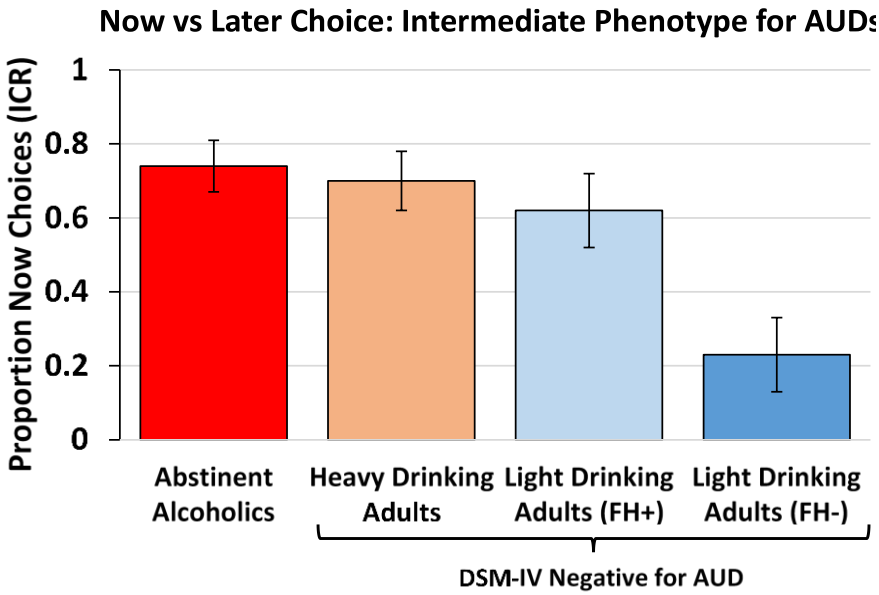
My research seeks to understand neural circuits associated with drug responsivity, how **dopamine (DA)** affects these circuits, and ultimately behavior. Combining behavioral, genetic, and neuroimaging measures including functional Magnetic Resonance Imaging (fMRI) and Positron Emission Tomography (PET), I seek to better characterize individual differences in DA signaling and risk factors associated with drug abuse. At **XXXX** University, I plan to build off work showing that both cortical and striatal DA signaling are associated with risk behaviors for drug addiction in differential ways. Cortical DA effects on decision making behaviors such as *NOW vs LATER* choice often follows an inverted-U model while we have found striatal DA affects trait measures of impulsivity and subjective responses to drugs of abuse in a more linear manner. The next step is integrating this work into a comprehensive model of DA's role in traits and behaviors that are also risk factors for substance abuse. Further characterization of both the biological bases and modulators of these risk factors **(*NOW choice bias, impulsivity, subjective experience*)** for substance use disorders will ultimately lead to improved intervention and treatment approaches. Furthermore, combining genetic and neuroimaging data with pharmacological studies can offer more thorough insights into how DA-targeted treatments may affect individuals differentially. This work may ultimately lead to more personalized and effective treatments for DA-related disorders including drug addiction, ADHD, schizophrenia, and Parkinson's disease.

Immediate Reward Selection Bias, Intermediate Phenotype for Alcohol Use Disorders

While work has demonstrated immediate reward selection bias (choosing a smaller, sooner reward (*NOW*) over a larger, later reward (*LATER*)) is elevated in individuals with substance use disorders, whether increased ***NOW* choice bias** is a cause or consequence of drug use remains unclear. We (Smith et al., 2015 *Frontiers in Human Neuroscience*) have shown *NOW* choice bias (indexed by an **impulsive choice ratio, ICR**) displays many qualities of an intermediate phenotype for alcohol use disorders (AUDs), including being elevated in heavy drinking adults without an AUD and in light drinking adults with a first degree relative with problematic alcohol use (family history/FH+, **see above Figure**). Furthermore, we observed that natural, age-related declines in *NOW* bias were not present in heavy drinkers suggesting heavy alcohol use may "lock in" a preference for *NOW*, potentially by affecting normal brain maturational processes. Importantly, we have also demonstrated this behavior is modulated by: 1) both age and putative prefrontal DA as assessed with the Val158Met ***COMT* (catechol-*O*-methyltransferase, an enzyme critical in metabolizing cortical DA)** single nucleotide polymorphism (SNP rs4680; Smith & Boettiger, 2012 *Psychopharmacology*), 2) estradiol by *COMT* effects in naturally cycling female participants (Smith et al., 2014 *J Neurosci*), and 3) putamen DA synthesis capacity as assessed with FMT PET (Smith et al., 2016 *J Neurophys*). These data suggest means by which individual differences in DA signaling may modulate *NOW* bias and offer insights into potential treatments to reduce elevated *NOW* bias in drug abusers.



