NEUROCOGNITIVE INVESTIGATION OF IMMEDIATE REWARD SELECTION BIAS, A PUTATIVE INTERMEDIATE PHENOTYPE FOR ALCOHOL USE DISORDERS

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ABSTRACT

CHRISTOPHER T. SMITH: Neurocognitive Investigation of Immediate Reward Selection Bias, A Putative Intermediate Phenotype for Alcohol Use Disorders
(Under the direction of Charlotte A. Boettiger)

Immediate reward selection (or "*Now"*) bias is defined as the tendency for individuals to preferentially select a smaller, sooner reward over a larger, later reward in a delay discounting task. This behavior has been suggested as an intermediate behavioral phenotype for alcohol use disorders (AUDs). While *Now* bias has been shown to be elevated in individuals with AUDs, we provide additional support for *Now* bias as an intermediate phenotype for AUDs by showing it is enhanced in heavy drinking adults (ages 26-40) with no reported AUDs. Furthermore, we found that *Now* bias is elevated in light drinking adults with at least one first degree relative with an AUD, a key criterion in categorizing a behavior as an intermediate phenotype.

Prior work has provided insight onto the genetic basis of *Now* bias with two studies reporting a role for a polymorphism associated with prefrontal cortex (PFC) dopamine (DA) tone: the Val^{158}Met single nucleotide polymorphism (SNP) in the catechol-*O*-methyltransferase (*COMT*) gene. One study in adolescent males (Paloyelis et al., 2010) found *Now* bias to be heightened in *COMT* Met/Met individuals while another in adult males and females found *Now* bias was elevated in Val/Val individuals. We sought to further investigate the role of *COMT* Val^{158}Met genotype in *Now* bias behavior to resolve the discrepancy between these previous studies. Here, we report data showing that variables that putatively affect frontal DA tone (age,

estradiol, and *COMT* genotype) can explain differences in *Now* bias according to an inverted-U function—in other words, those with low or high PFC DA display greater *Now* bias than do individuals with intermediate levels. Furthermore, we found that individuals with lower tonic DA (*COMT* Val allele carriers) benefited most from putative increases in DA signaling (associated with increasing estradiol levels). While, the neural bases of *Now* bias and the role of DA in this behavior remain to be studied in further detail, our data suggests that considering individual differences in DA signaling according to an inverted-U model may be critical in any future treatments aimed at reducing *Now* bias with dopaminergic drugs or other interventions targeted at PFC function.

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PREFACE

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LIST OF ABBREVIATIONS

ADHD	attention deficit hyperactivity disorder
ALLO	allopregnanolone
AUC	area under the curve
AUD	alcohol use disorder
AUDIT	alcohol use disorders identification test
AUDIT-c	alcohol use disorders identification test consumption subscale
BIS	Barratt impulsivity scale
BOLD	blood oxygenation level-dependent
COMT	catechol-*O*-methyltransferase enzyme
COMT	catechol-*O*-methyltransferase gene
CON	control (SOONER, LARGER) trial types in delay discounting task
d'	d-prime
DA	dopamine
DAST	drug abuse screening test
DAT	dopamine transporter
DD	delay discounting
dPFC	dorsal prefrontal cortex
dlPFC	dorsolateral prefrontal cortex
DRD1	D1-type dopamine receptor
DRD2	D2-type dopamine receptor
DRD3	D3-type dopamine receptor
DRD4	D4-type dopamine receptor
DSM	*Diagnostic and Statistical Manual of Mental Disorders*
DUSI	drug use screening inventory
DW	DON'T WANT trial type in delay discounting task
E+	estradiol
FH	family history
FHN	family history positive for alcohol use disorder
FHP	family history positive for alcohol use disorder
fMRI	functional magnetic resonance imaging
FMT	6-[18F]-fluoro-L-*m*-tyrosine

FP	follicular phase of menstrual cycle
FTPI	future time perspective inventory
FTQ	family tree questionnaire
*i*ICR	inferred ICR
ICR	impulsive choice ratio
LOC	Rotter's locus of control scale
LP	luteal phase of menstrual cycle
MM	motor mismatch
MP	menstrual phase of menstrual cycle
MRI	magnetic resonance imaging
NAS	neuroactive steroid
OFC	orbitofrontal cortex
PET	positron emission tomography
PFC	prefrontal cortex
PROG	progesterone
RAPI	Rutger's alcohol problem index
RT	reaction time
SES	socioeconomic status
SNP	single nucleotide polymorphism
SPM	statistical parametric mapping
SUD	substance use disorder
TMS	transcranial magnetic stimulation
VNTR	variable number of tandem repeat
W	WANT trial type in delay discounting task
WM	working memory

CHAPTER 1: GENERAL INTRODUCTION

Intermediate phenotypes and the study of complex psychiatric disorders

Many psychiatric disorders including schizophrenia and depression are complex and heterogenous. The highly heritable nature of these disorders, estimated from twin studies to be anywhere from 40 to 80% (Sullivan et al., 2000; Sullivan et al., 2003), suggests that some biological processes mediated by genetics must confer risk for developing the disorder. It has been proposed that the inability to isolate strong biological bases for how genetic variation leads to complex, highly heritable diseases lies in the fact that various intermediate behaviors or traits are more closely tied to genetics associated with the disease (Rasetti and Weinberger, 2011). For example, in schizophrenia left dorsolateral prefrontal cortex function (dlPFC) hyperactivity and poorer performance in an executive function task scales with *COMT* Val^{158}Met genotype more severely in those with the disorder (Egan et al., 2001). More sophisticated analyses creating a polygenic risk score for schizophrenia (identifying genetic variations in a large sample of those with and without the disease) have found that dlPFC activity during the Sternberg Item Recognition Paradigm scales with genetic risk for schizophrenia (Walton et al., 2013b). These studies suggest that the intermediate phenotype of dlPFC hyperactivity assessed during executive function tasks has a genetic basis that explains part of the variance in schizophrenia diagnosis. As dlPFC hyperactivity is a quantifiable trait associated with genetic risk, it has been proposed as an intermediate phenotype for schizophrenia in several studies (Manoach et al., 1999; Karlsgodt et al., 2007; Walton et al., 2013b; Walton et al., 2013a). Given that substance use disorders

(SUDs) are also complex disorders with heritability estimates ranging from 40 to 60% (Heath et al., 2001; Verweij et al., 2010; Bierut, 2011; Agrawal et al., 2012), the identification of intermediate phenotypes associated with risk for these disorders is a growing focus of recent research (Karoly et al., 2013). Behavioral candidates for SUD intermediate phenotypes include reduced response inhibition (Acheson et al., 2011a; Norman et al., 2011), increased risk taking behavior (Cservenka and Nagel, 2012; Schneider et al., 2012), aberrant reward responsivity (Wrase et al., 2007; Andrews et al., 2011), and increased discounting of delayed monetary rewards (Mitchell et al., 2005; Boettiger et al., 2007; Claus et al., 2011; MacKillop et al., 2011; MacKillop, 2013).

Criteria for categorizing a behavior as an intermediate phenotype

For an intermediate phenotype to be useful it must be a quantitative, continuously variable feature or behavior that can be consistently measured. Furthermore, as these intermediate phenotypes are thought to convey genetic risk for a disorder, they should be elevated in those affected with the disorder as well as in those individuals' close relatives. Importantly, the level of these phenotypes in affected individuals and their close relatives should be shifted away from a distribution of those otherwise unaffected with no familial risk (Gottesman and Gould, 2003). For example, Egan et al. (2001) found unaffected siblings of those with schizophrenia to display executive function deficits that fell between unaffected nonrelatives and individuals with schizophrenia. A variety of criteria have come to define an intermediate phenotype in psychiatry (Almasy and Blangero, 2001; Gottesman and Gould, 2003; Waldman, 2005; Meyer-Lindenberg and Weinberger, 2006). First, the phenotype should be sufficiently heritable with genetics explaining variance in the behavior. Second, the phenotype

should have good psychometric properties as it must be reliably measurable to be a useful diagnostic. Third, the phenotype needs to be related to the disorder and its symptoms in the general population (i.e., cognitive deficits associated with dlPFC function and schizophrenia). Fourth, the phenotype should be stable over time in that it can be measured consistently with repeated testing, potentially to assess treatment effects. Fifth, the behavior should show increased expression in unaffected relatives of those with the disorder as highlighted by Egan et al. (2001), above. Sixth, the phenotype should co-segregate with the disorder in families in that a family member with the disorder should show the behavior or trait to a greater degree than an unaffected sibling and that this unaffected sibling should display the trait to a greater degree than a distant unaffected relative. Finally, the phenotype should have common genetic influences with the disorder. For example, as schizophrenia is associated with poor performance (and dlPFC hyperactivity) on executive function tasks, genes affecting dlPFC activity and executive functions such as COMT should explain variation in schizophrenia risk (see Egan et al., 2001). A common factor in many of the criteria for categorizing a behavior or trait as an intermediate phenotype is that the trait or behavior itself be partially heritable and genetically mediated.

Immediate reward selection bias as an intermediate phenotype for alcohol use disorders

As delay discounting behavior has been shown to be highly heritable (Anokhin et al., 2011; Mitchell, 2011), suggesting a strong genetic component, and is elevated in a variety of addictive behaviors (MacKillop et al., 2011), we focused our current work on this behavior. Delay discounting (DD) behavior reflects the tendency for animals to discount the value of delayed rewards in comparison to those available immediately. DD has also been referred to as immediate reward selection ("*Now*") bias as the value of rewards available immediately

supersedes waiting for a larger, delayed reward in the future (Rachlin and Green, 1972; Mazur, 1987). This behavior has been suggested to display many of the necessary criteria of an intermediate phenotype for a variety of neurobehavioral disorders including substance use disorders (SUDs) (Becker and Murphy, 1988; Reynolds, 2006; Perry and Carroll, 2008; Rogers et al., 2010), attention deficit hyperactivity disorder (Barkley et al., 2001; Sonuga-Barke et al., 2008; Paloyelis et al., 2010), and pathological gambling (Alessi and Petry, 2003; Leeman and Potenza, 2012). As these behaviors often co-occur, they may share similar biological and genetic components (Wilens, 2007; Leeman and Potenza, 2012).

An overview of various intermediate phenotype criteria for SUDs met by *Now* bias has been recently outlined (MacKillop, 2013). Particularly relevant to the current work, individuals with alcohol use disorders (AUDs) consistently display greater *Now* bias behavior versus those without AUDs (Petry, 2001; Bjork et al., 2004; Mitchell et al., 2005; Boettiger et al., 2007; Mitchell et al., 2007; MacKillop et al., 2011). Thus, *Now* bias is elevated in those individuals with an AUD (intermediate phenotype criterion 3). Conceptually, *Now* bias can be thought to have some relation to AUDs, as every relapse or excess drink represents a decision favoring immediate over delayed benefits. Furthermore, *Now* bias behavior has been shown to be heritable and associates with substance use, suggesting common genetic influences with SUDs (Anokhin et al., 2011). Importantly, *Now* bias as assessed through delay discounting (DD) tasks, has good psychometric properties (responses are highly reliable (Matusiewicz et al., 2013; Weafer et al., 2013)), suggesting it is a trait that is robust to consistent measurement (intermediate phenotype criterion 2). This is further supported by the fact that DD behavior is stable over time (Kirby, 2009). Thus, *Now* bias satisfies many of the criteria for an intermediate phenotype for AUDs.

Under-investigated criteria for Now bias as an intermediate phenotype for AUDs

As *Now* bias is elevated in those with AUDs, we might expect to see this behavior heightened in those on a trajectory toward an AUD as well. Such demonstrations between elevated *Now* bias and AUD risk would add greatly to the utility of *Now* bias as an intermediate phenotype. As problematic alcohol use during the emerging adulthood may predict development of an AUD later in life (O'Neill et al., 2001; Merline et al., 2008; Dick et al., 2011), though many individuals mature out of problematic use (Bartholow et al., 2003; Costanzo et al., 2007; Lee et al., 2013), one might expect *Now* bias is enriched in problematic drinking emerging adults. Only one relatively small behavior study has looked at such a relationship with *Now* bias observed to be heightened among heavy versus lighter social drinking college students (Vuchinich and Simpson, 1998). This finding requires replication in a larger, more diverse sample as Vuchinich and Simpson (1998) focused on students with an average age of 19 to 20.

In addition to being elevated in problematic drinking emerging adults, to satisfy another intermediate phenotype criterion for AUDs, *Now* bias behavior should also be elevated in unaffected first-degree relatives of those suffering from AUDs (intermediate phenotype criterion 5). Elevated *Now* bias in first-degree relatives of those with AUDs has yet to be adequately demonstrated, however. Most of the intermediate phenotype literature considers the expression of the behavior or trait in first-degree relatives (siblings or parents) as critical in demonstrating that behavior as an intermediate phenotype. In the field of AUDs, however, positive family history of an AUD is often defined as having at least one parent with an AUD (Acheson et al., 2011b) or father with an AUD (Crean et al., 2002; Petry et al., 2002), or some combination of parental history or sufficient density of AUD history in second degree relatives (Herting et al.,

2010). In these previous studies, the effects of family history on *Now* bias was either only observed in females (Petry et al., 2002), was not found at all (Crean et al., 2002; Herting et al., 2010), or was not present when controlling for group differences in IQ and antisocial behavior (Acheson et al., 2011b). Measuring *Now* bias behavior in individuals with any first degree relatives with AUDs expands the classic family history positive AUD definition to include siblings, who display greater genetic concordance with a particular individual than their parents. To our knowledge, though, this definition of first degree family member positive or negative for AUDs has not been applied to the study of *Now* bias. Thus, while *Now* bias possesses many properties that suggest it could be a good intermediate phenotype for AUDs, further investigation of this possibility is warranted, particularly work focusing on examining whether *Now* bias is elevated in unaffected individuals with first degree relatives with AUDs.

Biological basis of Now bias – role of genetics and frontal dopamine

Pharmacological manipulations in rodents (Dalley et al., 2008; Doya, 2008; Winstanley, 2011) suggest that the neuromodulator dopamine (DA) is an important biological regulator of *Now* bias. Additional work in humans has suggested DA as a modulator of *Now* bias behavior (de Wit et al., 2002; Mitchell et al., 2007), though results have been inconsistent (Acheson et al., 2006; Hamidovic et al., 2008; Pine et al., 2010). Genetic variations in the human DA system may explain these heterogenous findings as they are associated with individual differences in *Now* bias (Boettiger et al., 2007; Eisenberg et al., 2007; Paloyelis et al., 2010; Kelm and Boettiger, 2013). Three of these genetic studies found variations in the gene encoding the catechol-*O*-methyltransferase (COMT) enzyme to be associated with *Now* bias. COMT is an important regulator of tonic DA in the prefrontal cortex (PFC) in animals (Karoum et al., 1994; Gogos et

al., 1998; Kaenmaki et al., 2010) and humans (Chen et al., 2004; Slifstein et al., 2008; Wu et al., 2012). A single nucleotide polymorphism (Val^{158}Met SNP) in the gene encoding the COMT enzyme results in higher tonic PFC but not striatal DA levels in those with the *COMT* Met/Met SNP (Wu et al., 2012). Recently, Kelm and Boettiger (2013) found that accounting for *COMT* Val^{158}Met genotype (hence referred to as *COMT* genotype) explained substantial variance in the effects of DA depletion on *Now* bias, emphasizing the critical role of this SNP in mediating dopaminergic modulation of *Now* bias. Earlier investigations of the role of *COMT* genotype in *Now* bias behavior have produced conflicting results, however (Boettiger et al., 2007; Paloyelis et al., 2010). Paloyelis et al. (2010) found greater *Now* bias in Met/Met individuals, and Boettiger et al. (2007) found that *COMT* Val/Val individuals displayed greater *Now* bias. Further exploration of the role of *COMT* genotype is needed to resolve these seemingly divergent findings.

Quantifying Now bias behavior using Impulsive choice ratio

To assess *Now* bias in the studies that follow, we employed a previously validated delay discounting task described in detail previously (Mitchell et al., 2005; Altamirano et al., 2011). All choices were hypothetical monetary amounts which have been shown to produce similar discounting behavior as real monetary choices (Johnson and Bickel, 2002; Madden et al., 2003; Madden et al., 2004; Lagorio and Madden, 2005). Participants chose their preferred option on W trials, their non-preferred option on the DW trials, and the side with the sooner time or larger monetary amount for SOONER and LARGER trials, respectively.

