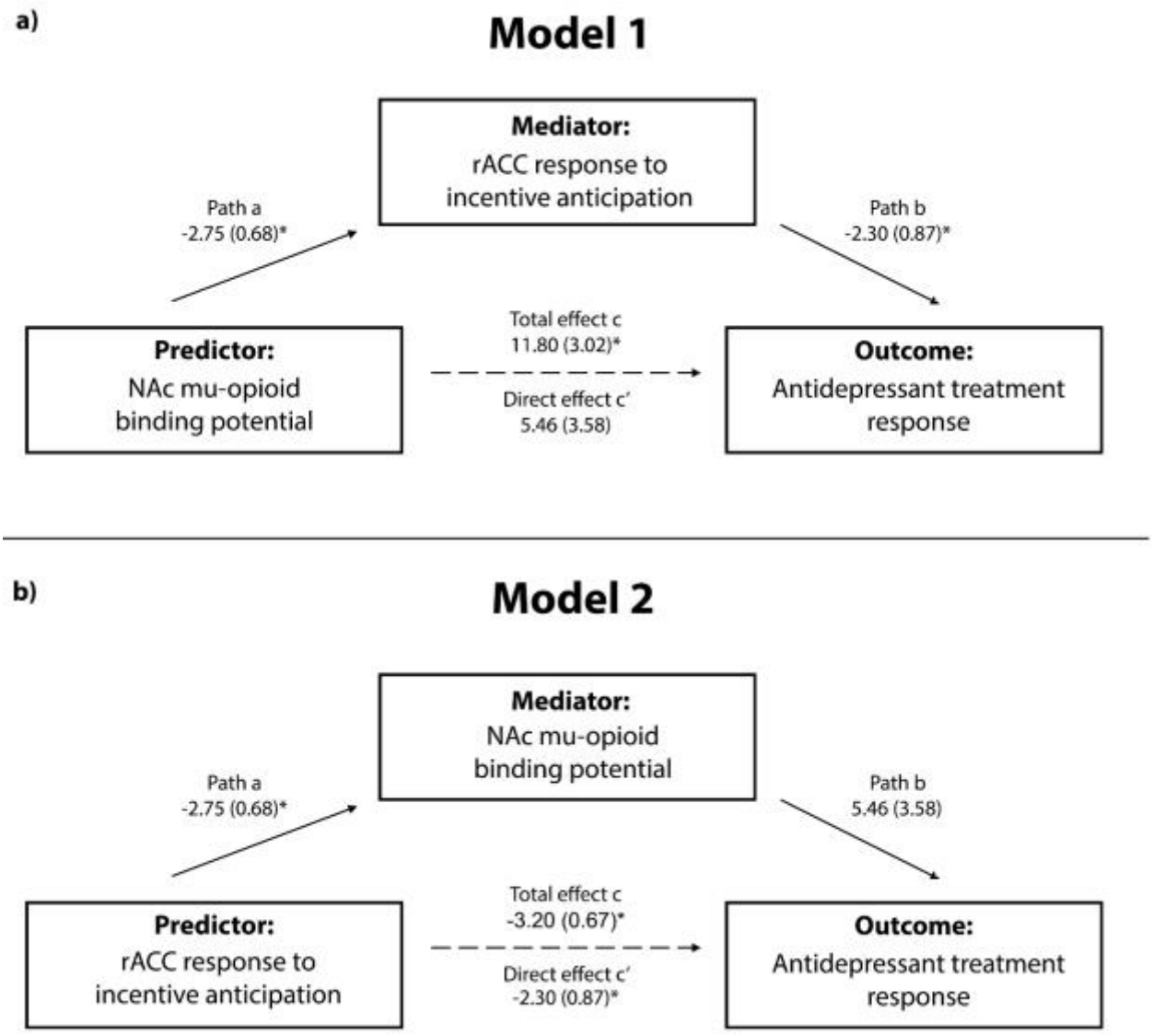


Figure 3.4. **Mediation analyses.** a) Model 1 tests whether rostral anterior cingulate cortex (rACC) BOLD response to incentive anticipation (mediator) mediates the relationship between μ-opioid binding potential in the nucleus accumbens (NAc) (predictor) and antidepressant treatment response (outcome). Path b denotes the relationship between rACC BOLD response to incentive anticipation and antidepressant treatment response, while controlling for the effect of μ-opioid binding in the NAc. Direct effect c' denotes the relationship between μ-opioid binding in the NAc and antidepressant treatment response, while controlling for the mediation effect. b) Model 2 tests whether μ-opioid binding potential in the NAc (mediator) mediates the relationship between rACC BOLD response to incentive anticipation (predictor) and antidepressant treatment response (outcome).