Future longitudinal work that investigates initial *NOW* bias level before alcohol use initiation and changes in *NOW* bias as a result of natural exposure to alcohol in college-aged individuals will begin to address the question of whether *NOW* bias is a potential risk factor for or cause of excessive alcohol use, a difficult relationship to disentangle without longitudinal studies. This work could involve assessing ICR in newly-enrolled freshman at **XXXX** and/or greater New York City area and then conducting follow-up tests over the course of their college careers. This would be coupled with the collection of alcohol and drug use measures in these individuals, which could be collected online via a system that de-identifies their responses and then links them to their ICR data collected in the lab.

Furthermore, a more complete characterization of the relationship between normal age-related brain maturation and changes in *NOW* bias among healthy college students will allow for an improved ability to isolate *abnormal* developmental processes in heavy alcohol drinkers. We have collected and are analyzing some of this data at UNC Chapel Hill, and have shown that differential activity in two functional brain networks (temporal lobe versus frontoparietal-striatal areas) relate to *Now* bias and current problematic alcohol use (Elton, Smith, et al., 2017 *JOCN*). More prognostic markers of future problematic alcohol use are needed, though. A longitudinal study like the one I propose to undertake at **XXXX** would address this gap in our knowledge. Furthermore, identifying individuals who are likely to transition to heavy alcohol use via behavioral, genetic, environmental, or other risk factors will be immensely powerful in planning early interventions.

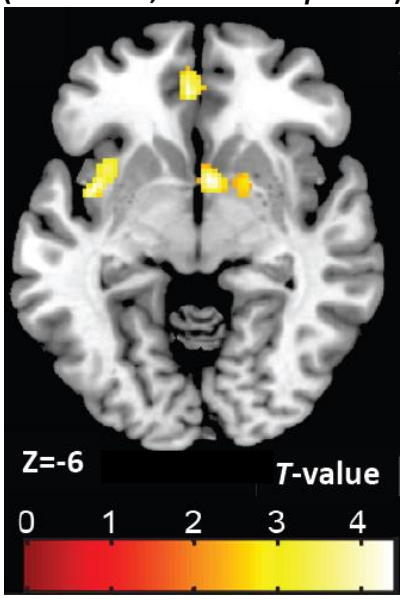
While the **COMT inhibitor, tolcapone**, has been shown to modulate *NOW* bias and its neural underpinnings, no one has tested its potential to treat *NOW*-focused drug-addicted individuals. Tolcapone may assist in cognitive or motivational therapies used to reframe drug abusers' goals and priorities beyond immediate drug use toward more constructive endeavors (work, health, social relationships). My research suggests that tolcapone's effectiveness may depend on *COMT* genotype, age, and estradiol levels (**see pg 5 for proposed model**). Thus, I plan to work with collaborators in **XXXX** Institute for Clinical & Translational Research to examine tolcapone's effects on *NOW* bias in both healthy individuals stratified by some of these modulating variables (age, COMT genotype, estradiol levels) and ultimately test in a properly targeted (based on which groups show effects in healthy individuals) AUD population whether a tolcapone intervention would be effective in improving their treatment outcomes.