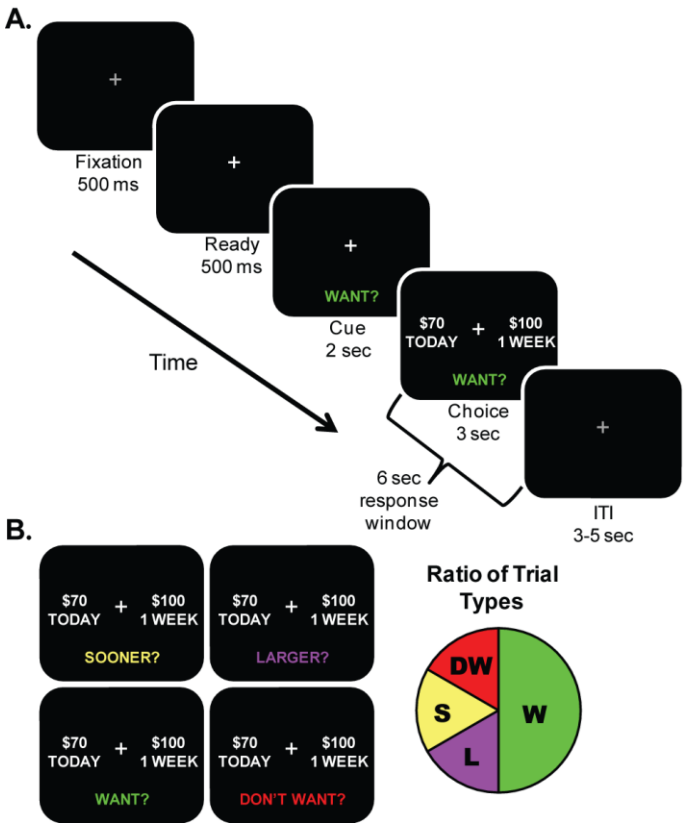

Figure 1.1: Overview of Delay Discounting (DD) Task. A. Order of events presented to participants during our delayed-discounting task. Participants are instructed to choose between two hypothetical monetary amounts: a smaller amount available Today (NOW) or a larger amount available at a delay in the future (LATER) based on the question prompt presented at the bottom of the screen. B. Question prompt types: SOONER?, LARGER?, WANT?, DON'T WANT? The proportion of question prompts presented over the course of the task is illustrated in the pie chart with WANT? trials occurring 50% of the time. From participants' WANT? trial responses, we calculate impulsive choice ration (ICR), the main dependent measure of *Now* bias in our studies. ICR is the ratio of Today WANT responses selected over the total WANT responses selected by each participant.

Our main dependent measure, the impulsive choice ratio (ICR) is calculated by dividing the number of W trials in which participants select the smaller, sooner *Now* reward over the total number of W responses made. Thus ICR can range from 0 to 1 with an ICR of 0 indicating a participant always chose the delayed, larger reward amount (*Later*) and an ICR of 1 resulting when a participant always chose the *Now* amount in the W trials.

This task has several methodological advantages to more common adjusting amount procedures (Madden et al., 1997; Richards et al., 1999) used to calculate individual indifference points for each participant. First, our task's control conditions allow us to remove participants not performing our task as instructed. Most discounting tasks employing only WANT choice prompts have no way to identify participants failing to follow task instructions. Using the discounting task employed here, each individual's reaction times (RTs) for all trial types can be plotted to determine whether they are actively evaluating the two choices during our W condition (Figure 1.2).

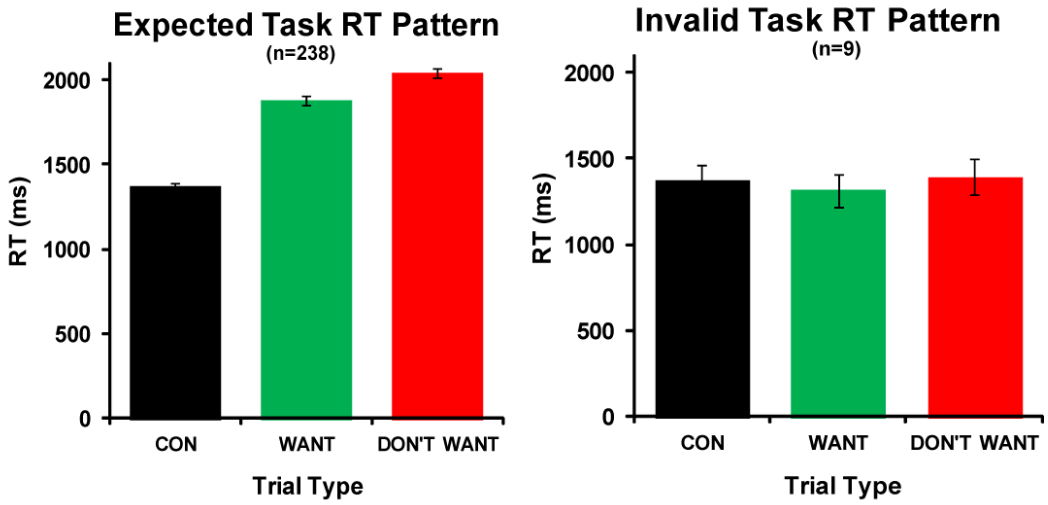

Figure 1.2: Expected versus invalid DD task reaction time patterns. Plots of expected and improper reaction time (RT) patterns from 247 participants completing the delay-discounting task used in our studies. RT for control trials are expected to be faster than for WANT and DON'T WANT trials. In addition, WANT RT is expected to be faster than DON'T WANT RT. Of the 247 participants data depicted here, ~96.4% (n=238) performed the task as expected with the remaining ~3.8% (n=9) not performing the task as expected. Participants with the invalid task RT pattern are excluded from our analyses. RT, reaction time; CON, Control (SOONER/LARGER) Trials

Participants performing the task as expected should take longer to make a response to a W trial than the control objective valuation CON trials. When participants do not show this expected pattern, we remove them from our analyses (Figure 1.2). Thus, the delay discounting task we employed insures that all *Now* bias data analyzed is from participants completing the

task as intended versus those responding reflexively and inconsistently during the task. Also, by randomly varying the delayed amount, time, and question trial types, this task allows participants to make their choices with less interference from previous choices. This differs from adjusting amount procedures where choices are titrated based on participants choices in such a way as to push them to more likely select the option they did not select previously (switching from *Now* to *Later* choices or vice versa by increasing the delayed amount or decreasing the delay time, for example). Thus, the task employed in the following studies allows for a more robust and consistent measurement of *Now* bias behavior by identifying participants not following task instructions and reduces biasing participants responses in the WANT condition by intermixing it with other choice conditions. As this task is also compatible with neuroimaging techniques (Boettiger et al., 2007; Boettiger et al., 2009), it easily also allows for investigation of the neural correlates of discounting behavior, an important future direction of the currently presented work. Importantly, ICR is highly correlated with a more traditional measure of delay discounting, *k* (Mitchell et al., 2005). In Mitchell et al. (2005), *k* was derived from the cumulative dollar ratio (CDR) for each delay time (D) according to the following equation (Mazur, 1987): CDR = 1/(1+kD) and taking the mean k across delay times. The model-free nature of ICR allows for the measurement of *Now* bias across a range of individuals whose subjective choice patterns may not conform to the selected discounting function, of which several have been proposed (Takahashi et al., 2008).

Rational for aims of current studies

Using the task outlined above to assess *Now* bias, quantified by ICR, the work presented here sought first to clarify the role of *COMT* Val^{158}Met genotype in *Now* bias behavior (Chapter

2). We hypothesized that due to age related declines in DA signaling (Mukherjee et al., 2002; Wahlstrom et al., 2010) and increases in COMT expression with age (Tunbridge et al., 2007) that *COMT*-related effects on *Now* bias may vary as a function of age. As the age range in Paloyelis et al. (2010) was 11 to 20 (average age 15.4±2) while Boettiger et al.'s (2007) average participant's age was 28.3±5.8, we reasoned age-related changes in tonic DA were the most parsimonious explanation for the divergent findings across these two studies. We recruited equal numbers of 18-21 and 22-40 year olds, determined their *COMT* genotype, and measured their *Now* bias behavior. We expected to find that *Now* bias was elevated in *COMT* Met/Met 18-21 year olds close in age to Paloyelis et al. (2010) and elevated in *COMT* Val/Val 22-40 year olds with a similar average age to Boettiger et al. (2007). We expected that taking into account age-related differences in DA tone across our groups, we could explain *Now* bias behavior across age and COMT groups via an inverted-U model.

Secondly, we sought to extend work suggesting *Now* bias to be a useful intermediate phenotype for AUDs (Chapter 3). We wanted to determine if heavy, problem drinking behavior in emerging adulthood (a risk factor for development of AUDs) was associated with elevated *Now* bias as seen by Vuchinich and Simpson (1998). Measuring *Now* bias in a larger sample of light and heavy drinkers across a wider age range (Vuchinich and Simpson (1998) ages were 19.1±1 for heavy and 19.7±2 for light drinkers) would allow us to assess if drinking status and age interact to affect *Now* bias. Additionally, age differences between Vuchinich and Simpson's (1998) two groups may have confounded their findings as the heavy drinkers tended to be younger than the light drinkers. As the Alcohol Use Disorders Identification Test (AUDIT) is an effective means of measuring problem drinking behavior (Fiellin et al., 2000; Barbor and Higgins-Biddle, 2001; Kokotailo et al., 2004), we recruited high and low AUDIT individuals

across a group of 18-40 year old social drinkers not reporting any AUD. We hypothesized that *Now* bias would be elevated in high but not low AUDIT emerging adults (defined as 18-21 or 18-24). Furthermore, we sought to test whether *Now* bias was elevated in those otherwise unaffected individuals (light/moderate social drinkers; low AUDIT) with a first degree relative with an AUD. We used the intermediate phenotype criteria of first degree biological relative status (father, mother, or sibling with AUD), excluding those with mothers with an AUD to rule out potential fetal alcohol effects. We hypothesized that *Now* bias would be elevated in low AUDIT individuals with a first degree relative with an AUD but not in those with no first degree AUD relative.

Proposed inverted-U function explaining role of PFC DA on Now bias

The interaction between age and *COMT* effects on *Now* bias that we observed in Chapter 2, led to an inverted-U model to describe the effect of prefrontal cortex (PFC) DA levels on *Now* bias that accounts for age and COMT genotype modulation of frontal DA. It has been well documented that another cognitive behavior associated with COMT genotype, working memory, follows such an inverted-U function (Malhotra et al., 2002; Cools and D'Esposito, 2011; Jacobs and D'Esposito, 2011). The proposed working model of COMT's effects on working memory posits that intermediate levels of PFC DA leads to optimal task performance and that insufficient or excess PFC DA impairs the ability of the PFC to maintain a goal representation in mind (Arnsten, 1997; Vijayraghavan et al., 2007; Arnsten, 2011). This prior work with working memory is especially relevant to *Now* bias as assessed in the current DD task as a network of PFC and posterior parietal brain structures (Boettiger et al., 2007) are more active in individuals with greater *Now* bias. These structures are also utilized in working memory tasks (Owen et al.,

2005) and show heightened activity with increasing task difficulty (Braver et al., 1997; Manoach et al., 1997). At least one study has suggested that working memory is negatively correlated with *Now* bias (Shamosh et al., 2008) and depletion of DA effects on working memory performance correlated with its effects on *Now* bias (Kelm and Boettiger, 2013). Thus, examining the role of PFC DA on *Now* bias according to models from the working memory literature seemed to be an important concept to probe further. Specifically, we hypothesized that the elevated *Now* bias associated with inefficient PFC function (Boettiger et al., 2007) could be partially explained by PFC DA levels according to an inverted-U model with insufficient or excess PFC DA resulting in elevated *Now* bias.

Testing the inverted-U model as a predictor of Now bias behavior change

To test the implications of the proposed inverted-U as a potential means of predicting dopaminergic modulation of *Now* bias, in Chapter 4, we investigated whether a dynamic factor that interacts with frontal DA can shift *Now* bias within individuals. Specifically, we took advantage of the fact that DA signaling increases with acute estradiol administration (Becker, 1990) and with naturally varying estradiol over the estrus cycle (Xiao and Becker, 1994) in rodents. In naturally cycling human females, it has been shown that increasing estradiol and associated dopaminergic signaling can impair working memory performance in *COMT* Met/Met individuals with high tonic PFC DA while improving performance in those with *COMT* Val/Val genotype (Jacobs and D'Esposito, 2011). Thus, estradiol's effects on a PFC DA-dependent process follows an inverted-U function when taking tonic PFC DA levels into account. In our final study, we assessed *Now* bias behavior in naturally-cycling female participants at the menstrual and follicular phase in their menstrual cycle in a counterbalanced, within-subject

design. We hypothesized that COMT Val^{158}Met genotype would moderate the relationship between increasing dopaminergic signaling associated with rising estradiol from menstrual to follicular phase and changes in *Now* bias. We reasoned our COMT and estradiol effects on *Now* bias would follow an inverted-U model as seen with estradiol × COMT effects on working memory (Jacobs and D'Esposito, 2011). This demonstration of an inverted-U model for the role of PFC DA on *Now* bias would have important implications for developing individualized treatments for reducing *Now* bias. We reason that taking account of where an individual's PFC DA tone sits on the inverted-U curve would affect which treatment options should be considered – either augmentation or suppression of PFC DA signaling – to reduce *Now* bias behavior.

CHAPTER 2: AGE MODULATES THE EFFECT OF COMT GENOTYPE ON DELAY DISCOUNTING BEHAVIOR[1]

INTRODUCTION

Humans and other animals tend to discount the value of delayed, relative to immediate rewards, a phenomenon known as delay-discounting (Ainslie, 1975; Mazur, 1987; Frederick et al., 2002; Green and Myerson, 2004). Delay-discounting is heightened among individuals with a history of substance use disorders (Bickel and Marsch, 2001; Reynolds, 2006), as well as other impulse control disorders, such as attention deficit/hyperactivity disorder (ADHD) (Sagvolden and Sergeant, 1998; Winstanley et al., 2006; Paloyelis et al., 2009). Such immediate reward bias represents one facet of the multi-dimensional construct of impulsivity (Evenden, 1999). A variety of evidence links delay-discounting to dopamine (DA) and DA-modulated frontostriatal circuits (Boettiger et al., 2007; Doya, 2008; Kobayashi and Schultz, 2008; Lee et al., 2009; Paloyelis et al., 2010; Pine et al., 2010; Altamirano et al., 2011; Adriani et al., 2012). Such evidence includes data showing that variation in the gene encoding catechol-*O*-methyltransferase (COMT) is associated with differences in the tendency to choose immediate over delayed rewards (Boettiger et al., 2007; Paloyelis et al., 2010). COMT is an enzyme that regulates DA levels in the prefrontal cortex (Gogos et al., 1998; Tunbridge et al., 2004; Yavich et al., 2007;

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Kaenmaki et al., 2010), where it is the primary regulator of DA levels (Karoum et al., 1994; Kaenmaki et al., 2010). A polymorphism in the COMT gene (*COMT Val^{158}Met; rs4680*) causing a valine (Val)-to-methionine (Met) substitution at codon 158 results in a 4-fold reduction of COMT enzymatic activity (Lachman et al., 1996), which is presumed to result in reduced cortical DA in Val/Val homozygotes relative to the Met/Met genotype (Chen et al., 2004).