Works Cited

Arns, M., Etkin, A., Hegerl, U., Williams, L. M., DeBattista, C., Palmer, D. M., … Gordon, E. (2015). Frontal and rostral anterior cingulate (rACC) theta EEG in depression: Implications for treatment outcome? *European Neuropsychopharmacology*, *25*(8), 1190–1200. https://doi.org/10.1016/j.euroneuro.2015.03.007

Chen, C.-H., Ridler, K., Suckling, J., Williams, S., Fu, C. H. Y., Merlo-Pich, E., & Bullmore, E. (2007). Brain Imaging Correlates of Depressive Symptom Severity and Predictors of Symptom Improvement After Antidepressant Treatment. *Biological Psychiatry*, *62*(5), 407–414. https://doi.org/10.1016/j.biopsych.2006.09.018

Clark, C. P., Brown, G. G., Frank, L., Thomas, L., Sutherland, A. N., & Gillin, J. C. (2006). Improved anatomic delineation of the antidepressant response to partial sleep deprivation in medial frontal cortex using perfusion-weighted functional MRI. *Psychiatry Research: Neuroimaging*, *146*(3), 213–222. https://doi.org/10.1016/j.pscychresns.2005.12.008

Cox, R. W. (1996). AFNI: Software for Analysis and Visualization of Functional Magnetic Resonance Neuroimages. *Computers and Biomedical Research*, *29*(3), 162–173. https://doi.org/10.1006/cbmr.1996.0014

Ehrich, E., Turncliff, R., Du, Y., Leigh-Pemberton, R., Fernandez, E., Jones, R., & Fava, M. (2015). Evaluation of Opioid Modulation in Major Depressive Disorder. *Neuropsychopharmacology*, *40*(6), 1448–1455. https://doi.org/10.1038/npp.2014.330

Fields, H. L., & Margolis, E. B. (2015). Understanding opioid reward. *Trends in Neurosciences*, *38*(4), 217–225. https://doi.org/10.1016/j.tins.2015.01.002

Garriock, H. A., Tanowitz, M., Kraft, J. B., Dang, V. C., Peters, E. J., Jenkins, G. D., … Hamilton, S. P. (2010). Association of Mu-Opioid Receptor Variants and Response to Citalopram Treatment in Major Depressive Disorder. *American Journal of Psychiatry*, *167*(5), 565–573. https://doi.org/10.1176/appi.ajp.2009.08081167

Greenberg, P. E., Kessler, R. C., Birnbaum, H. G., Leong, S. A., Lowe, S. W., & Corey-Lisle, P. K. (2003). The Economic Burden of Depression in the United States: How Did It Change Between 1990 and 2000? *The Journal of Clinical Psychiatry*, *64*(12), 1465–1475.

Hamilton, M. (1960). A RATING SCALE FOR DEPRESSION. *Journal of Neurology, Neurosurgery, and Psychiatry*, *23*(1), 56.

Jewett, D. M. (2001). A simple synthesis of [11C]carfentanil using an extraction disk instead of HPLC. *Nuclear Medicine and Biology*, *28*(6), 733–734. https://doi.org/10.1016/S0969-8051(01)00226-8

Karp, J. F., Butters, M. A., Begley, A. E., Miller, M. D., Lenze, E. J., Blumberger, D. M., … Iii, C. F. R. (2014). Safety, Tolerability, and Clinical Effect of Low-Dose Buprenorphine for Treatment-Resistant Depression in Midlife and Older Adults. *The Journal of Clinical Psychiatry*, *75*(8), 785–793. https://doi.org/10.4088/JCP.13m08725

Keitner GI, Ryan CE, Miller IW, & Norman WH. (1992). Recovery and major depression: factors associated with twelve-month outcome. *American Journal of Psychiatry*, *149*(1), 93–99. https://doi.org/10.1176/ajp.149.1.93