Variation in Subjective and Neural Signaling Responses to d-Amphetamine

Individual differences exist in subjective responses to drugs of abuse. Some individuals find drug use to be a pleasurable experience and will be more likely to use again while others don't experience many subjective effects after their first drug use episode(s). We (Smith et al., 2016 *J Psychopharmacology*) have found that across two independent datasets totaling over 450 young adults exposed to oral d-amphetamine (dAMPH), all individuals experienced heightened physiological responses (blood pressure and heart rate) to dAMPH but subjective ratings of dAMPH (High, Like, Feel, Want More) versus placebo varied. A subset of individuals did not report many subjective effects to dAMPH (Nonresponders). Of those reporting subjective effects, some participants' subjective ratings peak relatively quickly. We found these Early Peak Responders show faster peripheral absorption of the drug (higher plasma levels at 60 minutes post dAMPH administration versus Late Peak & Nonresponders) and that time to peak subjective response correlated negatively with novelty seeking scores on the Tridimensional Personality Questionnaire (higher for those whose subjective effects peaked earlier; not reported in paper as TPQ was only available on part of the dataset). These data suggest that individual differences in timing of peak subjective drug effects may be useful measures to consider as potential risk factors for psychostimulant abuse. Coupled with our PET work demonstrating relationships between baseline D2 receptor availability in vmPFC and subsequent self-reported dAMPH high as well as relationships between Want More ratings and dAMPH-induced DA

release in vmPFC, insula, and ventral striatum (VS; Smith et al., 2016 *Neuropharmacology*; **see Figure, at right**), we are beginning to understand the relationship between individual differences in DA signaling and initial responses to psychostimulant drugs. Currently, we are exploring the role of DA transporter levels (DAT, measured via PET) in explaining differences in personality, dAMPH subjective effects, and DA release. We have recently found that DAT levels in VS are negatively related to trait impulsivity (**Smith et al., 2018**; in revision at *Translational Psychiatry*). This work has implications for understanding DA's role in traits present in both drug abusers and those with attention-deficit/hyperactivity disorder (ADHD).

DA release in vmPFC, right VS, & left insula associate with wanting more dAMPH (Smith et al., 2016 *Neuropharm.*)



Future work that measures the neural correlates (fMRI BOLD activity, PET measures of DA release) of subjective responses to dAMPH and other drugs of abuse will allow for a more thorough characterization of brain areas and processes critical in these experiences. Furthermore, if early dAMPH subjective response is a marker of addiction risk, as we propose, we would expect individuals with a family history of drug abuse to be more likely to show an early peak response. Identifying if early peak response or DA signaling responsivity in general is associated with any genetic or neural markers may allow identification of at risk individuals. For instance, we have found that the C957T SNP (rs6277) in the DRD2 gene affects putamen and ventral striatum DA D2 receptor availability (Smith et al., 2017 *Translational Psychiatry*). We propose individuals with lower levels of D2 receptor availability (CC genotype) would be at increased risk for addiction based on previous PET studies but whether C957T confers addiction risk or differences in responsivity to drugs of abuse remains unknown. In addition, understanding the biological bases of why some individuals *don't respond* to dAMPH may offer insights into targets for ameliorating the positive subjective effects of drugs of abuse. Our work also suggests DA release in a vmPFC-insula-VS network (**see Figure, above**) is associated with drug wanting (Smith et al., 2016 *Neuropharmacology*) and, presumably, drug craving. Neuroimaging work investigating the structural and functional links between these nodes in response to dAMPH exposure in naïve individuals and at baseline in drug abusers with years of drug use (and varying degrees of drug craving) will offer greater insights into changes in this corticolimbic network over the course of drug use. Ultimately, this work will provide insights into the critical biological nodes of change that could be targeted by future treatments for psychostimulant abusers.

Role of Hormones in Modulating Value, Choice, and DA-Dependent Processes

Many psychiatric disorders including schizophrenia, depression, ADHD, and drug addiction are known to exhibit considerable sex differences in terms of onset, prevalence, severity, and treatment outcomes. However, our understanding of mechanistic neurobiological differences between males and females is limited. In response to this, the National Institutes of Health has taken steps to emphasize the importance of sex as a biological variable. As a researcher interested in studying the role of DA in human behavior, the female sex-linked hormone estradiol is particularly interesting to study as it has been shown in rats to increase striatal DA synthesis and levels of tyrosine hydroxylase, the rate limiting enzyme in the DA biosynthetic pathway. Drug-seeking behavior has also been linked with estradiol as it increases cocaine self-administration rates when administration to ovariectomized rats. Furthermore, estradiol has been associated with increased DA release in response to the dAMPH in rats and, along with progesterone, differential subjective responses to dAMPH in humans. Thus,

there is strong evidence that female hormones can modulate the pleasurable effects of drugs of abuse in the brain. Indeed, stress- and cue-induced craving for cocaine in women are affected by progesterone and estradiol.