We have previously reported that *COMT Val^{158}Met* genotype predicts variation in delay-discounting behavior in adult humans, including those with a history of alcoholism; specifically, those with the Val/Val genotype demonstrate greater delay discounting than do met-allele carriers (Boettiger et al., 2007). In contrast, a recent study of male adolescents with and without ADHD found that those with the Met/Met genotype demonstrate greater delay-discounting than do Val-allele carriers (Paloyelis et al., 2010). The sample size in our 2007 study was rather small, thus, in the present study, we sought to confirm our earlier finding in a larger sample. In addition, we sought to determine whether the relationship between *COMT* genotype and impulsive choice changes from late adolescence to adulthood. Several measures of frontal DA neurotransmission decrease from adolescence to adulthood (see (Wahlstrom et al., 2010) for recent review). Moreover, COMT expression increases with age in humans (Tunbridge et al., 2007), which should contribute to reduced frontal DA signaling from adolescence to adulthood. Behaviors that depend on frontal DA commonly operate within a range of optimal functioning, with both excessive and deficient levels of DA impairing behavioral performance (Goldman-Rakic, 1998). Thus, an increase in COMT with age could mean that the low activity *COMT* genotype could yield an "overdose" of DA in adolescence, but a more optimal level in adulthood, whereas the high activity *COMT* genotype may compensate for other aspects of enhanced DA signaling in adolescence, but produce a DA deficit in adulthood, as DA signaling

declines. Specifically, we hypothesized that the effects of genetically determined variation in COMT function on delay-discounting behavior are oppositely modulated by age, specifically from late adolescence to young adulthood. To test this hypothesis, we genotyped late adolescent and adult participants for the *COMT Val*^{158}*Met* polymorphism, measured their delay-discounting behavior, and tested for interacting effects of age group and *COMT* genotype on discounting behavior. As cognitive studies commonly consider participants 18 and over to be adults, we were particularly interested in testing this hypothesis in late adolescents that are frequently assumed to be adults (ages 18-21 years).

METHODS

Subjects

Participants (n = 142) were recruited from the University of North Carolina, Chapel Hill (UNC) and surrounding community. Participants were healthy individuals 18-40 years old with no known past or present neurological or psychiatric diagnoses, no history of substance use disorders, and no current use of psychoactive medications or other psychoactive substances aside from moderate caffeine, nicotine or alcohol. All subjects were native English speakers, had at least a high-school education, and reported having consumed alcohol at least once in their lifetime. Participants were recruited into one of two age groups: late adolescents (18-21 years; n = 72) or adults (22-40 years; n = 70). These age group criteria were based on preliminary results from other studies in our lab indicating behavioral differences in our task between these two age groups. This age cutoff is supported by a recent large scale investigation of functional brain maturation that indicated that brain maturation asymptotes at approximately age 22 (Dosenbach et al., 2010). Information regarding participants' personal and parental occupation and education

was collected via a questionnaire and quantified as Hollingshead socioeconomic status SES scores (Hollingshead, 1975). Participants gave written informed consent, as approved by the UNC Office of Human Research Ethics. Subjects received monetary compensation for participating.

Delay Discounting Task

The paradigm was based on a previously described task (Mitchell et al., 2005; Boettiger et al., 2007; Mitchell et al., 2007; Altamirano et al., 2011). Briefly, in each session, subjects completed a short (~4 min) practice run and then 8 full runs of approximately 42 or 43 trials each (~7 min). There were four trial types: WANT (W), DON'T WANT (DW), SOONER, and LARGER. Trial types were randomly ordered and weighted such that 50% were W condition trials and the remaining trials were evenly divided between the other conditions. Trials began with an instruction cue, followed by two options, each of which was a monetary value and a time. Subjects were asked to evaluate the options as if they would actually receive the specified amounts at the corresponding times. The options consisted of one of five "full" amounts ($2, $5, $10, $20, or $100) at one of five future delays (1 week, 2 weeks, 1 month, 3 months, or 6 months) and a discounted amount (70, 85, 90, or 95% of the "full" amount) offered at no delay ("TODAY"). Subjects were instructed to make a choice in each trial, according to the trial type: preferred option on W trials, non-preferred option on DW trials, and the side with the sooner time or larger amount of money for SOONER and LARGER trials, respectively. These latter two conditions are considered together as control ("CON") trials. The order of trial types was the same for all subjects; however, the delayed amount, delay time, and discount were pseudorandomly ordered. The length of the task (~56 min) could raise the concern that choice behavior is affected by fatigue or other temporally-dependent effects. This could be a particular

concern if such effects varied with age or genotype. These concerns can be dismissed on the basis of the following analyses. First, a repeated measures ANOVA found no significant effect of block number on ICR ($F_{(7, 917)} = 0.49$, $p = 0.84$), nor any significant ICR by block interaction with age ($F_{(7, 917)} = 0.31$, $p = 0.95$) or genotype ($F_{(14, 917)} = 0.22$, $p = 1$). Second, when we calculated the split-half ICR for odd and even blocks, the correlation across all subjects was $r = 0.98$ ($p < 0.001$). Similarly, the correlation between first half ICR and second half ICR was $r=0.96$ ($p < 0.001$). Finally, for the sample as a whole, Cronbach's $\alpha=0.99$. Furthermore, Cronbach's α was also 0.99 when calculated separately for each age group and each genotype. We note that these reliability measures are well above the standard criterion for adequate reliability of 0.70 (Kline, 2000).

Genotyping

COMT Val^{158}Met (rs4680) genotyping was performed on DNA extracted from saliva samples (DNA Genotek, Kanata, Ontario, Canada) using TaqMan technology (Applied Biosystems, Foster City, CA), as described previously (Boettiger et al., 2007). Genotyping was performed by the UNC Mammalian Genotyping Core and/or the Duke Center for Human Genetics. Genotyping was performed in duplicate for n=42 samples and compared to ensure validity of the data. The genotype concordance rate was 100% both within (n = 42) and across (n = 32) genotyping facilities. Allele frequencies in this sample did not deviate from Hardy–Weinberg equilibrium ($\chi^2 = 0.098$, df = 2, $p = 0.95$).

Data Analysis

Our index of temporal discounting was the proportion of "TODAY" choices in W trials, which we have termed the impulsive choice ratio (ICR). Although this value was calculated

separately according to delay time and delayed amount, here we focus on the ratio collapsed across all W trials.

To test the significance of across group comparisons, we used unpaired two-tailed *t*-tests for continuous measures and χ^2 tests for categorical measures. For multi-factorial comparisons, we used regular or mixed repeated measures ANOVA in SPSS (SPSS Inc., Chicago, IL), with age group and genotype as between subjects factors. When necessary, a Greenhouse-Geisser non-sphericity correction was applied. Post-hoc paired comparisons were performed where indicated using two-tailed *t*-tests. When data were not normally distributed, appropriate arcsine-root transformations were applied in Excel (Microsoft Corp., Redmond, WA) prior to making statistical comparisons to ensure the validity of parametric statistical tests. Simple regression analyses were performed in SPSS.

RESULTS

Demographic and psychometric data

To test whether *COMT* $Val^{158}Met$ genotype differentially predicts ICR among late adolescents versus adults, we genotyped two groups of subjects: late adolescents (18-21 years; $n = 72$) and adults (22 - 40 years; $n = 70$). There were no significant differences between the two groups in terms of gender, ethnicity, or parental socio-economic status (SES; see Table 2.1). As expected, the late adolescent group was significantly younger than the adult group, and also reported lower personal SES levels (driven primarily by a lower level of education), and slightly greater alcohol use (AUDIT score) than did the adult group (Table 2.1).

Table 2.1: Demographic data by age group

	Late Adolescent (ages 18-21) (n = 72)	Adult (ages 22-40) (n = 70)	$t_{(140)}$	p value
Age (yrs)	20 ± 1	27 ± 5	11.53	*<0.001*
Education (yrs)	14 ± 1	17 ± 2	11.90	*<0.001*
Subject Hollingshead SES	40 ± 3	47 ± 7	7.49	*<0.001*
Parent Hollingshead SES	54 ± 9	54 ± 11	0.13	*ns*
Sex (% female)	53	54		*ns*†
Ethnicity (% white)	62	66		*ns*†
Black (%)	13	19		*ns*†
Hispanic (%)	4	4		*ns*†
Asian (%)	14	7		*ns*†
Other/mixed (%)	7	4		*ns*†
AUDIT score	8.9 ± 6.3	7.2 ± 4.1	1.99	0.049

Values are reported as mean ± standard deviation. Reported p-values reflect the results of unpaired two-tailed comparisons between groups. Exact p-values reported unless $p < 0.001$. AUDIT, Alcohol Use Disorders Identification Test; SES, socioeconomic status. †p-value represents results of χ^2 test.

Interaction between age and COMT genotype on frequency of impulsive choices

On the basis of *COMT Val*^{158}*Met* genotype, participants were subdivided into Met-homozygotes (Met/Met; n = 33), heterozygotes (Val/Met; n = 69) and Val/Val homozygote individuals (n=40). *COMT* genotype groups did not differ significantly in terms of demographic features (education, age, sex, ethnicity, SES, and alcohol use), or task performance (control reaction times and control trial accuracy; Table 2.2). We did observe a main effect of *COMT* genotype on WANT trail reaction time, however (Table 2.2). On the basis of our *a priori* hypothesis for an age×genotype interaction in delay-discounting behavior, we conducted a factorial ANOVA with age group and *COMT Val*^{158}*Met* genotype as between-subjects factors, taking an index of delay-discounting, the impulsive choice ratio (ICR; see Methods), as the dependent measure. Although our groups were matched for sex (Tables 2.1 and 2.2), we included sex as a factor in our analyses as a means of detecting sexually dimorphic effects of *COMT* on

delay discounting, as sex-dependent effects on COMT enzyme activity have been reported (Chen et al., 2004). We included SES, alcohol use (AUDIT score), and WANT trial reaction time as covariates due to the fact that we observed significant differences in SES and AUDIT scores between our age groups (Table 2.1) and WANT trial RT between our *COMT* groups (Table 2.2).

Table 2.2: COMT genotype groups: demographics and task performance

	V/V (n=40)	V/M (*n* = 69)	M/M (*n* = 33)	$F_{(2,139)}$	*p*-value
Demographics					
Age (yrs)	24 ± 5	23 ± 6	23 ± 5	0.57	*ns*
Education (yrs)	16 ± 2	15 ± 2	15 ± 2	1.85	*ns*
Hollingshead SES	44 ± 7	43 ± 6	45 ± 7	0.35	*ns*
Sex (% female)	45	54	64		*ns*†
Ethnicity (%white)	50	68	73		*ns*†
Black (%)	25	15	6		*ns*†
Hispanic (%)	7.5	1	6		*ns*†
Asian (%)	12.5	10	9		*ns*†
Other/mixed (%)	5	6	6		*ns*†
AUDIT Score	8.5 ± 5.0	7.5 ± 5.0	8.6 ± 6.6	0.67	*ns*
Task performance					
CON Trial Acc	96.6 ± 3.9	97.6 ± 2.5	97.1 ± 2.7	1.60	*ns*
CON Trial RT	1325 ± 306	1363 ± 289	1375 ± 314	0.29	*ns*
WANT Trial RT	1701 ± 380	1890 ± 420	1904 ± 377	3.41	*0.036*

Values are reported as mean ± standard deviation. Reported *p*-values reflect the results of unpaired two-tailed comparisons between groups. Exact *p*-values reported unless $p < 0.001$. Acc, accuracy; AUDIT, Alcohol Use Disorders Identification Test; COMT, catechol-*O*-methyltransferase; CON, control; M/M, methionine/methionine; RT, reaction time; SES, socioeconomic status V/M, valine/methionine; V/V, valine/valine. †*p*-value represents results of χ^2 test.

In a 2×2×3 ANOVA (age group × *COMT* genotype × sex), we did not detect significant main effects of age group ($F_{(1, 127)} = 0.81$, $p = 0.371$, $\eta^2 = 0.005$), *COMT Val*^{158}*Met* genotype ($F_{(2, 127)} = 0.10$, $p = 0.908$, $\eta^2 = 0.001$) or sex ($F_{(1, 127)} = 1.22$, $p = 0.272$, $\eta^2 = 0.008$) on ICR. Moreover, we observed no significant interaction between sex and *COMT* genotype ($F_{(2, 127)} = 1.00$, $p = 0.371$, $\eta^2 = 0.013$), or three-way interaction between sex, *COMT* genotype, and age

group ($F_{(2, 127)}$ = 0.43, p = 0.650, η^2 = 0.006). In contrast, consistent with our hypothesis, there was a significant age-by-*COMT Val*^{158}*Met* interaction effect on delay-discounting behavior. ($F_{(2, 127)}$ = 5.12, p= 0.007, η^2 = 0.069; Figure 2.1). We also detected a smaller interactive effect between sex and age-group ($F_{(1, 127)}$ = 4.03, p = 0.047, η^2 = 0.027).

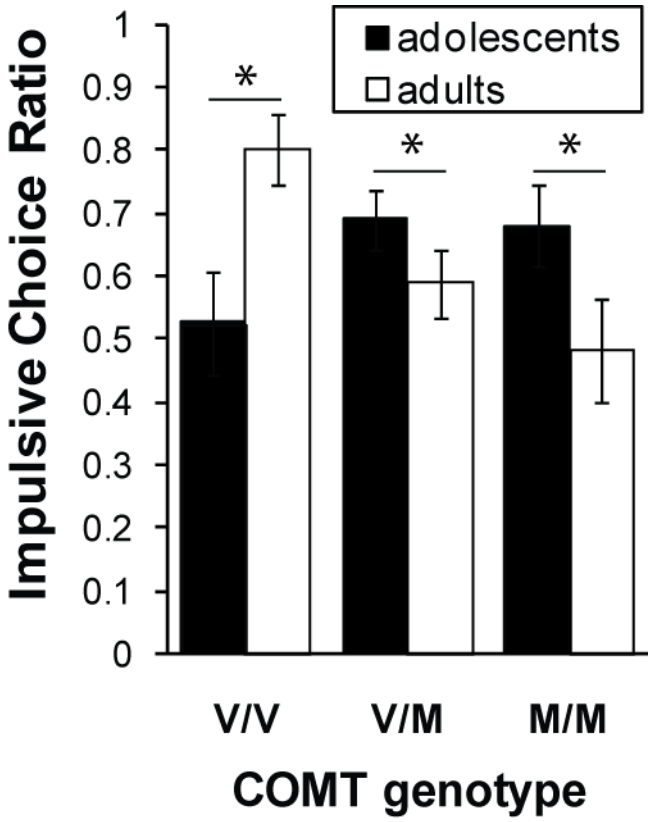

Figure 2.1: Age interacts with *COMT* genotype to influence impulsive decision-making. (a) Plot of impulsive choice ratio (ICR) as a function of *COMT* genotype, showing a significant age by genotype interaction ($F_{(2,127)}$ = 5.12, p=0.007). This effect reflects significant age-related changes in ICR for all three genotypes. M/M, methionine/methionine; V/M, valine/methionine; V/V, valine/valine. *p < 0.05.

Given our somewhat ethnically mixed sample (see Tables 2.1 and 2.2) and the reported racial differences in *COMT Val*^{158}*Met* allele frequencies (e.g. (McLeod et al., 1994; McLeod et al., 1998), it is worth noting that the age-by-*COMT Val*^{158}*Met* interaction was also seen in our white participants, the largest ethnic group included ($F_{(2, 82)}$ = 11.77, p < 0.001, η^2 = 0.209). Among non-white participants, we observed a similar, albeit non-significant, trend ($F_{(2, 42)}$ = 1.61,

p = 0.21, η^2 = 0.065), likely due to the heterogeneity within this smaller group. This interaction effect reflects a significant age-related increase in delay-discounting among Val-homozygotes ($t_{(38)}$ = 2.48, p = 0.018) and a significant age-related decline in delay-discounting among both Met-homozygotes ($t_{(31)}$ = 2.20, p = 0.036) and *COMT Val*^{158}*Met* heterozygotes ($t_{(67)}$ = 2.01, p = 0.048). Considering age as a continuous variable, we observed a negative correlation between age and ICR among Met-allele carriers (r = -0.31, p = 0.001). In contrast, among Val/Val individuals we observed a significant positive correlation between age and ICR (r = 0.32, p = 0.047).

While we have previously reported that choice behavior in this task does not correlate with education or SES (Mitchell et al., 2005; Mitchell et al., 2007), the present study included a somewhat younger demographic. Thus, we carried out bivariate correlations to assess whether demographic factors predicted decision-making behavior in this cohort of participants. We found that the tendency to choose a smaller immediate reward did not correlate with years of education (r = -0.09, t = -1.04, p = 0.299). Similarly, SES was not significantly correlated with ICR (r = -0.14, t = -1.66, p=0.1). We also observed no correlation between age and ICR (r = -0.14, t = -1.62, p = 0.107), which is not unexpected based on the opposing age effects for Met-carriers and Val homozygotes.