Kennedy SE, Koeppe RA, Young EA, & Zubieta J. (2006). Dysregulation of endogenous opioid emotion regulation circuitry in major depression in women. *Archives of General Psychiatry*, *63*(11), 1199–1208. https://doi.org/10.1001/archpsyc.63.11.1199

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K. R., … Wang, P. S. (2003). The Epidemiology of Major Depressive Disorder: Results From the National Comorbidity Survey Replication (NCS-R). *JAMA*, *289*(23), 3095. https://doi.org/10.1001/jama.289.23.3095

Knutson, B., Bhanji, J. P., Cooney, R. E., Atlas, L. Y., & Gotlib, I. H. (2008). Neural Responses to Monetary Incentives in Major Depression. *Biological Psychiatry*, *63*(7), 686–692. https://doi.org/10.1016/j.biopsych.2007.07.023

Knutson, B., Westdorp, A., Kaiser, E., & Hommer, D. (2000). FMRI Visualization of Brain Activity during a Monetary Incentive Delay Task. *NeuroImage*, *12*(1), 20–27. https://doi.org/10.1006/nimg.2000.0593

Langenecker, S. A., Kennedy, S. E., Guidotti, L. M., Briceno, E. M., Own, L. S., Hooven, T., … Zubieta, J.-K. (2007). Frontal and Limbic Activation During Inhibitory Control Predicts Treatment Response in Major Depressive Disorder. *Biological Psychiatry*, *62*(11), 1272–1280. https://doi.org/10.1016/j.biopsych.2007.02.019

Leuchter, A. F., Cook, I. A., Marangell, L. B., Gilmer, W. S., Burgoyne, K. S., Howland, R. H., … Greenwald, S. (2009). Comparative effectiveness of biomarkers and clinical indicators for predicting outcomes of SSRI treatment in Major Depressive Disorder: Results of the BRITE-MD study. *Psychiatry Research*, *169*(2), 124–131. https://doi.org/10.1016/j.psychres.2009.06.004

Logan, J., Fowler, J. S., Volkow, N. D., Wang, G.-J., Ding, Y.-S., & Alexoff, D. L. (1996). Distribution Volume Ratios without Blood Sampling from Graphical Analysis of PET Data. *Journal of Cerebral Blood Flow & Metabolism*, *16*(5), 834–840. https://doi.org/10.1097/00004647-199609000-00008

Mayberg, H. S., Brannan, S. K., Mahurin, R. K., Jerabek, P. A., Brickman, J. S., Tekell, J. L., … Fox, P. T. (1997). Cingulate function in depression: a potential predictor of treatment response. *Neuroreport*, *8*(4), 1057–1061.

Narendran, R., & Martinez, D. (2008). Cocaine abuse and sensitization of striatal dopamine transmission: a critical review of the preclinical and clinical imaging literature. *Synapse (New York, N.Y.)*, *62*(11), 851–869. https://doi.org/10.1002/syn.20566

Nestler, E. J., & Carlezon Jr, W. A. (2006). The Mesolimbic Dopamine Reward Circuit in Depression. *Biological Psychiatry*, *59*(12), 1151–1159. https://doi.org/10.1016/j.biopsych.2005.09.018

Peciña, S., Smith, K. S., & Berridge, K. C. (2006). Hedonic Hot Spots in the Brain. *The Neuroscientist*, *12*(6), 500–511. https://doi.org/10.1177/1073858406293154

Peciña M, Bohnert AB, Sikora M, & et al. (2015). Association between placebo-activated neural systems and antidepressant responses: Neurochemistry of placebo effects in major depression. *JAMA Psychiatry*, *72*(11), 1087–1094. https://doi.org/10.1001/jamapsychiatry.2015.1335

Phillips, M. L., Chase, H. W., Sheline, Y. I., Etkin, A., Almeida, J. R. C., Deckersbach, T., & Trivedi, M. H. (2015). Identifying Predictors, Moderators, and Mediators of Antidepressant Response in

Major Depressive Disorder: Neuroimaging Approaches. *American Journal of Psychiatry*, *172*(2), 124–138. https://doi.org/10.1176/appi.ajp.2014.14010076

Pizzagalli, D. A. (2011). Frontocingulate Dysfunction in Depression: Toward Biomarkers of Treatment Response. *Neuropsychopharmacology*, *36*(1), 183–206. https://doi.org/10.1038/npp.2010.166