Estradiol goes beyond acting on affective systems. It can also modulate cognitive processes that are often compromised in individuals with drug abuse disorders. For instance, estradiol has been shown to affect effort-based decision making in rats and **working memory (WM)** and temporal discounting choices (Smith et al., 2014 *J Neurosci*) in human subjects, dependent on putative levels of prefrontal cortical DA. These data suggest that estradiol affects DA-related processes across the brain from deep limbic/motivational structures to higher order cortical areas.

Future work needs to focus on measuring the effects of female hormones on DA signaling and behavior. While data suggests activity in functional brain networks relate to *Now* bias and problematic alcohol use (Elton, Smith, et al., 2017 *JOCN*), it is not clear how estradiol levels, which modulate the behavior (Smith et al., 2014 *J Neurosci*), affect neural activity associated with *Now* bias. One proposed study from my group would involve female subjects completing our fMRI discounting task at multiple points in their menstrual cycle to measure whether hormonal fluctuations affect brain activity associate with choosing *NOW* over *LATER* (or vice versa).

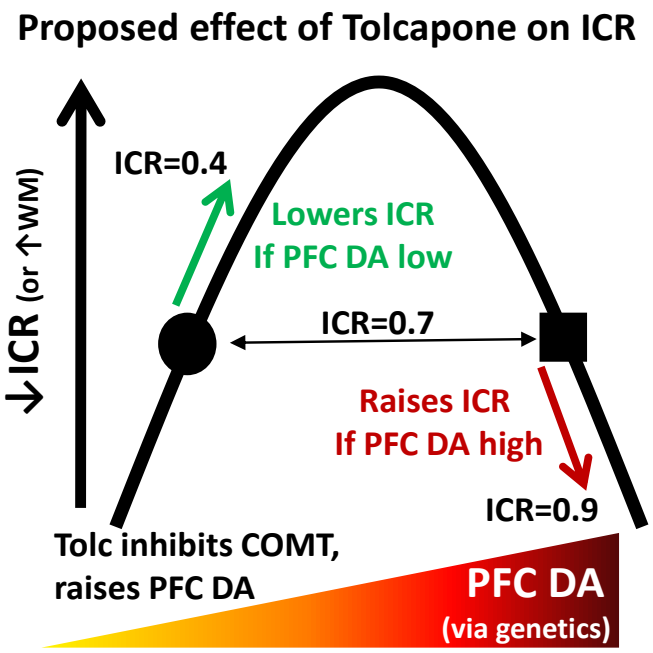
In addition, my lab will make use of natural fluctuations in female hormones over the menstrual cycle to investigate how not only *Now* Choice (ICR) and WM change over the cycle (to replicate and build off previous reports) but also explore the effects of hormonal fluctuation on effort-based decision making using the EEfRT task (to build off the animal work mentioned earlier) developed in my postdoctoral lab at Vanderbilt. As ICR, WM, and EEfRT are modulated by DA signaling, we will be able to probe the effects of estradiol and progesterone in modulating these processes by measuring these tasks at multiple points in the cycle when each hormone is relatively low and high. Coupling these measurements with genetic measures of putative DA signaling (*COMT*, *DARPP-32*, *DRD2*, *DAT1*) will allow us to investigate whether the inverted-U model applies to estradiol's effects on effort-based decision making (as it does for WM and ICR).

Furthermore, birth control affects estradiol and progesterone levels which can affect components of the DA system and thus, DA-dependent processes. However, little work has systematically investigated the effects of birth control on decision making processes. Thus, my lab will work to categorize the effects of the most common forms of birth control (progestin only pill or injection, combination estrogen+progestin pill, combination extend-cycle pill) on decision making behavior by assessing ICR, WM, and EEfRT performance of females on and off birth control as well as age-matched males, controlling for *COMT* genotype (which differentially effects fMRI and ICR measures in males and females: Elton, Smith et al., 2017 *Frontiers in Human Neuroscience*). A more complete understanding of female hormone's effects on value, choice, and DA could aid in the development of more effective therapies for women and men suffering from DA-related diseases.