The mean overall ICR values (shown in Figure 2.1), including SD, were as follows for the Late Adolescent group: Val/Val, 0.56 ± 0.39; Val/Met, 0.70 ± 0.24; Met/Met, 0.70 ± 0.28. Corresponding values for the Adult group were: Val/Val, 0.79 ± 0.21; Val/Met, 0.57 ± 0.31; Met/Met, 0.45 ± 0.32. We have previously reported that discounting in this task is modulated by reward magnitude (Mitchell et al., 2005; Mitchell et al., 2007), such that participants show greater discounting for smaller rewards. This finding was replicated in the present study (Figure

2.2) as shown by a 3-way ANOVA (delayed reward amount × age group × genotype), which found a significant main effect of delayed reward amount ($F_{(2.15, 292.96)} = 146.24$, $p < 0.001$, $\eta^2 = 0.512$).

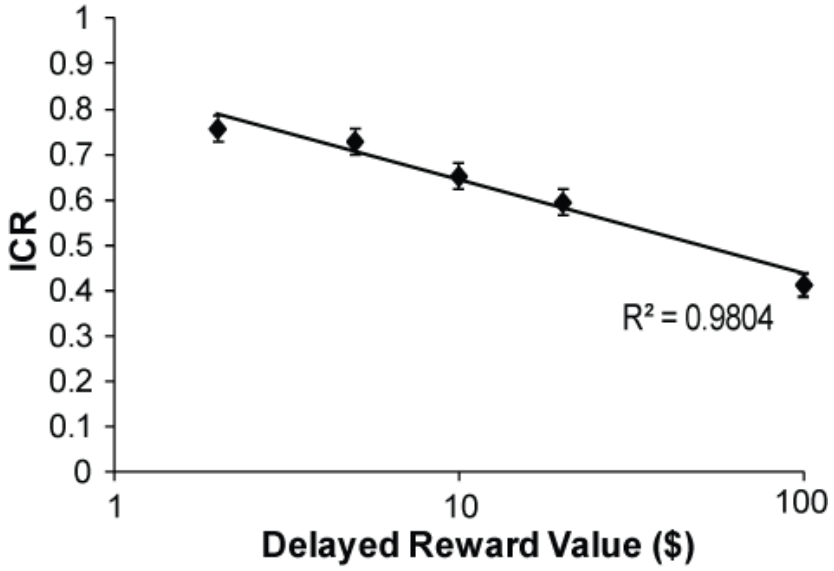

Figure 2.2: Comparison of reward magnitude discounting functions. Semi-log plot of ICR as a function of the delayed reward amount. Data reflect mean ± SEM. Curves represent logarithmic fit the group averaged data.

We did not observe significant interactions between delayed reward amount and either age group ($F_{(2.15, 292.96)} = 0.79$, $p = 0.465$, $\eta^2 = 0.003$) or genotype ($F_{(4.31, 292.96)} = 0.67$, $p = 0.624$, $\eta^2 = 0.005$). Likewise, we observed no significant three-way interaction ($F_{(4.31, 292.96)} = 0.66$, $p = 0.632$, $\eta^2 = 0.005$). Thus the genotype × age group interaction that we observed does not appear to be specific to certain magnitudes of reward.

DISCUSSION

The present results confirm our previous finding of enhanced delay-discounting among *COMT Val*^{158}*Met* Val/Val adults relative to Met-allele carriers (Boettiger et al., 2007). Moreover, these results help account for the discrepancy between our earlier findings and the results of

Paloyelis et al. (2010) showing enhanced delay-discounting among *COMT Val^{158}Met* Met/Met adolescent males. Furthermore, as our study included females and found no main effects of sex, nor any interaction of sex with our genotype by age effect, these data extend the previous finding in adolescent males of Paloyelis et al. (2010) to late adolescent females.

Relationship between alcohol use and delay-discounting

We previously found a significant positive relationship between ICR and alcohol use in studies including both control subjects and those with a history of alcoholism (Mitchell et al., 2005; Boettiger et al., 2007; Mitchell et al., 2007). We have also reported a positive correlation between ICR and the dependence and harm subscales of the AUDIT (Mitchell et al. 2005). However, consistent with the present data, we have not found a significant relationship between ICR and AUDIT scores in studies restricted to those with no history of alcoholism (Altamirano et al., 2011). This discrepancy may reflect inadequate power to detect an effect due to insufficient variance in AUDIT dependence and harm scores in samples excluding AUDs. For example, the median AUDIT dependence and harm score here was 2 (interquartile range: 1 - 4.75). In contrast, the median in Mitchell, et al. (2005) was the same, but the interquartile range was more >3× larger (0.75 - 16). Alternatively, the relationship between ICR and AUDIT may be weak.

Cortical dopamine regulation of delay-discounting

Consistent with our hypothesis, we found that age modulates *COMT Val^{158}Met* genotype effects on delay-discounting behavior. Adult met-allele carriers showed significantly less delay-discounting relative to late adolescent met-carriers, while Val/Val adults showed significantly

more delay-discounting relative to late adolescent Val/Val individuals. To account for both the adolescent and adult data we report, we propose a single U-shaped model of the relationship between frontal DA levels and impulsive choice (Figure 2.3).

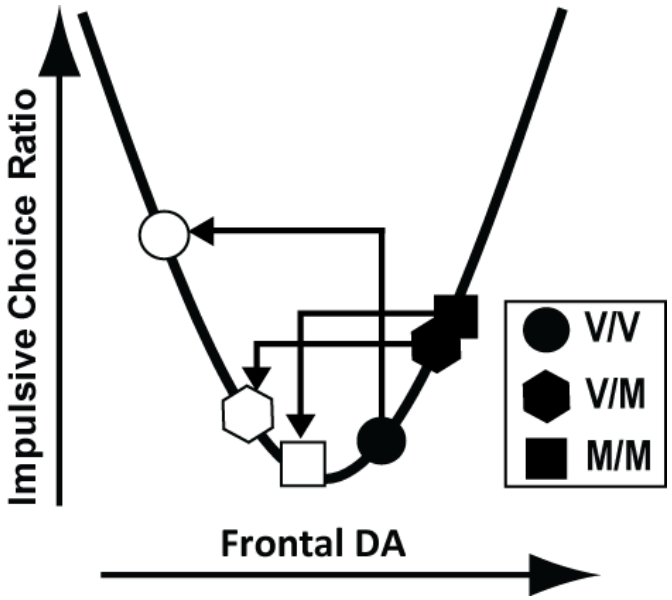

Figure 2.3: Model depicting hypothetical regulation of impulsive choice by frontal dopamine. Closed symbols represent adolescents, whole open symbols represent adults. Arrows indicate the effects of a developmental decline in frontal dopamine function for each genotype. Model posits that reduced frontal DA signaling in adulthood relative to late adolescence results in opposing effects on impulsive choice that vary with *COMT* genotype, based on an underlying U-shaped relationship. An age-dependent drop in frontal DA signaling in Val/Val individuals is predicted to yield greater ICR in adults relative to adolescents. In contrast, an equivalent age-dependent drop in frontal DA signaling is predicted to result in reduced ICR in Met-carrier adults relative to adolescents. For simplicity, equivalent declines in frontal DA signaling are proposed for all COMT genotypes, although differential changes may occur. *COMT*, catechol-*O*-methyltransferase; DA, dopamine; M/M, methionine/methionine; V/M, valine/methionine; V/V, valine/valine.

Such a model is supported by evidence that dopaminergic modulation of frontal functions often follows a U-shaped curve, where deficient *or* excess DA can impair frontal functioning (Arnsten, 1997; Zahrt et al., 1997; Goldman-Rakic et al., 2000; Williams and Castner, 2006). Our model posits that reduced frontal DA signaling in adulthood relative to late adolescence results in opposing effects on impulsive choice in different *COMT* genotypes, based on this U-

shaped relationship. Specifically, an age-dependent drop in frontal DA signaling in Val/Val individuals yields greater ICR in adults relative to adolescents. In contrast, an equivalent age-dependent drop in frontal DA signaling results in reduced delay-discounting in Met-carrier adults relative to adolescents. For simplicity, we have proposed equivalent declines in frontal DA signaling across *COMT* genotypes; however, developmental declines in frontal DA signaling may vary by *COMT* genotype. Support for this possibility comes from recent data showing *COMT Val*^{158}*Met* genotype-dependent methylation resulting in reduced Val allele expression (Ursini et al., 2011). Developmental regulation of methylation is one mechanism by which declines in frontal DA signaling could vary by *COMT* genotype. An important prediction of this model is that within typical "healthy young adult" samples (ages18-40), the admixture of late adolescents and young adults would tend to obscure *COMT* genotype effects. While this model is currently hypothetical, future PET studies may test the validity of this U-shape model of the effects of age-dependent differences in frontal DA signaling on impulsive choice.

One remaining important question is whether age-dependent differences in *COMT* genotype effects on decision-making behavior differ among different ethnic groups. While the present data conclusively find an age by *COMT* genotype interaction among white participants, they lack sufficient power to draw this same conclusion for other ethnic groups. Heterogeneity within the non-white sample may contribute to this lack of power. Moreover, the relationship between age and discounting behavior could vary with ethnicity, introducing another source of variance. Larger explicit studies of the effects of ethnicity may resolve this question.

Broader implications of age-dependent differences in COMT genotype effects

In addition to playing a role in modulating impulsive choice, the Val/Val genotype is also associated with poorer performance on executive tasks and greater frontal activation relative to Met-carriers (Egan et al., 2001; Blasi et al., 2005; Minzenberg et al., 2006; Winterer et al., 2006a; Winterer et al., 2006b; Tan et al., 2007), which is thought to reflect frontal processing inefficiency, particularly during tasks requiring maintenance of stable representations (Bilder et al., 2004; Nolan et al., 2004; Tunbridge et al., 2006). However, published data regarding the role of the *COMT Val*^{158}*Met* genotype in cognition is mixed (Barnett et al., 2008; Dennis et al., 2010). Thus, in addition to reconciling the literature regarding the effect of *COMT Val*^{158}*Met* genotype on delay-discounting behavior, these data also suggest an explanation for discrepancies in the literature regarding *COMT* genotype effects on executive function.

We note that the present study was cross-sectional in nature; a prospective study is required to determine whether the age modulation of *COMT* genotype effects on delay-discounting reflects developmental processes. Such processes may specifically affect delay-discounting behavior or may also impact linked behaviors, such as working memory (Shamosh and Gray, 2008; Shamosh et al., 2008). Specificity of underlying neural circuits may result in task-dependent sensitivity to frontal DA levels, whereby the optimal level for certain tasks is sub-optimal for other tasks (Cools and Robbins, 2004; Nolan et al., 2004). Moreover, frontal circuit maturation remains incomplete until the early-to-mid twenties (Sowell et al., 1999; Casey et al., 2000; Sowell et al., 2001; Giedd, 2004; Gogtay et al., 2004; Lenroot and Giedd, 2006),, and components of this circuitry critical for particular tasks may mature at different rates.

Implications for effects of manipulating DA signaling

As noted earlier, several measures of frontal DA signaling decrease from adolescence to adulthood (see (Wahlstrom et al., 2010) for recent review). COMT expression increases across the lifespan in humans (Tunbridge et al., 2007), which should result in an age-dependent decrement in frontal DA. An important implication of the age-dependent differences in *COMT* genotype effects on delay-discounting is that accounting for both age and COMT genotype may be required to accurately predict the effects of medications that alter frontal DA. Relevant clinical disorders are those associated with impaired frontal DA function, such as schizophrenia, addiction, and ADHD. As these disorders frequently onset in late adolescence (or sooner, in the case of ADHD), understanding how age may impact medication response could help to optimize clinical outcomes for these conditions.

Study limitations

A limitation of the present study is that it cannot completely reconcile the differences between the findings of Paloyelis et al. (2010) and Boettiger et al. (2007), since the adolescent group in the present study did not include participants younger than 18, as did that of Paloyelis and colleagues. Another limitation is the lack of investigation of other genetic variations that may impact delay-discounting behavior, a substantially heritable trait (Anokhin et al., 2011; Mitchell, 2011). For example, the DA D_4 receptor (DRD4) and D_2 receptor (DRD2) genes have been linked to variation in delay-discounting behavior (Eisenberg et al., 2007), although these findings are not unequivocal (White et al., 2008; White et al., 2009; Paloyelis et al., 2010). As such it is important to consider these results primarily as further evidence that proxy indicators of frontal DA signaling can predict some of the individual differences in delay-discounting. In

addition, our results highlight the importance of considering age as a possible confounding factor in future studies evaluating genetic contributions to delay-discounting behavior. Future studies designed to test for interactions between *COMT* and other polymorphisms in adults may help to clarify the interacting roles for frontal and striatal DA signaling in regulating delay discounting behavior; such studies will also require larger sample sizes than that reported here. Beyond age, we did not find additional environmental variables that accounted for substantial variance in discounting behavior within our sample. However, future larger-scale studies that explicitly test for effects of alcohol use, gender, as well as related cognitive phenotypes may allow for a more complete understanding of the neurobiology of discounting behavior. In particular, measures of working memory, reward sensitivity, and response inhibition may each be regulated by separate DA-regulated networks, which in turn make differing contributions to delay-discounting behavior.

CHAPTER 3: INTERTEMPORAL CHOICE BEHAVIOR IN EMERGING ADULTS AND ADULTS: EFFECTS OF AGE INTERACT WITH ALCOHOL USE AND FAMILY HISTORY STATUS

INTRODUCTION

Adults with addictive disorders, including alcohol use disorders (AUDs), tend to choose smaller, sooner over larger, delayed rewards in the context of delay-discounting (DD) tasks more frequently than do adults with no addiction history (Petry, 2001; Mitchell et al., 2005; MacKillop et al., 2011). This immediate reward selection (or "*Now*") bias persists even after years of abstinence and does not correlate with abstinence duration (Mitchell et al., 2005), suggesting irreversible consequences of chronic alcohol abuse and/or a pre-existing risk trait, or intermediate phenotype (Meyer-Lindenberg and Weinberger, 2006; MacKillop, 2013). If the latter were true, we would predict heightened *Now* bias among young people who engage in at-risk drinking but who do not meet clinical criteria for alcohol dependence, relative to age-matched moderate drinkers. We would also predict heightened *Now* bias among moderate drinkers with problem-drinking first degree relatives.

We have previously found marked *Now* bias among emerging adults (18-25 yrs), regardless of drinking behavior (Kelm et al., 2010). This suggests elevated DD generally among individuals transitioning from adolescence to adulthood. The observation that adult controls (average age of 26-28) with no AUD diagnosis display reduced *Now* bias compared to abstinent alcoholic adults (Mitchell et al., 2005; Boettiger et al., 2007) suggests that this bias should decline between emerging adulthood and adulthood, at least among moderate, non-problem

drinkers. While emerging adults are widely regarded as impulsive (Chambers and Potenza, 2003; de Wit, 2009), and DD normally decreases from childhood to the early 30's (Green, 1994; Scheres et al., 2006; Olson et al., 2007; Eppinger et al., 2012), little is known about specific changes in DD from late adolescence to adulthood. Some data show trait impulsivity declining linearly with age from early adolescence to age 30 (Steinberg et al., 2008). Thus, given positive correlations between DD and trait impulsivity (Mitchell et al., 2005; de Wit et al., 2007), DD should decline with age from adolescence into the 30s, but, to our knowledge, no prior studies have explicitly investigated age effects on DD in detail from ages 18 to 40. Moreover, we do not know whether heavy alcohol use moderates any such age-related changes in DD.

Thus, we tested whether an age-related decrease in DD occurs from emerging adulthood to adulthood, and whether any such age effect on DD is absent among sub-clinical heavy drinkers. To do so, we recruited individuals with no substance use disorder (SUD) history who had consumed alcohol at least one time previously into one of two age groups: late adolescents (18-21) and adults (22-40). We initially recruited across these age groups as we have found that a genetic regulator of *Now* bias differentially affects DD behavior in these groups (Smith and Boettiger, 2012). We wanted to recruit in such a way as to control for this effect. Within each age group, we recruited equal numbers of light/moderate drinkers and heavy, possible problem drinkers, based on Alcohol Use Disorders Identification Test (AUDIT) scores. Low AUDIT individuals were defined as AUDIT scores <8 for males and <5 for females , and high AUDIT individuals were defined as AUDIT scores ≥8 for males and ≥5 for females (Neumann et al., 2004). We quantified DD using a previously validated task (Mitchell et al., 2005; Altamirano et al., 2011) and assessed the effects of age, alcohol use, their interaction, and family history (FH) of alcoholism on *Now* bias.