Pizzagalli, D. A., Holmes, A. J., Dillon, D. G., Goetz, E. L., Birk, J. L., Bogdan, R., … Fava, M. (2009). Reduced Caudate and Nucleus Accumbens Response to Rewards in Unmedicated Individuals With Major Depressive Disorder. *American Journal of Psychiatry*, *166*(6), 702–710. https://doi.org/10.1176/appi.ajp.2008.08081201

Pizzagalli, D., Pascual-Marqui, R. D., Nitschke, J. B., Oakes, T. R., Larson, C. L., Abercrombie, H. C., … Davidson, R. J. (2001). Anterior Cingulate Activity as a Predictor of Degree of Treatment Response in Major Depression: Evidence From Brain Electrical Tomography Analysis. *American Journal of Psychiatry*, *158*(3), 405–415. https://doi.org/10.1176/appi.ajp.158.3.405

Preacher, K. J., & Hayes, A. F. (n.d.). Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behavior Research Methods*, *40*(3), 879–891. https://doi.org/10.3758/BRM.40.3.879

Rush, A. J. (2007). Limitations in Efficacy of Antidepressant Monotherapy. *The Journal of Clinical Psychiatry*, *68*(suppl 10), 8–10.

Salvadore, G., Cornwell, B. R., Colon-Rosario, V., Coppola, R., Grillon, C., Zarate Jr., C. A., & Manji, H. K. (2009). Increased Anterior Cingulate Cortical Activity in Response to Fearful Faces: A Neurophysiological Biomarker that Predicts Rapid Antidepressant Response to Ketamine. *Biological Psychiatry*, *65*(4), 289–295. https://doi.org/10.1016/j.biopsych.2008.08.014

Scott, D. J., Stohler, C. S., Egnatuk, C. M., Wang, H., Koeppe, R. A., & Zubieta, J.-K. (2007). Individual Differences in Reward Responding Explain Placebo-Induced Expectations and Effects. *Neuron*, *55*(2), 325–336. https://doi.org/10.1016/j.neuron.2007.06.028

Appendix 3.1: SPSS output of mediation analysis for Model 1 (rACC mediator)

```
Run MATRIX procedure:

************* PROCESS Procedure for SPSS Release 2.16.1 *****************

          Written by Andrew F. Hayes, Ph.D.       www.afhayes.com
    Documentation available in Hayes (2013). www.guilford.com/p/hayes3

**************************************************************************
Model = 4
    Y = improve
    X = NAC
    M = rACC

Sample size
         22

**************************************************************************
Outcome: rACC

Model Summary
          R       R-sq        MSE          F        df1        df2          p
      .6723      .4520      .6399    16.4978     1.0000    20.0000      .0006

Model
              coeff         se          t          p       LLCI       ULCI
constant     8.0253     1.9946     4.0236      .0007     3.8645    12.1861
NAC         -2.7548      .6782    -4.0618      .0006    -4.1696    -1.3399

**************************************************************************
Outcome: improve

Model Summary
          R       R-sq        MSE          F        df1        df2          p
      .7646      .5846     9.7832    13.3709     2.0000    19.0000      .0002

Model
              coeff         se          t          p       LLCI       ULCI
constant   -11.6077    10.4907    -1.1065      .2823   -33.5664    10.3511
rACC        -2.3022      .8743    -2.6331      .0164    -4.1322     -.4721
NAC          5.4607     3.5824     1.5243      .1439    -2.0377    12.9592

************************ TOTAL EFFECT MODEL ****************************
Outcome: improve

Model Summary
          R       R-sq        MSE          F        df1        df2          p
      .6581      .4331    12.6855    15.2766     1.0000    20.0000      .0009

Model
              coeff         se          t          p       LLCI       ULCI
constant   -30.0831     8.8806    -3.3875      .0029   -48.6089   -11.5574
NAC         11.8026     3.0197     3.9085      .0009     5.5032    18.1019

***************** TOTAL, DIRECT, AND INDIRECT EFFECTS ********************
```

```
Total effect of X on Y
     Effect         SE          t          p       LLCI       ULCI
    11.8026     3.0197     3.9085      .0009     5.5032    18.1019

Direct effect of X on Y
     Effect         SE          t          p       LLCI       ULCI
     5.4607     3.5824     1.5243      .1439    -2.0377    12.9592

Indirect effect of X on Y
         Effect    Boot SE   BootLLCI   BootULCI
rACC     6.3419     3.1363     1.2510    13.5193

Partially standardized indirect effect of X on Y
         Effect    Boot SE   BootLLCI   BootULCI
rACC     1.3738      .6177      .2468     2.6518

Completely standardized indirect effect of X on Y
         Effect    Boot SE   BootLLCI   BootULCI
rACC      .3536      .1545      .0885      .6984

Ratio of indirect to total effect of X on Y
         Effect    Boot SE   BootLLCI   BootULCI
rACC      .5373      .3197      .0839     1.3108

Ratio of indirect to direct effect of X on Y
         Effect    Boot SE   BootLLCI   BootULCI
rACC     1.1614   159.4378    -3.3576    46.9584

R-squared mediation effect size (R-sq_med)
         Effect    Boot SE   BootLLCI   BootULCI
rACC      .3823      .1309      .1316      .6275

Normal theory tests for indirect effect
     Effect         se          Z          p
     6.3419     2.9309     2.1638      .0305

******************** ANALYSIS NOTES AND WARNINGS *************************

Number of bootstrap samples for bias corrected bootstrap confidence
intervals:
     5000

Level of confidence for all confidence intervals in output:
    95.00

NOTE: Some cases were deleted due to missing data.  The number of such cases
was:
  38

NOTE: Kappa-squared is disabled from output as of version 2.16.

------ END MATRIX -----
```