Integrating Genetics, Pharmacology, and PET to Study Brain DA Transmission

Given that drug use initiation often begins in the teenage years, studying the factors that predispose adolescents and young adults to try and continue to use drugs is essential. While PET allows for quantification of DA signaling, it cannot be used experimentally on those ages<18, limiting the study of adolescents with this technique. Thus, another goal of my work is to identify genetic variants that explain differences in DA signaling as observed with PET or that affect reward related responses in behavioral tasks. To this end, we have found the *COMT* Val158Met SNP (rs4680) to interact with age to affect *NOW* choice bias (Smith & Boettiger, 2012 *Psychopharmacology*) and interact with estradiol to explain changes in *NOW* choice bias over the menstrual cycle (Smith et al., 2014 *J Neurosci*). Both these findings suggest *NOW* choice bias (**ICR**) follows an inverted U-shaped function of DA effects

(intermediate DA levels lead to more optimal decisions, less *NOW* choice bias). This model has recently been extended to show inverted U-shaped effects on **ICR**, neural activation during choice, and resting state functional connectivity in an fMRI study by our group (Elton, Smith et al., 2017 *Front in Hum Neurosci*). Based on this inverted U-shaped model, individuals can be impulsive from either too little or too much DA. Thus, understanding these individuals' endogenous DA levels via genetic markers will be critical in implementing the correct treatment (DA agonists versus antagonists or COMT inhibitors such as tolcapone) to reduce their *NOW* choice bias (**see Proposed Model, right**). Furthermore, COMT interacts with a SNP in DARPP-32 (rs907094) to impact working memory (**WM**) performance (Smith et al., 2014 *JOCN*). The WM finding supports the ability to use genetic polymorphisms to measure primarily D1 DA receptor signaling in the cortex. This is important because good D1 receptor radiotracers are lacking for PET. Utilizing genetic proxies along with pharmacological manipulations can allow us to gain a more complete understanding of the D1 DA signaling thought to be critical in executive function. Certain pharmacological agents, like tolcapone, would be expected to target cortical DA and genetic variation in cortical DA related signaling genetic polymorphisms (*COMT*) would be expected to modulate tolcapone's effects.



PFC: prefrontal cortex; WM: working memory; ICR: impulsive choice ratio; DA: Dopamine

Age, estradiol, COMT methylation, and striatal DA may also influence effects of DA treatments

Future work using tolcapone will allow for the hypothesis of *COMT* x *DARPP-32* genotype indexing D1 DA-related signaling to be tested by asking if tolcapone's effects modulate working memory according to an inverted-U pattern based on genetically determined D1 DA signaling (**see Proposed Model, above**). This work will offer greater insights into the key modulators of cortical DA signaling, which is thought to underlie a variety of psychiatric disorders and may suggest that *COMT* x *DARPP-32* genotype serves as a biomarker for such disorders.

I also plan to analyze a large collection of Fallypride PET data from Vanderbilt to identify genetic predictors of D2/3 receptor availability (n>200) and dAMPH-induced DA (n>80) release in the brain. The genetic variants we identify in the PET data could then serve as candidate polymorphisms to test in behavioral and fMRI data collected previously and to be collected at **XXXX**. I also plan to build on my prior PET work into the drivers of individual differences in DA signaling using the facilities at the **XXXX** PET Center.

My proposed research program is consistent with the funding priorities at NIDA and NIAAA, which include the study of sex differences in drug abuse, neural changes associated with drug abuse/dependence, and potential genetic variants (*COMT, C957T*) associated with drug abuse risk. In addition, much of my fundamental work on individual differences in DA signaling effects would be of funding interest to NIA given that DA signaling is known to decline with age (see also Smith et al., 2017 *JCBFM*) but how this ultimately affects cognitive and decision making processes is an under-explored area of research.