METHODS

Participants

Participants (*n*=246; ~50% female) aged 18-40 were recruited from the University of North Carolina (UNC) and surrounding community. We recruited participants based on AUDIT (Saunders et al., 1993) scores, age, and sex. High AUDIT groups were defined by AUDIT scores ≥8 for males, and ≥5 for females (*n*=142, mean: 11.8±4.7), and low AUDIT groups had AUDIT scores <8 for males and <5 for females (*n*=104, mean: 3.3±2.1). Our "late adolescent" group included participants ages 18-21 and our "adult" group included participants ages 22-40, based on preliminary findings from two other studies in our lab, as well as data showing brain maturation asymptoting ~22 yrs (Dosenbach et al., 2010). Participants had consumed alcohol one or more times in their lifetime, had no known history of any neurological, SUD, or other psychiatric disorders, and no current psychoactive drug use, excluding nicotine, caffeine, and alcohol. Although no participants self-reported any AUD, *post-hoc* evaluation of responses in the Rutgers Alcohol Problem Index (RAPI) indicated probable alcohol dependence among 43 recruited subjects (17.5%; 91% High AUDIT), based on *Diagnostic and Statistical Manual of Mental Disorders* (4th ed.; *DSM–IV*; (American Psychiatric Association, 1994) criteria; however, excluding these participants from our analyses did not qualitatively change our findings. Therefore, we did not exclude these participants, although we do additionally report data with these subjects excluded where noted. Nine subjects were excluded from all analyses due to unreliable task performance (see below). Thus, 237 participants (*n*=118 male) are included in our analyses; sex ratios were purposely balanced within each recruitment group. Subjects provided written, informed consent, as approved by the UNC Office for Human Research Ethics.

Behavioral Inventories

We administered standard questionnaires to quantify personal substance use, alcoholism familial history (FH), and behavioral traits. These included the AUDIT, the RAPI (White and Labouvie, 1989), the Drug Abuse Screening Test (DAST) (Skinner, 1982), and the Drug Use Screening Inventory, Domain I (DUSI-I); (Tarter, 1990). DUSI-I scores reported as % affirmative answers from Domain I, part B. We calculated density of familial alcohol abuse from the Family Tree Questionnaire (FTQ) (Mann et al., 1985), and classified participants reporting a problem drinking father or sibling as FH positive for alcoholism (FHP; *n*=76). Those reporting a problem-drinking mother (*n*=22) were excluded from our FH analyses to avoid potential confounds from fetal alcohol exposure. Those reporting no problem drinking first degree relatives were classified as family history negative (FHN; *n*=161). The Barratt Impulsiveness Scale-11 (BIS); (Patton et al., 1995) was used as a subjective measure of trait impulsiveness. Socio-economic Status (SES) was quantified as Hollingshead scores, following the Barratt Simplified Measure of Socioeconomic Status method (Hollingshead, 1975; Barratt, 2006).

Delay Discounting Task

The task has been described in detail previously (Altamirano et al., 2011; Smith and Boettiger, 2012). In brief, subjects practiced, then completed 8 blocks of 42 trials. There were four conditions: WANT (W), DON'T WANT (DW), SOONER, and LARGER; the latter two are considered together as control (CON) trials. Trial types were pseudorandomly ordered. Each trial displayed two monetary reward options, one ($2 - $100) available at a delay (1 wk – 6 mos) and a lesser amount (5 – 30% less) available "TODAY". All choices were hypothetical. Participants chose their preferred option on W trials, their non-preferred option on the DW trials, and the side with the sooner time or larger monetary amount for SOONER and LARGER trials, respectively.

The delayed amount, delay time, percent discount, and left/right position were pseudorandomly selected for each trial. We also collected reaction time (RT) for each trial. Nine subjects were excluded based on faster RT in the W and/or DW trials than in the CON trials, indicating lack of subjective consideration of options.

Genotyping

We previously found that a polymorphism in the catechol-*O*-methyltransferase (COMT) gene (*COMT Val^{158}Met; rs4680)* interacts with age to affect ICR (Smith and Boettiger, 2012). To control for this potential confound, participants were genotyped for the *COMT Val^{158}Met* polymorphism as previously described (Boettiger et al., 2007; Smith and Boettiger, 2012). Although *COMT* genotype distribution did not differ across recruitment groups (see Tables 3.1-3.3), we included a *COMT**age covariate in our analyses to account for the COMT by age effect we previously observed.

Data Analysis

Our primary index of DD was the proportion of smaller, sooner choices made in the W condition, the impulsive choice ratio (ICR). We also calculated ICR as a function of delay time and of delayed reward amount, and we calculated area under the ICR by delay time curve (AUC). Inferred ICR (*i*ICR) at each delay time was calculated based on the non-selected option in DW trials. We calculated the absolute difference between ICR and *i*ICR at each delay time, and averaged this value across all delay times as a gross index of motor control (motor mismatch, MM). Also, criterion interest rate was calculated as reported previously (Mitchell et al., 2007) as a measure of how much more the delayed reward needed to be valued over the now reward to be accepted ~75% of the time.

For single factor statistical comparisons between groups, we used unpaired two-tailed *t*-tests for continuous measures and χ^2 tests for categorical measures. For multi-factorial comparisons, we used standard or repeated measures mixed model ANOVAs with group as a between subjects factor, using SPSS (IBM, Montauk, NY). When necessary, a Greenhouse-Geisser non-sphericity correction was applied. When data were not normally distributed, arcsine-root transformations were applied in Excel to ensure the validity of parametric statistical tests. All analyses performed in SPSS unless otherwise noted. Effect sizes for ANOVA are reported as η^2, while effect sizes for *t*-tests are reported as Cohen's *d*.

Exploratory analyses to define optimal future age and alcohol-use groups

For exploratory analyses focused on the effect of age and AUDIT consumption (AUDIT-c) scores on ICR, we calculated *d*-prime (*d'*), a discriminability index derived from signal detection theory (Green & Swets, 1966). To identify, *post-hoc*, age and AUDIT-c cutoff scores producing the largest group difference in ICR, we calculated *d'* for ICR group differences as:

$$d' = \frac{2 \times (ICR_{group1} - ICR_{group2})}{\sqrt{(SD_{ICRgroup1})^2 + (SD_{ICRgroup2})^2}}$$

Where ICR_{groupn}=average ICR for group *n*, $SD_{ICRgroupn}$=standard deviation of ICR for group *n*. Cutoffs for group determinations were set as the grouping criteria that produced a d′ for our ICR group difference comparison of 1 or more. To confirm our *d'* findings, we also calculated Cohen's *d* effect sizes (Cohen, 1988) for each grouping, using a pooled measure of SD (Hartung et al., 2008).

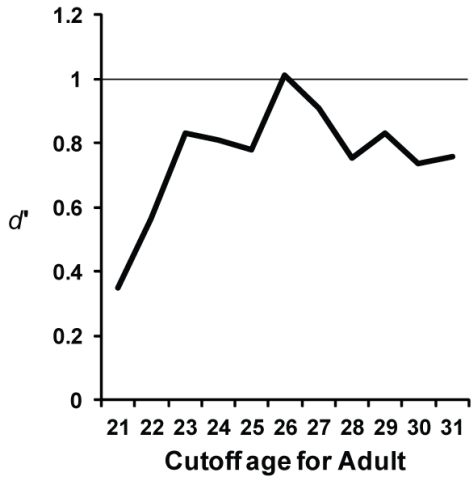

Figure 3.1: Systematic analysis of adult age cutoff on ICR age group effects among moderate drinkers. Plot depicts the group discrimination index (*d'*) as a function of adult age cutoff. Among moderate drinkers in this sample, *d'* is maximal with an adult age cutoff of 26 years of age. Note that a gap year was included in each age group comparison.

Analysis of age group effect size in Low AUDIT individuals

Our recruitment groups were empirically based, but the age cut-offs between groups may not be optimal for detecting age effects on ICR. To inform future studies requiring smaller sample sizes, we used signal detection theory to evaluate the ability of different age cutoffs to discriminate the mean ICR of emerging adults from "full" adults (within low AUDIT individuals), taking *d'* as our discrimination index (see above). We focused this analysis on the Low AUDIT group to investigate the effects of age on ICR without the additional effect of heavy, problematic alcohol use on this behavior. Grouping participants into older and younger age groups, leaving a gap year between groups, we used a sliding window to calculate ICR means and SDs for each grouping. We then quantified the discriminability (*d'*) between each age group pair for each age cutoff (from ages 21-31). Maximal discrimination of ICR between age groups occurred with an adult age cutoff of 26 (i.e. comparing ages 18-24 to ages 26-40; Figure 3.1). In confirmation, Cohen's *d* effect sizes for each age grouping found the largest age group

effect with an age cutoff of 26 (Cohen's *d*=0.82). Based on these discriminability results, for our subsequent age group analyses, we classified participants as emerging adults (ages 18-24, *n*=184; mean age=20.8±1.7) or adults (ages 26-40, *n*=39; mean age=31.4±4.0).

RESULTS

Demographic and psychometric data by recruitment groups

Based on preliminary data from our lab, we initially recruited late adolescent (ages 18-21) and young adult (ages 22-40) subjects, with roughly equal ratios of high AUDIT individuals within each group. Details on the demographic differences between these recruited age groups can be found in Table 3.1. Importantly, our recruited age groups did not differ in terms of ethnicity, sex, SES, family history of alcohol abuse (FTQ density), *COMT* genotype distribution, nor in terms of several measures of substance use (DUSI, DAST; Table 3.1).

Considering the subjects according to AUDIT recruitment groups, we observed no significant differences between groups in terms of age, education, SES, sex, ethnicity, *COMT* genotype distribution, FH of alcohol abuse, or FTPI scores (Table 3.2).

Considering demographic, substance use, and psychometric measures across our age*AUDIT recruitment groups, we found that these four groups did not differ in terms of sex, *COMT* genotype, or ethnicity (Table 3.3). Moreover, we found no significant age*AUDIT interactions on any measure except AUDIT scores and DUSI scores (Table 3.3). This result reflects ~26% higher AUDIT scores and ~21% higher DUSI scores among 18-21 year olds relative to 22-40 year olds within the high AUDIT group (Table 3.3).

Demographic and psychometric data: Emerging Adults vs Adults

We investigated whether any demographic, substance use, or psychometric measures varied across our emerging adult (18-24), adult (26-40) and low and high AUDIT recruitment groups via a 2x2 ANOVA. We found that these four groups did not differ in terms of sex, *COMT* genotype, or ethnicity (Table 3.4). Moreover, we found no significant age*AUDIT group interactions on any measure except AUDIT and DUSI scores (Table 3.4). This result reflects ~47% higher AUDIT scores and ~39% higher DUSI scores among 18-24 year olds relative to adults within the high AUDIT group (Table 3.4).

Table 3.1: Demographic, substance use, and psychometric data by age recruitment group

	Ages 18-21 (n = 111)	Ages 22-40 (n = 126)	$t_{(235)}$	*p value*
General				
Age (yrs)	19.7 ± 1.2	25.5 ± 4.6	-13.58	<0.001
Education (yrs)	13.8 ± 1.2	16.5 ± 1.7	-13.17[a]	<0.001
SES	51.4 ± 8.2	51.1 ± 8.9	0.20	0.84
Gender (% female)	49.5	50.8		0.85[†]
Ethnicity (% non-white)	33.3	24.6		0.14[†]
COMT genotype (% ValVal)	22.5	31.7		0.32[†]
Substance use-related				
AUDIT - total	9.2 ± 6.5	7.4± 4.6	2.41	0.017
AUDIT consumption	4.9 ± 2.6	4.8 ± 2.2	0.44[b]	0.66
AUDIT D/H	4.2 ± 4.4	3.0 ± 3.1	2.34[b]	0.02
RAPI	8.8 ± 8.0	6.7 ± 7.4	2.16	0.032
DUSI	0.3 ± 0.2	0.3 ± 0.2	1.60	0.11
DAST	2.1 ± 2.5	2.0 ± 2.4	0.43	0.67
FTQ density (%)	16.9 ± 18.0	14.8 ± 16.6	0.93	0.356
Psychometric				
BIS - total	60.5 ± 9.6	57.8 ± 9.6	2.17[c]	0.031
BIS Attention	15.9 ± 3.7	15.3 ± 3.5	1.3[c]	0.19
BIS Motor	22.1 ± 3.5	21.3 ± 3.8	1.74[c]	0.084
BIS Non-Planning	22.4 ± 4.6	21.2 ± 4.6	2.12[c]	0.035
FTPI mean extension (yrs)	8.6 ± 5.4	6.6 ± 5.4	2.89	0.004
FTPI max extension (yrs)	31.1 ± 23.3	24.4 ± 19.9	2.37	0.018

Values are reported as mean ± standard deviation. Reported p-values reflect the results of unpaired two-tailed comparison between groups. Exact p-values reported unless $p < 0.001$. AUDIT, Alcohol Use Disorders Identification Test; AUDIT D/H, AUDIT dependence/harm subscales; RAPI, Rutgers Alcohol Problem Index; DUSI, Drug Use Screening Inventory, part I-B; DAST, Drug Abuse Screening Test; SES, Socioeconomic Status; BIS, Barratt Impulsiveness Scale; FTPI, Future Time Perspective Inventory. [†]p-value represents results of χ^2 test. [a]df=233, [b]df=215; [c]df=234

Table 3.2: Demographic, substance use, and psychometric data by AUDIT recruitment group

	Low AUDIT (<8 M, <5 F, n=99)	High AUDIT (≥8 M, ≥5 F, n=138)	$t_{(235)}$	*p value*
General				
Age (yrs)	23.0 ± 4.8	22.7 ± 4.3	0.55	0.58
Education (yrs)	15.4 ± 2.0	15.2 ± 2.0	0.76[a]	0.45
SES	51.1 ± 9.4	51.4 ± 8.0	-0.27	0.79
Gender (% female)	44.4	54.3		0.13[†]
Ethnicity (% non-white)	29.3	28.3		0.86[†]
COMT genotype (% ValVal)	24.2	29.7		0.63[†]
Substance use-related				
AUDIT - total	3.4 ± 2.1	11.8 ± 4.7	-18.77	<0.001
AUDIT consumption	2.9 ± 1.5	6.3 ± 1.9	-13.4[a]	<0.001
AUDIT D/H	0.8 ± 1.2	5.7 ± 3.7	-14.0[a]	<0.001
RAPI	2.6 ± 4.0	11.3 ± 7.7	-11.5	<0.001
DUSI	0.1 ± 0.1	0.4 ± 0.2	-13.72	<0.001
DAST	1.0 ± 1.3	2.8 ± 2.8	-6.7	<0.001
FTQ density (%)	14.9 ± 16.5	16.5 ± 17.8	-0.72	0.475
Psychometric				
BIS - total	56.7 ± 8.9	60.8 ± 9.9	-3.23[b]	0.001
BIS Attention	15.2 ± 3.3	15.9 ± 3.9	-1.55[b]	0.122
BIS Motor	21.2 ± 3.4	22.1 ± 3.9	-1.88[b]	0.062
BIS Non-Planning	20.4 ± 4.4	22.8 ± 4.5	-4.05[b]	<0.001
FTPI mean extension (yrs)	7.8 ± 5.3	7.3 ± 5.6	0.62	0.54
FTPI max extension (yrs)	27.9 ± 20.9	27.3 ± 22.4	0.21	0.84

Values are reported as mean ± standard deviation. Reported *p*-values reflect the results of unpaired two-tailed comparison between groups. Exact *p*-values reported unless $p < 0.001$. Conventions as per Table 3.1. M, males; F females. [†]*p*-value represents results of χ^2 test. [a]df=233, [b]df=215; [c]df=234

AUDIT does not predict ICR in emerging adults

Among our emerging adults (ages 18-24), we found no significant ICR differences between high AUDIT (0.63±0.32) and low AUDIT (0.68±0.25) groups ($t_{(180.448)}$=1.29, p=0.20, d=0.18). Considering AUDIT as a continuous variable, we also found no significant correlation between AUDIT and ICR among emerging adults ($r_{(182)}$= 0.014, p=0.42, β=0.001). Furthermore, ICR did not correlate with any other substance use measures in this age group (max r=0.065, min p=0.383). Thus, the relationship between ICR and alcohol use robustly observed in adult samples (Mitchell et al., 2005; Boettiger et al., 2007; Mitchell et al., 2007) is not detectable in an emerging adult population.