Appendix Table 3.2. **SPSS output of mediation analysis for Model 1 (rACC mediator)**

Chapter Four.
Conclusion

Summary of Results

In Chapter 2, we demonstrate three key relationships. First, striatal response to reward anticipation is associated with striatal dopamine release and D2/3 receptor availability. Second, reduced striatal response to reward outcome is associated with increased thalamic mu-opioid receptor availability. Third, greater anticipation response-associated DA D2/3 receptor availability in the striatum is associated with increased scores of anhedonia and apathy. These findings reinforce evidence from animal literature linking dopamine function with reward motivation and mu-opioid function with reward hedonics. The medial thalamus has not been a major focus of investigations into disrupted reward function in depression, but as a part of the cortico-striato-thalamo-cortical reward loop and a site with dense expression of mu-opioid receptors, the relationship with striatal response to reward outcome merits further investigation.

In Chapter 3, we demonstrate that rostral anterior cingulate (rACC) response to reward anticipation predicts antidepressant treatment response, and furthermore mediates the relationship between NAc mu-opioid receptor availability and antidepressant treatment response. Previous research had identified NAc mu-opioid receptor availability (Peciña et al., 2015) and rACC activity as predictors of antidepressant treatment response (see Pizzagalli, 2011 for

review). For the first time, however, the current findings provide evidence which joins those two predictors in a single model, and further emphasize the role of cortico-striatal interactions in major depression.

Common Discussion

Dopamine/opioid interaction. In the present work, we used measures of dopamine and mu-opioid receptor availability to separately examine relationships with anticipatory and hedonic reward response. However, these systems interact in the ventral tegmental area and striatum, adding an additional layer of complexity onto interpretations of the above results. Opioid binding regulates the mesolimbic and mesocortical dopamine pathways by disinhibition. More specifically, endorphins and enkephalins bind to mu-opioid receptors on GABAergic interneurons in the VTA which tonically inhibit the VTA dopaminergic neurons. Activating the MOR inhibits GABA release, stopping the tonic inhibition of the dopaminergic neurons and facilitating dopamine release in their limbic and cortical projections. So while motivation may be generally associated with dopamine receptor binding and hedonics with MOR binding, interactions between the two systems suggest a more nuanced relationship guiding neurotransmitter modulation of reward response. Similarly, dopamine and mu-opioid receptor binding are part of a larger reward network involving many neurotransmitters such as GABA, glutamate, and serotonin which may modulate, or be modulated by, dopamine and opioid activity.