Table 3.3: Demographic, Substance Use Related, and Psychometrics Measures Across Age and AUDIT groups

	Low AUDIT (<8 M, <5 F, n=99)		High AUDIT (≥8 M, ≥5 F, n=138)			
	Ages 18-21 (*n* = 45)	Ages 22-40 (*n* = 54)	Ages 18-21 (*n* = 66)	Ages 22-40 (*n* = 72)	Significant Age*AUDIT interaction	
					F_{233}	p
Demographic						
Age (yrs)	19.6 ± 1.2	25.8 ± 4.9	19.8 ± 1.1	25.3 ± 4.4	0.85	0.36
Education (yrs)	13.8 ± 1.4	16.7 ± 1.4	13.9 ± 1.2	16.3 ± 2.0	1.08[a]	0.30
SES	51.6 ± 9.2	50.6 ± 9.6	51.2 ± 7.6	51.5 ± 8.4	0.31	0.58
Ethnicity (% non-white)	31.1	27.8	34.8	22.2		0.42[†]
Sex (% female)	42.2	46.3	54.5	54.2		0.49[†]
COMT genotype (% ValVal)	20	27.8	24.2	34.7		0.59[†]
AUDIT	3.3 ± 2.3	3.4 ± 1.9	13.2 ± 5.2	10.5 ± 3.6	7.91	0.005
AUDIT consumption	2.7 ± 1.4	3.1 ± 1.5	6.5 ± 2.0	6.0 ± 1.8	2.98[b]	0.086
AUDIT D/H	0.8 ± 1.1	0.7 ± 1.2	6.8 ± 4.2	4.8 ± 2.9	5.92[b]	0.016
RAPI	3.3 ± 4.7	1.9 ± 3.1	12.6 ± 7.5	10.2 ± 7.7	0.38	0.54
DUSI	0.12 ± 0.13	0.13 ± 0.12	0.41 ± 0.15	0.34 ± 0.16	3.97	0.048
DAST	1.02 ± 1.5	0.91 ± 1.1	2.8 ± 2.9	2.7 ± 2.8	0.003	0.96
FTQ density (%)	15.1 ± 16.6	14.7 ± 16.6	18.2 ± 18.9	15 ± 16.7	0.359	0.55
Psychometric						
BIS - total	57.6 ± 8.8	56 ± 9.1	62.5 ± 9.7	59.2 ± 9.9	0.44[c]	0.51
BIS Attention	15.3 ± 3.3	15.1 ± 3.3	16.4 ± 3.9	15.5 ± 3.7	0.69[c]	0.41
BIS Motor	21.7 ± 3.3	20.7 ± 3.4	22.4 ± 3.7	21.8 ± 4.0	0.11[c]	0.74
BIS Non-Planning	20.7 ± 4.1	20.1 ± 4.7	23.7 ± 4.5	22 ± 4.4	1.004	0.32
FTPI mean ext (yrs)	9.0 ± 5.0	6.7 ± 5.4	8.3 ± 5.8	6.4 ± 5.3	0.134	0.72
FTPI max ext (yrs)	32.3 ± 22.1	24.2 ± 19.3	30.2 ± 24.3	24.6 ± 20.4	0.188	0.67

Values are reported as mean ± standard deviation. Reported *p*-values reflect the results of unpaired two-tailed comparison between groups. Exact *p*-values reported unless $p < 0.001$. Conventions as per Table 3.1. [†]*p*-value represents results of χ^2 test. [a]df=231; [b]df=213, [c]df=232

Effects of age and AUDIT group on ICR

Based on our hypothesized age-related decrease in ICR specific to moderate drinkers, we conducted a 2-way (age group*AUDIT group) ANOVA, covarying for DUSI scores (see Table

3.4). This analysis did not detect a significant main effect of AUDIT group ($F_{(1,217)}$=0.042, p=0.84, η^2<0.001). However, a trend toward a main effect of age group ($F_{(1,217)}$=3.66, p=0.057, η^2=0.016) and a significant age*AUDIT interaction ($F_{(1,217)}$=5.17, p=0.024, η^2=0.023; Figure 3.2) on ICR was present. Post-hoc analyses of age-related changes in ICR by AUDIT recruitment groups found that among low AUDIT individuals (AUDIT <8 males, <5 females), mean ICR was ~48% higher in 18-24 year olds (0.68±0.25) relative to 26-40 year olds (0.46±0.37; $F_{(1, 91)}$=8.46, p=0.005, η^2=0.085). In contrast, in the high AUDIT group, mean ICR was ~1.6% higher in 18-24 year olds (0.63±0.32) relative to those 26-40 (0.62±0.32; $F_{(1,124)}$=0.009, p=0.93, η^2<0.001). This finding is suggestive that ICR may decline more with age in low versus high AUDIT individuals.

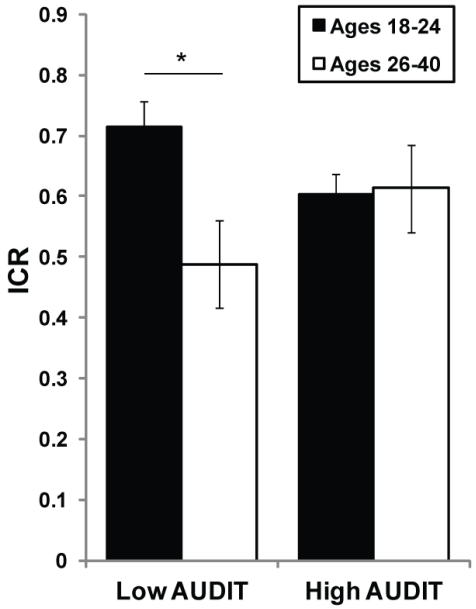

Figure 3.2: Interacting effects of age and alcohol use on delay discounting behavior. Plot depicts the ratio of immediate reward choices (ICR) in the delay-discounting task as a function of *Low and High* AUDIT and adult and emerging adult age groups, demonstrating a significant age*AUDIT interaction *($F_{(1,217)}$=5.17, p=0.024, η^2=0.023)*. This finding reflects the fact that among low AUDIT individuals (AUDIT <8 for males, <5 for females), ICR was significantly higher in emerging adults relative to adults ($F_{(1, 91)}$=8.46, p=0.005, η^2=0.085), but among possible problem drinkers (AUDIT ≥8 for males, ≥5 for females), ICR did not differ between age groups. AUDIT, Alcohol Use Disorders Identification Test. *$p<05$.

Table 3.4: Demographic, Substance Use Related, and Psychometrics Measures Across Age and High/Low AUDIT groups

	Low AUDIT (<8M, <5 F)		High AUDIT (≥8 M, ≥5 F)			
	Ages 18-24 (*n* = 75)	Ages 26-40 (*n* = 20)	Ages 18-24 (*n* = 109)	Ages 26-40 (*n* = 19)	Age*AUDIT interaction	
					F_{219}	*p*
Demographic						
Age (yrs)	20.7 ± 1.7	31.2 ± 4.0	20.9 ± 1.7	31.5 ± 4.2	0.02	0.89
Education (yrs)	14.7 ± 1.6	17.6 ± 1.5	14.7 ± 1.5	17.6 ± 2.8	<0.001[a]	0.998
SES	50.6 ± 10	53.1 ± 7.7	51.4 ± 7.7	52.9 ± 9.6	0.13	0.71
Ethnicity (% non-white)	25.3	40.0	29.4	26.3		0.63[†]
Sex (% female)	42.7	55.0	52.3	63.2		0.34[†]
COMT genotype (% ValVal)	22.7	30.0	29.4	36.8		0.73[†]
Substance use-related						
AUDIT	3.5 ± 2.2	3.0 ± 1.9	12.5 ± 4.8	8.5 ± 2.5	7.19	0.008
AUDIT consumption	3.0 ± 1.4	2.7 ± 1.4	6.6 ± 1.9	4.8 ± 1.1	6.53[b]	0.011
AUDIT D/H	0.9 ± 1.2	0.6 ± 1.2	6.2 ± 3.8	3.7 ± 2.3	4.77[b]	0.03
RAPI	2.9 ± 4.0	1.5 ± 3.7	12.3 ± 7.5	7.9 ± 7.7	1.77	0.19
DUSI	0.12 ± 0.13	0.12 ± 0.13	0.39 ± 0.15	0.28 ± 0.19	4.44	0.04
DAST	1.0 ± 1.4	0.85 ± 0.9	2.7 ± 2.7	2.6 ± 3.5	<0.001	0.99
FTQ density (%)	13.9 ± 15.5	21.5 ± 19.5	15.4 ± 16.8	22.2 ± 20.8	0.019	0.89
Psychometric						
BIS - total	56.9 ± 8.7	55.7 ± 9.8	61.7 ± 9.4	55.3 ± 9.9	2.55[c]	0.11
BIS Attention	15.4 ± 3.0	14.4 ± 4.0	16.1 ± 3.7	14.6 ± 3.8	0.16[c]	0.69
BIS Motor	21.2 ± 3.5	20.8 ± 3.1	22.4 ± 3.8	20.0 ± 3.3	2.45[c]	0.12
BIS Non-Planning	20.3 ± 4.3	20.6 ± 5.0	23.2 ± 4.5	20.7 ± 4.6	2.95[c]	0.09
FTPI mean ext (yrs)	8.6 ± 5.3	5.1 ± 4.0	7.5 ± 5.8	6.9 ± 5.0	2.24	0.14
FTPI max ext (yrs)	30.7 ± 21.8	19.8 ± 15.2	28.0 ± 23.6	23.1 ± 14.2	0.62	0.43

Values are reported as mean ± standard deviation. Reported F and *p*-values reflect the results testing for significant age by AUDIT group interactions. Exact *p*-values reported unless $p < 0.001$. Conventions as per Table 3.1. [†]*p*-value represents results of χ^2 test. [a]error=217, [b]error=202; [c]error=218

Among low AUDIT individuals, ICR decreases with age

While we found a trend toward a main effect of age group on ICR that was significant in post-hoc tests in the low AUDIT, treating age as a continuous variable provides greater insight

on the effect of increasing age on ICR. Overall, a significant negative correlation between ICR and age was observed ($r_{(235)}$=-0.134, p=0.019, β=-0.012) indicating a ~1.1% ICR decrease for each year of age >18. Examining the relationship between ICR and age by AUDIT recruitment groups, we found a striking difference between the high and low AUDIT groups. The low AUDIT group showed a ~2.2% decline in ICR with each year of age >18 ($r_{(197)}$=-0.276, p=0.003, β=-0.022). In contrast, we observed no relationship between ICR and age within the high AUDIT group ($r_{(136)}$=-0.025, p=0.39, β=-0.002). Thus, the age effect on ICR in the whole sample was driven by the low AUDIT group.

Examination of secondary measures of DD behavior within the low AUDIT group produced qualitatively similar results. Specifically, the average area under the ICR by delay time curve (AUC) of 18-24 year olds (123.1±52.5) was ~22% larger, relative to that of 26-40 year olds (101.0±64.3; $t_{(49.287)}$=2.011, p=0.05, d=0.41), indicating greater *Now* bias in emerging adults. Moreover, among low AUDIT individuals, we found a significant negative correlation between AUC and age ($r_{(97)}$=-0.309, p=0.001). No such decline in AUC with age was seen in the high AUDIT group ($r_{(136)}$=-0.034, p=0.345).

Other aspects of DD task performance

Importantly, we found no significant main or interacting effects of age or AUDIT group on basic measures of DD task performance, including accuracy in CON trials, and RTs for any condition (Table 3.5). Moreover, we found no significant main or interacting effects of age or AUDIT group on unintentional motor responding (MM; see Methods; max F=0.785, min p=0.38). Thus, group differences in MM cannot explain the group differences in ICR observed here. A recent study found greater DD in adolescents (ages 13-15) relative to adults (ages 19-50)

that was associated with greater choice inconsistency (Ripke et al., 2012). Here, we found that the consistency of ICR across task blocks (8 blocks of the task were administered over ~50 minutes) did not differ between age groups (Cronbach's α, ages 18-24: 0.984, ages 26-40: 0.990). Furthermore, a two-way repeated measures ANOVA (age group*block) found no significant main effect of block ($F_{(7,1491)}$=0.96, p=0.46) nor block*age group interaction ($F_{(7,1491)}$=0.58, p=0.77) on ICR. Thus, our observed age effects on ICR are not attributable to age–related changes in response consistency. Task related performance and these other measures of discounting are reported across low and high AUDIT and emerging adults versus adults in Table 3.5. Note that there was no significant age*AUDIT group interaction on any performance related measure and that the only significant interaction was observed with our AUC measure, which confirms the AUDIT*age group interaction we observed with our ICR measure (see *Effects of age and AUDIT group on ICR* above).

Relationship between AUDIT and ICR: moderation by age

We further investigated the lack of an age effect on ICR among high AUDIT individuals by evaluating the relationship between ICR and AUDIT scores within our age groups. In contrast to 18-21 year olds, among 22-40 year olds, we found a trend toward a positive correlation between AUDIT score and ICR (Table 3.6). This relationship appears to be driven by a relationship between ICR and AUDIT consumption subscale (AUDIT-c) scores, as we observed no significant relationship between ICR and AUDIT dependence/harm subscales in adults (Table 3.6). Among 18-21 year olds, we found no significant relationship between ICR and AUDIT subscale scores (Table 3.6). Repeating these analyses using our *post-hoc* emerging adult and adult age groups (see Methods), we found a significant positive relationship between ICR and

AUDIT scores among adults (ages 26-40), an effect driven mostly by AUDIT-c scores (Table 3.6). In adults 26-40, each additional AUDIT-c point was associated with a 15.6% increase (β=0.117) in ICR (Table 3.6). In contrast, increases in AUDIT-c scores had no significant effect on ICR among emerging adults (18-24; Table 3.6). In this sample, then, it is alcohol consumption (as measured via the AUDIT-c) that is related to ICR and the relationship between AUDIT-c and ICR becomes apparent when the effects of young age (which we observe is naturally associated with high ICR) have diminished after age 25.

Exploratory d' analysis of AUDIT-c cutoffs

As ICR correlated better with AUDIT-c than with full AUDIT scores in adults, we sought to identify the AUDIT-c score cutoff that produced the largest group difference in ICR so that recruitment for future studies investigating this effect could be adequately powered. As for our *post hoc* age group analysis (see Methods), we used a sliding cutoff value to identify the maximal between group *d'*, starting with an AUDIT-c score cutoff of 2. The ICRs of AUDIT-c groups began to be maximally discriminated using a cutoff score of 5. Cohen's *d* effect size for ICR difference across AUDIT-c groups with a cutoff of 5 was (0.73).

Testing for interacting effect of age and alcohol use in AUDIT-c groups

The fact that we observed age related differences in ICR that were driven by low AUDIT individuals and that AUDIT-c appeared to be the primary predictor of ICR in adults 26-40 suggested that ICR may vary as a function of emerging adult (18-24) versus adult (26-40) and low versus high AUDIT-c groups. We conducted a 2-way (age group*AUDIT-c) ANOVA, with ICR as our dependent measure. We classified participants as emerging adults (ages 18-24,

n=184; mean age=20.8±1.7) or adults (ages 26-40, *n*=39; mean age=31.4±4.0), and moderate drinkers (AUDIT-c <4, *n*=60; mean AUDIT: 2.8±2.2, mean AUDIT-c: 2.1±0.9) or heavy drinkers (AUDIT-c ≥5, *n*=102; mean AUDIT: 12.1±5.1, mean AUDIT-c: 6.8±1.6). Participants with AUDIT-c scores of 4 were excluded, as scores ≥4 may be associated with alcohol misuse in females but not males (Bradley et al., 2007). Demographic data across these AUDIT-c and age groups are displayed in Table 3.7.