Multiple reward processes. We have framed the observed reward processing in the context of reward anticipation (or motivation) and outcome (hedonics). However, reward processing

encompasses many more psychological processes, some of which may also influence response in our task. Our ability to identify the neural underpinnings of a psychological process can only be as good as our isolation of that process. Here, it is difficult to parse out response to anticipation of reward (i.e. looking forward to something) and motivation (i.e. drive to earn something). These processes are often tightly interconnected, and may be further influenced by other reward processing like reinforcement and reward learning. In progressing toward a fuller understanding of the biology of reward processing, progress will also need to be made in clarifying and delineating the many psychological processes that comprise reward response.

Interpreting BOLD and PET signal. In discussing measures of binding potential and BOLD response, we must be cognizant how these measures are derived. Binding potential is the ratio between receptor density (B_{max}) and the radioligand equilibrium dissociation constant (K_D). Therefore, individual differences in binding potential between participants could reflect differences in the number of receptors, binding affinity (the inverse of K_D) or the endogenous tone of the ligand. So while binding potential provides a measure of available receptors across subjects, the specific neurobiological mechanism facilitating individual differences is not demonstrated. BOLD signal reflects changes in the concentration of deoxyhemoglobin, which changes as a result of increased cerebral blood flow to a given location in the brain in response to neural activity. Increases in BOLD response are therefore often interpreted as a proxy for increased neural firing, but may most closely reflect the summation of postsynaptic potentials (Logothetis et al., 2001). While postsynaptic activity and action potentials may be highly correlated in many cases, changes in regional cerebral blood flow can occur independently of neural firing. As the field's understanding of the neurobiological basis of PET and BOLD

signals continue to develop, our ability to confidently interpret these signals and the individual differences they reflect will improve.

Skilled vs unskilled reward. In our implementation of the Monetary Incentive Delay task, we use a null condition as the baseline for our contrast, in which the subject is neither rewarded nor punished regardless of performance. This is compared to a condition in which potential reward is dependent on subject performance, which we believe allows us to capture a measure of motivated or anticipatory response. However, what if reward outcome was determined not by performance, but by luck? How would anticipation and hedonic response change if rewards were meted out for reasons beyond the participant's control, and how would that affect relationships with dopamine and mu-opioid receptor activity? This question of skilled compared to unskilled anticipation is particularly interesting in the context of major depression, as it deals with the concept of locus of control. One might hypothesize that healthy controls would show a greater difference in response to skilled versus unskilled anticipation, compared to MDD patients who may believe that receiving the reward is outside of their control in both conditions and therefore have demonstrate similar neural response in each.

Limitations

While the current findings identify relationships between PET binding and BOLD reward response in patients with major depression, it is difficult to say how these relationships reflect disordered reward processing. Having a sample of healthy control subjects with both PET and fMRI scans to directly compare would allow a cleaner interpretation of whether the relationships observed in MDD are aberrant.

We experienced large signal dropout in the OFC and vmPFC of our fMRI data. This dropout is an unfortunately persistent characteristic of data acquired with the echo planar imaging (EPI) pulse sequence, as our data was. Given the putative role of these regions in reward processing, and particularly in hedonic reward, the relationships tested here must be considered incomplete.

The correlative nature of these neuroimaging techniques provide description of associations between neural reward response and associated receptor function, but prevent us from drawing conclusions about causation and mechanisms. Any such conclusions drawn from this data would be speculative.

Future Directions

Molecular and clinical correlates of reward network connectivity. While we have used BOLD response from individual regions as covariates in the present analyses, we know that cognitive processes are not facilitated by individual brain regions in isolation. Instead, networks of brain circuitry interact to give rise to behaviors like reward processing. Investigations into disrupted reward processing in major depression have identified aberrant cortico-striatal functional connectivity (Admon et al., 2015). Given that the interactions of the reward network are a level of complexity beyond what is examined here, a logical next step would be to investigate how individual differences in the functional connectivity of reward circuitry are related to neurotransmission, apathy and anhedonia, and treatment response.

Moderators of differential treatment response. The present work identifies rACC response to reward anticipation as a predictor of SSRI treatment response. Future work may benefit from

attempting to identify modulators of differential treatment diagnosis (Phillips et al., 2015); that is, rather than isolating a biomarker which predicts response to a single treatment, attempt to find biomarkers that facilitate more effective recovery from depressive symptoms in one treatment compared to another, such as antidepressant medication vs psychotherapy, or SSRIs vs dopamine and norepinephrine reuptake inhibitors.