Table 3.5: Delay Discounting Task Behavioral Measures Across Age and High/Low AUDIT groups

	Low AUDIT (<8 M, <5 F)		High AUDIT (≥8 M, ≥5 F)			
	Ages 18-24 (*n* = 75)	Ages 26-40 (*n* = 20)	Ages 18-24 (*n* = 109)	Ages 26-40 (*n* = 19)	Age*AUDIT interaction	
					F_{219}	*p*
Task Performance						
Control Trial Acc	97.0 ± 3.3	96.7 ± 3.5	96.6 ± 3.6	98.1 ± 2.0	2.22	0.14
Control Trial RT	1364 ± 339	1347 ± 315	1401 ± 329	1233 ± 276	1.68	0.20
WANT Trial RT	1920 ± 383	1743 ± 388	1915 ± 396	1711 ± 389	0.04	0.84
DON'T WANT Trial RT	2064 ± 399	1859 ± 385	2086 ± 449	1888 ± 369	0.002	0.96
ICR by Delay Curve Measures						
Area Under Curve	130.6 ± 42.9	86.8 ± 69.2	118.0 ± 57.8	116.0 ± 56.7	4.74	0.031
Delay Curve Slope	0.13 ± 0.09	0.09 ± 0.11	0.09 ± 0.08	0.10 ± 0.08	1.50	0.22
Delay Curve Intercept	0.21 ± 0.46	0.10 ± 0.44	0.27 ± 0.41	0.24 ± 0.44	0.23	0.63
Other Task Measures						
Criterion Int. Rate	19.0 ± 18.7	8.3 ± 10.2	17.7 ± 30.5	18.4 ± 19.9	1.61[a]	0.21
Motor Mismatch	0.11 ± 0.06	0.10 ± 0.07	0.11 ± 0.06	0.12 ± 0.07	0.61	0.44
ICR consistency (α)	0.978	0.991	0.987	0.988	0.86[†]	0.51[†]

Values are reported as mean ± standard deviation. Reported F and *p*-values reflect the results testing for significant age by AUDIT group interactions. Exact *p*-values reported unless $p < 0.001$. Conventions as per Table 3.1. [a]error=210 [†]result of ICR by Block by Age by AUDIT Group ANOVA, Greenhouse-Geisser corrected: F (7,1477)

Table 3.6: AUDIT Correlates with ICR More Strongly in Adults and is Driven by AUDIT Consumption Subscale.

Recruited Age Groups

	AUDIT Total	**AUDIT Consumption**	**AUDIT Dependence/Harm**
18-21 (n=111)	$r_{(109)}$=-0.017, p=0.43 β=0	$r_{(97)}$=0.059, p=0.28 β=0.008	$r_{(97)}$=-0.012, p=0.45 β=0
22-40 (n=126)	$r_{(124)}$=0.141, p=0.057 β=0.013	$r_{(116)}$=0.158, p=0.044 β=0.031	$r_{(116)}$=0.101, p=0.137 β=0.014

***Post-hoc* Age Groups**

	AUDIT Total	**AUDIT Consumption**	**AUDIT Dependence/Harm**
18-24 (n=184)	$r_{(184)}$=0.014, p=0.42 β=0.001	$r_{(166)}$=0.018, p=0.41 β=0.003	$r_{(166)}$=0.017, p=0.41 β=0.002
26-40 (n=39)	$r_{(37)}$=0.30, p=0.032 β=0.04	$r_{(36)}$=0.398, p=0.007 β=0.117	$r_{(36)}$=0.24, p=0.073 β=0.048

Table reflects Pearson correlation r, p, and beta values from linear regression analyses of AUDIT score and AUDIT subscales score effects on ICR by recruited age groups and age groups proposed for further analysis of age effect.

Running our AUDIT-c by age group ANOVA, we found, as expected, significant main effects of age ($F_{(1,157)}$=6.12, p=0.014, η^2=0.035) and AUDIT-c ($F_{(1,157)}$=4.37, p=0.038, η^2=0.025) on ICR. Critically, we also observed a significant age*AUDIT-c interaction on ICR ($F_{(1,157)}$=8.32, p=0.004, η^2=0.047; Figure 3.3). Among moderate drinkers, mean ICR was 80% higher in emerging adults (n=44, mean: 0.72±0.21) relative to adults (n=16, mean: 0.41±0.35; $t_{(18.679)}$=3.38, p=0.003, d=1.28). Whereas among heavy drinkers, mean ICR did not differ between age groups ($t_{(100)}$=-0.21, p=0.84, d=-0.06). Moreover, among adults, the mean ICR of heavy drinkers (0.69±0.32, n=13) was 72.5% higher than that of moderate drinkers (0.41±0.35, n=16; $t_{(27)}$=-2.28, p=0.031, d=-0.88; Figure 3.3). In contrast, among emerging adults, mean ICR did not differ between moderate and heavy drinkers ($t_{(115.216)}$=1.018, p=0.311, d=0.17). Considering age and ICR as continuous variables, ICR was negatively related to age in the low AUDIT-c group ($r_{(62)}$=-0.46, p<0.001, β=-0.034), reflecting a 2.9% decrease in ICR with each year of age >18. No such age effect was detected in the high AUDIT-c group ($r_{(107)}$=-0.033,

p=0.37, β=-0.003). Qualitatively similar results were obtained with AUC values (data not shown). As in *a priori* age groups, we found no age effect on response consistency (data not shown).

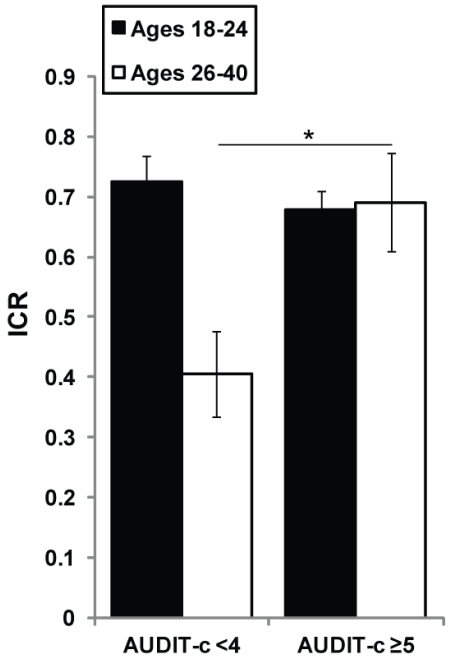

Figure 3.3: Interacting effects of age and alcohol consumption on delay discounting behavior. Plot depicts the ratio of immediate reward choices (ICR) in the delay-discounting task as a function of *post hoc* defined AUDIT-c and age groups. There was a significant age by AUDIT consumption group interaction on ICR, $F_{(1, 157)}$=8.32, p=0.004, η^2=0.047. *ICR is higher in heavy (AUDIT consumption ≥5) versus light/moderate drinking adults ($t_{(27)}$=-2.28, p=0.031, d=-0.88). In emerging adults (ages 18-24), ICR does not differ based on alcohol consumption ($t_{(115.216)}$=1.018, p=0.311, d=0.17). AUDIT-c, AUDIT consumption subscale. *$p<05$.

High ICRs in late adolescents is not driven by underage drinkers

Both our *a priori* late adolescent and *post hoc* emerging adult groups included participants under the U.S. legal drinking age (21 yrs), raising the concern that the high ICRs of the adolescent/emerging adult groups are driven by underage drinkers. Thus, we tested for a relationship between age and ICR within the low and high AUDIT groups after excluding participants (n=71) <21. In the low AUDIT (AUDIT<8 Males, <5 Females) group, we still

observed a significant negative relationship between ICR and age ($r_{(66)}$=-0.283, p=0.010; β=-0.025), reflecting a -2.4% drop in ICR with each year of age >21. Likewise, in the high AUDIT group, we still observed no relationship between age and ICR ($r_{(96)}$=-0.031, p=0.38; β=-0.003). Moreover, among low AUDIT individuals, 21-24 year olds had a mean ICR 24.9% higher than that of participants >25 yrs (0.67±0.24 vs. 0.46±0.37; $t_{(25.414)}$=2.36, p=0.026). In addition, among 21-24 year olds, we found no significant difference in mean ICR between high AUDIT (0.62±0.32, n=69) and low AUDIT groups (0.67±0.24, n=44; $t_{(108.566)}$=1.069, p=0.29), and no relationship between AUDIT and ICR ($r_{(111)}$=0.056, p=0.55). Qualitatively similar effects were found using AUDIT-c scores in correlation analyses and group definition (data not shown). Thus, *Now* bias observed here among the late adolescents/emerging adults are not driven by current underage drinkers.

Age and Age x AUDIT-c effects are not confounded by college student status

Our participants consisted of a large portion of undergraduate and graduate students (43.5%) with the proportion of students in our emerging adult (18-24) group (48.4%) significantly greater than that in our adult (26-40) age group (28.2%; χ^2=5.29, df=1, p=0.021). We covaried for student status in our key analyses: the Low vs High AUDIT group x age group ANOVA resulted in similar effects to those observed without this covariate (see: *Effects of age and AUDIT group on ICR, above):* a trend toward a main effect of age group ($F_{(1,216)}$=3.57, p=0.06, η^2=0.016) and a significant age*AUDIT interaction ($F_{(1,216)}$=5.08, p=0.025, η^2=0.022). Revisiting our AUDIT-c by age group ANOVA, covarying for student status did not alter our results: significant effect of age group ($F_{(1,156)}$=5.94, p=0.016, η^2=0.034), AUDIT-c group ($F_{(1,156)}$=4.34, p=0.039, η^2=0.025), and age*AUDIT-c group interaction ($F_{(1,156)}$=8.14, p=0.005,

η^2=0.047) on ICR. Thus differences in the number of students in our age groups cannot explain the age and age x AUDIT-c effects we observed on ICR.

Table 3.7: Demographic, Substance Use Related, and Psychometrics Measures Across Age and High/Low AUDIT-c groups

	Light/Moderate Drinkers (AUDIT-c < 4)		Heavy Drinkers (AUDIT-c ≥ 5)			
	Ages 18-24 (n = 44)	Ages 26-40 (n = 16)	Ages 18-24 (n = 89)	Ages 26-40 (n = 13)	Age*AUDIT-c interaction	
					F_{158}	p
Demographic						
Age (yrs)	20.5 ± 1.8	31.4 ± 3.8	21.1 ± 1.7	32.2 ± 4.3	0.06	0.80
Education (yrs)	14.5 ± 1.7	17.3 ± 1.3	14.8 ± 1.5	17.5 ± 3.4	0.00	0.97
SES	47.5 ± 11.6	52.2 ± 7.6	51.6 ± 7.5	51.7 ± 10	1.53	0.22
Ethnicity (% non-white)	20.5	31.3	30.3	38.5		0.52†
Sex (% female)	59.1	62.5	44.9	62.5		0.26†
COMT genotype (% ValVal)	20.5	18.8	28.1	46.2		0.58†
Substance use-related						
AUDIT	2.7 ± 2.2	2.9 ± 2.2	12.6 ± 5.2	8.7 ± 2.7	5.71	0.018
AUDIT consumption	2.1 ± 0.9	2.2 ± 0.9	7.0 ± 1.6	5.5 ± 0.7	7.62	0.006
AUDIT D/H	0.8 ± 1.5	0.8 ± 1.8	5.9 ± 4.0	3.2 ± 2.5	4.23	0.041
RAPI	2.3 ± 3.2	2.1 ± 7.5	12.1 ± 7.7	6.2 ± 4.3	4.59	0.034
DUSI	0.09 ± 0.11	0.10 ± 0.14	0.39 ± 0.16	0.25 ± 0.20	6.64	0.011
DAST	0.66 ± 0.83	1.0 ± 1.2	3.0 ± 2.8	2.8 ± 4.1	0.23	0.63
FTQ density (%)	14.2 ± 16.5	25.9 ± 20.6	15.9 ± 17.3	20.3 ± 23.5	0.95	0.33
Psychometric						
BIS - total	56.1 ± 9.3	55.8 ± 7.6	62.0 ± 9.7	54.5 ± 9.8	3.38	0.068
BIS Attention	15.3 ± 3.2	14.3 ± 3.3	16.2 ± 3.7	13.9 ± 3.5	0.69	0.41
BIS Motor	20.9 ± 3.7	20.8 ± 2.1	22.7 ± 3.8	19.5 ± 3.6	4.39	0.038
BIS Non-Planning	19.9 ± 4.5	20.7 ± 4.4	23.1 ± 4.7	21.1 ± 4.7	2.11	0.149
FTPI mean ext (yrs)	8.4 ± 5.3	6.0 ± 4.5	8.0 ± 6.1	6.6 ± 5.6	0.16	0.69
FTPI max ext (yrs)	28.3 ± 19.8	22.2 ± 16.1	30.4 ± 24.3	22.8 ± 15.1	0.03	0.86

Values are reported as mean ± standard deviation. Reported F and p-values reflect the results testing for significant age by AUDIT group interactions. Exact p-values reported unless $p < 0.001$. Conventions as per Table 3.1. †p-value represents results of χ^2 test.

Family history of alcoholism and DD

Our findings thus far do not point to heightened *Now* bias as a pre-existing trait associated with AUD risk, as it was present generally among emerging adults. However, another way to identify intermediate phenotypes for complex neurobehavioral disorders is to compare the behavior of unaffected people with and without 1° relatives with the disorder (Meyer-Lindenberg and Weinberger, 2006). As our sample included a significant proportion of FHP participants (~23%), we conducted a 3-way ANOVA, with the following factors: post-hoc age group, AUDIT-c group, and FH (see Methods). We found a trend toward a main effect of FH on ICR with higher ICRs among FHP individuals (0.69±0.24) relative to FHN individuals (0.66±0.3; $F_{(1,137)}$=3.635, p=0.059, η^2=0.022). We found a significant FH*age interaction ($F_{(1,137)}$=5.333, p=0.022, η^2=0.033), reflecting a trend toward higher ICRs among FHP adults (ages 26-40; 0.65±0.26) relative to FHN adults (0.46±0.37; $F_{(1,31)}$=3.769, p=0.061, η^2=0.10), and no significant difference in ICR between FHP (0.70±0.23) and FHN (0.69±0.28; $F_{(1,166)}$=0.424, p=0.516, η^2=0.003) emerging adults. While, we found no significant FH*AUDIT-c interaction ($F_{(1,\ 137)}$=0, p=0.995, η^2=0), we did observe a near-significant 3-way interaction effect on ICR ($F_{(1,137)}$=3.537, p=0.062, η^2=0.022). This reflects the fact that among emerging adults, we observed a significant FH*AUDIT-c group effect on ICR ($F_{(1,116)}$=5.034, p=0.027, η^2=0.042) but no main effect of either factor (max. F=0.23). Among heavy drinking emerging adults, ICRs were not different between FHP individuals (0.75±0.22) relative to FHN individuals (0.65±0.31; $F_{(1,78)}$=1.73, p=0.192). However, in light drinking emerging adults, we observed a trend toward higher ICRs in FHN individuals (0.77±0.19) relative to FHP individuals (0.62±0.25; $F_{(1,37)}$=3.89, p=0.056, η^2=0.092). In contrast, among adults, we observed significantly higher ICRs in heavy versus light drinkers ($F_{(1,\ 20)}$=7.23, p=0.014, η^2=0.20) and in FHP compared to FHN individuals

($F_{(1,\ 20)}$=6.40, p=0.020, η^2=0.177), but no FH*AUDIT-c interaction ($F_{1,\ 20)}$=0.46, p=0.51, η^2=0.013).

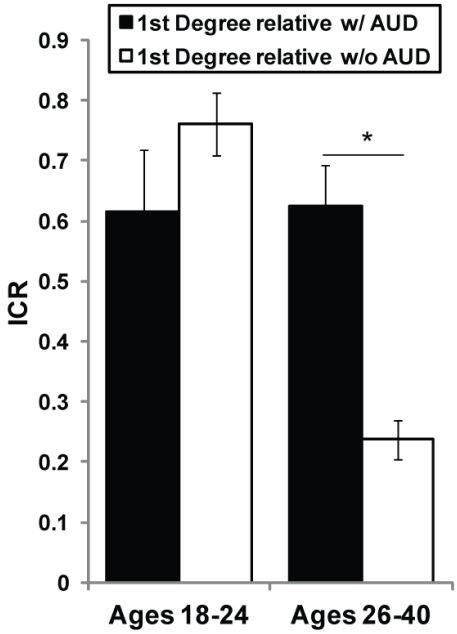

Figure 3.4: Family history of alcohol use disorders is associated with greater Now bias in light/moderate drinking adults but not emerging adults. Age group and family history interacted to affect ICR ($F_{(1,50)}$=16.40, p<0.001, η^2=0.203). This interaction reflects dramatically higher ICRs among FHP adults relative to FHN adults (*$F_{(1,12)}$=7.21, p=0.020, η^2=0.366) and smaller, opposing effects of FH among emerging adults ($F_{(1,37)}$=3.89, p=0.056, η^2=0.092).