Common or differential reward disruptions across disorders. One of the benefits of the RDoC approach is the opportunity to investigate impairments of functional domains across diagnoses. Disordered reward function is a particularly good process for transdiagnostic investigation, and it seems to be a critical facet of many psychiatric conditions (Baskin-Sommers & Foti, 2015; Hägele et al., 2014), such as substance abuse and the negative symptoms of schizophrenia. Investigating reward across these diagnoses would help identify commonalities and differences in reward dysfunction, and provide a clearer, more complete picture of how motivation and hedonics can be disrupted.

Inflammation and disrupted reward in MDD. Many MDD patients have increased peripheral levels of inflammatory cytokines, indicating increased inflammation. Recent research has begun to investigate the relationship between inflammation and reward circuitry in depression, and has found that increased levels of inflammatory biomarkers were associated with decreased corticostriatal functional connectivity during resting-state fMRI (Felger et al., 2015). With this link established, a next step would be to test the relationship between inflammatory biomarkers and corticostriatal connectivity in response to reward.

Concluding remarks

Major depression is a significant burden on affected individuals and on society as a whole. There remains much that we do not yet understand about the complex biology underlying this heterogeneous disorder, leaving the field ill-equipped to improve on current treatments which prove inadequate for many patients. Elucidating the disruptions which drive impairments in functional domains across psychiatric illnesses (such as impaired reward processing in depression, schizophrenia, addiction, etc), rather than looking for a single etiology that covers all patients within a diagnosis, represents a promising path to cope with heterogeneity in mental health research. Identifying effective predictors of treatment response will help reduce the burden associated with failed treatment and bring about more timely recovery. This work reinforces links between reward process and neurotransmitter function previously supported by the animal literature, and identifies a mediation effect integrating two previously identified predictors of antidepressant treatment response.

Works Cited

Admon, R., Nickerson, L. D., Dillon, D. G., Holmes, A. J., Bogdan, R., Kumar, P., … Pizzagalli, D. A. (2015). Dissociable cortico-striatal connectivity abnormalities in major depression in response to monetary gains and penalties. *Psychological Medicine*, *45*(1), 121–131. https://doi.org/10.1017/S0033291714001123

Baskin-Sommers, A. R., & Foti, D. (2015). Abnormal reward functioning across substance use disorders and major depressive disorder: Considering reward as a transdiagnostic mechanism. *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology*, *98*(2 Pt 2), 227–239. https://doi.org/10.1016/j.ijpsycho.2015.01.011

Felger, J. C., Li, Z., Haroon, E., Woolwine, B. J., Jung, M. Y., Hu, X., & Miller, A. H. (2015). Inflammation is associated with decreased functional connectivity within corticostriatal reward circuitry in depression. *Molecular Psychiatry*. https://doi.org/10.1038/mp.2015.168

Hägele, C., Schlagenhauf, F., Rapp, M., Sterzer, P., Beck, A., Bermpohl, F., … Heinz, A. (2014). Dimensional psychiatry: reward dysfunction and depressive mood across psychiatric disorders. *Psychopharmacology*, *232*(2), 331–341. https://doi.org/10.1007/s00213-014-3662-7

Logothetis, N. K., Pauls, J., Augath, M., Trinath, T., & Oeltermann, A. (2001). Neurophysiological investigation of the basis of the fMRI signal. *Nature*, *412*(6843), 150–157. https://doi.org/10.1038/35084005

Peciña M, Bohnert AB, Sikora M, & et al. (2015). Association between placebo-activated neural systems and antidepressant responses: Neurochemistry of placebo effects in major depression. *JAMA Psychiatry*, *72*(11), 1087–1094. https://doi.org/10.1001/jamapsychiatry.2015.1335

Phillips, M. L., Chase, H. W., Sheline, Y. I., Etkin, A., Almeida, J. R. C., Deckersbach, T., & Trivedi, M. H. (2015). Identifying Predictors, Moderators, and Mediators of Antidepressant Response in Major Depressive Disorder: Neuroimaging Approaches. *American Journal of Psychiatry*, *172*(2), 124–138. https://doi.org/10.1176/appi.ajp.2014.14010076

Pizzagalli, D. A. (2011). Frontocingulate Dysfunction in Depression: Toward Biomarkers of Treatment Response. *Neuropsychopharmacology*, *36*(1), 183–206. https://doi.org/10.1038/npp.2010.166