Specifically looking at the effect of FH on ICR among moderate drinkers, stratified by age group, we found a trend toward a main effect of FH with higher ICRs in FHP (0.62±0.25) relative to FHN (0.50±0.31; $F_{(1,50)}$=3.521, p=0.066, η^2=0.044) participants, but we also found a significant age*FH interaction ($F_{(1,50)}$=16.40, p<0.001, η^2=0.203; Figure 3.4). This interaction reflects dramatically higher ICRs among FHP adults (0.61±0.27) relative to FHN adults (0.23±0.32; $F_{(1,12)}$=7.21, p=0.020, η^2=0.366) and smaller, opposing effects of FH among emerging adults, as reported above. In fact, ICR values of light drinking FHP adults are quantitatively similar to that seen in heavy drinking adults in this study and in adults with AUDs (Mitchell et al., 2005; Boettiger et al., 2007).

Removing participants with potential DSM alcohol dependence did not alter our results

As mentioned above, *post-hoc* evaluation of responses in the Rutgers Alcohol Problem Index (RAPI) indicated probable alcohol dependence among 43 recruited subjects (~18%), based on *Diagnostic and Statistical Manual of Mental Disorders* (4th ed.; *DSM–IV*; (American Psychiatric Association, 1994) criteria. To ensure that these individuals were not confounding our results, we removed them from our key analyses. The Low vs High AUDIT group x age group ANOVA resulted in similar effects to those observed in the larger dataset (see: *Effects of age and AUDIT group on ICR, above). We observed* a main effect of age group ($F_{(1,177)}$=5.98, p=0.015, η^2=0.031) and a significant age*AUDIT interaction ($F_{(1,177)}$=5.27, p=0.023, η^2=0.028). The AUDIT-c group x age group ANOVA (see: *Testing for interacting effect of age and alcohol use in AUDIT-c groups, above)* produced significant age ($F_{(1,126)}$=6.57, p=0.012, η^2=0.045). and AUDIT-c ($F_{(1,126)}$=4.80, p=0.03, η^2=0.033) effects on ICR, as seen with these possibly dependent participants included in the analyses. We also still observed a significant age group x AUDIT-c group interaction on ICR ($F_{(1,126)}$=7.79, p=0.006, η^2=0.054). Investigating our key family history x age group ANOVA in the low AUDIT-c group (see: *Family history of alcoholism and DD,* above), we observed a trend toward an effect of family history on ICR ($F_{(1,48)}$=3.49, p=0.068, η^2=0.046) and a significant age group x family history interaction on ICR ($F_{(1,48)}$=15.279, p<0.001, η^2=0.199). Again, in our adult low AUDIT-c individuals, FH positive individuals displayed significantly higher ICRs (0.62±0.29) than those without a family history of AUDs (0.23±0.32; $F_{(1,11)}$=7.12, p=0.022, η^2=0.378). We also observed a significant effect of family history in the opposite direction in our emerging adult group with the family history negative individuals displaying significantly higher ICRs (0.76±0.19) than those FHP (0.62±0.25, $F_{(1,36)}$=4.35, p=0.044, η^2=0.105). The fact that our key findings are observed when removing those participants with potential alcohol dependence suggests that these effects are robust and

point to high Now bias (ICR) as a potentially useful intermediate phenotype for AUDs as it is observed in heavy drinking adult and family history positive light drinking adult individuals not meeting DSM-IV alcohol dependence.

DISCUSSION

DD across the lifespan

We hypothesized based on our earlier work (Mitchell et al., 2005; Kelm et al., 2010) and previous DD studies (Green, 1994; Scheres et al., 2006; Olson et al., 2007; Eppinger et al., 2012) that Now bias would decline in light/moderate drinkers during the transition from emerging adulthood to adulthood. Here we demonstrate that DD indeed negatively correlates with age well into the second decade of life among moderate FHN drinkers, with reduced *Now* bias typically observed by the mid-twenties. It should be noted that a study by Dom et al (2006) found a significant negative relationship between discounting behavior and age in a control, non-problem drinking group that was not observed in early or late onset problem drinkers. Thus, there is some existing evidence in the literature that age may be related to discounting behavior in non-problem drinkers and that problem drinking may uncouple this relationship. Our findings lend further support for this hypothesis and warrant further investigation. If emerging adults (18-24) tend to show heightened *Now* bias, the utility of this behavior as an intermediate phenotype may be restricted to older individuals.

DD and alcohol use

Investigators consistently find greater *Now* bias in participants with AUDs versus those without (Petry, 2001; Bjork et al., 2004; Mitchell et al., 2005; Boettiger et al., 2007; Mitchell et al., 2007), and greater DD among heavy versus lighter social drinkers was reported in a small college

age sample (Vuchinich and Simpson, 1998). A recent meta analysis of DD behavior across addictions (MacKillop et al., 2011) reported modest effects of subclinical heavy alcohol use on increased discounting behavior. Other than the Vuchinich and Simpson (1998) study, all remaining subclinical findings reported in that meta analysis consisted of questionnaire-type measures of discounting behavior, which could be less reliable than behavioral-based methods to assess discounting. Thus, our study focused on subclinical problematic drinking behavior in a relatively large sample to address the limited information we have on behavioral discounting in problematic drinkers not identifying themselves as having an alcohol use disorder. Here, we observed greater DD with heavy alcohol use specifically among adults (>25 yrs) but not emerging adults (18-24). It should be noted that Vuchinich and Simpson (1998) was a relatively small study (n=31) using different problem drinking determination and DD measures than those used here. Also, nearly all of Vuchinich and Simpson's problem drinkers were under age 20 while the social drinking group's average age >20. Thus, if age is negatively related to DD in general, as we propose in this study, the drinking status effects observed in Vuchinich and Simpson could have been confounded by age differences across their groups. Further investigation of the interaction of age and problem alcohol use on discounting behavior is warranted to resolve our findings with Vuchinich and Simpson (1998).

While previous studies that included participants with AUDs found ICR to positively correlate with AUDIT dependence and harm subscales (Mitchell et al., 2005), but not AUDIT-c; in this larger sample of participants self reporting no drinking problems, AUDIT-c score best predicted ICR. Taking an AUDIT-c cutoff score of 5, which fairly specifically identifies heavy drinkers (Bush et al., 1998), we found substantially greater *Now* bias among heavy drinking adults relative to lighter drinking adults, with the ICR's of heavy drinking adults on par with

those of adults with AUDs (Mitchell et al., 2005; Boettiger et al., 2007). Thus, heightened *Now* bias in adults may be an early indicator of AUD risk. Among emerging adults, in contrast, our findings suggest that developmental effects on DD behavior generally occlude a relationship between alcohol use and ICR, except in FHP individuals. In that particular at-risk population, heightened *Now* bias is associated with heavy, sub-clinical alcohol use, and may have practical utility in identifying at-risk individuals. Future longitudinal studies are required to further investigate this issue.

Impulsivity in emerging adults & heavy drinking adults: shared mechanisms?

Our data demonstrates equivalent DD between two groups at risk for developing AUDs: emerging adults and heavy drinking adults. The fact that heavy drinking emerging adults did not show higher ICRs than moderate drinking emerging adults suggests the possibility of shared underlying mechanisms in these populations. For example, both groups might have dysfunction in the frontal circuitry engaged during *Now/Later* decision-making (Boettiger et al., 2007). A variety of evidence indicates that frontal development is incomplete until the early-to-mid twenties (Sowell et al., 1999; Casey et al., 2000; Sowell et al., 2001; Giedd, 2004; Gogtay et al., 2004; Lenroot and Giedd, 2006), and functional maturation of brain circuits begins to plateau at ~22 yrs, with continuing maturation into the mid-to-late twenties (Dosenbach et al., 2010). Such maturational effects are consistent with our finding that "adult-like" DD emerges sometime between ages 22-26. Indeed, a structural neuroimaging study of individuals aged 9-23, showed that age-related increases in frontal white matter integrity negatively correlate with *Now* bias (Olson et al., 2009); however, that study included very few individuals >18, precluding conclusions regarding changes from late adolescence to early adulthood. Recent functional neuroimaging data show that DD decreases from early adolescence (age 11) to early adulthood (age 31) are

associated with changes in corticostriatal circuit function (Christakou et al., 2011). Thus, structural and functional changes in frontal circuits could underlie the age-related decline in DD we observed among moderate FHN drinkers. Not only do frontal circuits mature late, they are especially prone to insult by heavy alcohol consumption, especially binge drinking (De Bellis et al., 2005; Miguel-Hidalgo et al., 2006; Jacobus et al., 2009; McQueeny et al., 2009), and decreased frontal metabolism is observed in AUDs (Volkow et al., 1997; Catafau et al., 1999). Thus, we speculate that *Now* bias reflects immature frontal function in emerging adults, and dysfunction in similar circuits in heavy drinking adults.

Now bias as an AUD intermediate phenotype

Determining the biological basis of risk for complex neurobehavioral disorders like AUDs is difficult due to multiple interacting risk factors. Moreover, genes do not encode for psychopathology, hence the association of gene effects should be greater at the level of simpler, intermediate phenotypes (Meyer-Lindenberg and Weinberger, 2006). We argue that *Now* bias in adults shows promise as an intermediate phenotype for AUDs, as has been recently suggested of discounting behavior by others (MacKillop, 2013). First, *Now* bias is obviously related to AUDs, as every relapse or excess drink represents a decision favoring immediate over delayed benefits. Second, DD behavior has good psychometric properties; as we demonstrate here, responses are highly reliable (Chronbach's $\alpha > .98$). Third, DD behavior is stable over time (Kirby, 2009). Fourth, as we show here, *Now* bias occurs to a greater degree in unaffected relatives. Fifth, DD behavior is heritable and associates with substance use, suggesting common genetic influences with SUDs (Anokhin et al., 2011). Evidence regarding cosegregation of *Now* bias with AUDs within families is currently lacking, but every other criteria appears to be met (Meyer-

Lindenberg and Weinberger, 2006; MacKillop, 2013). Other factors may impact DD in adolescence, obscuring the utility of this behavioral intermediate phenotype in late adolescents/emerging adults, however, future neuroimaging studies may identify a neural signature associated with *Now* bias that is enhanced in FHP adolescents.

Family history of AUDs and Now bias

With regards to earlier studies investigating the effect of family history status on discounting behavior, it is worth noting that most of the studies focused on individuals under the age of 26 (Crean et al., 2002; Herting et al., 2010) found very modest to no effect of family history status on discounting behavior. A study by Petry et al. (2002) which found effects of family history on discounting in females had an average age around 26, thus, this study potentially contained enough adult participants (using the definition of 26-40 proposed in our current study) to observe an effect of family history. The fact that Petry et al. observed family history effects in females only (Petry et al., 2002) supports the idea that developmental "maturity" may be needed to see family history effects. Female brains are believed to reach functional maturity a few years earlier than males (Lenroot et al., 2007; Lenroot and Giedd, 2010). Thus, Petry et al.'s female participants may have been mature enough for FH effects to be observed while the effect in males could have been obscured by the fact that their brains could have still been developing during the early-to-mid twenty age range of participants sampled in Petry et al. (2002). In the current study, we observed a family history effect when focusing on 26-40 year old light/moderate drinkers. Thus, otherwise non-affected adults (light/moderate drinking; no reported AUDs) with at least one first degree relative with an AUD show heightened Now bias relative to those without a family history of AUDs, a critical demonstration

of heightened Now bias as an intermediate phenotype of AUDs (Meyer-Lindenberg and Weinberger, 2006). We believe our findings here point to the importance of considering how age effects may occlude the identification of factors such as family history on delay discounting behavior. Future studies should explore the effect of family history on discounting behavior in adult samples to confirm our findings that Now bias meets this important intermediate phenotype criteria.

Study limitations

First, while our recruitment group age ranges were evidence-based and yielded a main effect of age group on ICR in moderate drinkers, our data indicate that ICR decreases substantially with age until ~26 yrs. Our sample size in the 26-40 group limited our power to investigate effects of other factors on ICR (e.g. sex) in adults. Second, we sought to investigate ICR in groups at risk for AUDs, thus excluding individuals with AUDs, however, we did not conduct clinical interviews to directly establish AUD diagnoses. Third, our measure of alcohol consumption, the AUDIT-c score, has the limitations of any self-report measure and is a coarse measure of alcohol use. More detailed measures of drinking behavior will be needed to determine how the quantity, frequency, and pattern of alcohol consumption may relate to ICR. For example, binge drinking may be a stronger predictor of ICR, as it is most damaging to the brain and frontal cortices in particular.

Our results are suggestive that Now bias decreases with age in light/moderate but not heavy, problem drinkers. The cross-sectional nature of this study, however, prevents us from being able to directly test whether Now bias relates to aging within individuals differentially

based on their drinking behavior. Longitudinal studies are needed to directly confirm our hypotheses.

Conclusions

Here, we found that three AUD risk factors strongly predict *Now* bias: age, heavy alcohol use, and FH status. We show that *Now* bias declines with age in FHN moderate drinkers, with ICR declining to adult levels around the mid-twenties. In adults, we also found that either heavy alcohol use or a positive FH for alcoholism is associated with *Now* bias equivalent to that seen in abstinent alcoholics. Thus, *Now* bias may be an intermediate phenotype common to groups at higher risk for AUDs that is present before an AUD. While the underlying neural mechanisms of elevated ICR in emerging adults, heavy drinking adults, and FHP individuals remains to be investigated, *Now* bias represents a promising intermediate phenotype for AUD risk. As such, modification of this behavior may prove an effective AUD prevention strategy in at-risk groups.

CHAPTER 4: OVARIAN CYCLE EFFECTS ON IMMEDIATE REWARD BIAS IN HUMANS: A ROLE FOR ESTRADIOL

INTRODUCTION

All animals, including humans, discount delayed rewards (Mazur, 1987; Rachlin, 2000), a tendency variously known as delay discounting (DD), temporal discounting, or immediate reward bias. While some degree of DD is normal, excessive immediate reward, or *"Now",* bias is associated with multiple clinical conditions, including substance abuse (Becker and Murphy, 1988; Reynolds, 2006; Perry and Carroll, 2008; Rogers et al., 2010), attention deficit hyperactivity disorder (Barkley et al., 2001; Sonuga-Barke et al., 2008; Paloyelis et al., 2010), pathological gambling (Alessi and Petry, 2003; Leeman and Potenza, 2012), and obesity (Weller et al., 2008; Epstein et al., 2010). Thus, understanding the biological bases of such *Now* bias may have wide-reaching impact. Evidence from both humans and animals indicates that individual DD tendency is highly heritable (Anokhin et al., 2011; Mitchell, 2011). However, animal studies also indicate that *Now* bias can be pharmacologically modulated, particularly by dopamine (DA) (Dalley et al., 2008; Doya, 2008; Winstanley, 2011).

Accumulating work in humans also suggests DA as a key regulator of *Now* bias. First, genetic variations in the DA system are associated with individual differences in *Now* bias (Boettiger et al., 2007; Eisenberg et al., 2007; Paloyelis et al., 2010; Smith and Boettiger, 2012), particularly variations in the gene encoding the catechol-*O*-methyltransferase (COMT) enzyme, which regulates tonic frontal DA (Karoum et al., 1994; Gogos et al., 1998; Slifstein et al., 2008;

Kaenmaki et al., 2010; Wu et al., 2012); putative lower tonic frontal DA is associated with greater *Now* bias among adults (Boettiger et al., 2007; Smith and Boettiger, 2012). Second, human pharmacology studies suggest that DA modulates *Now* bias, albeit with inconsistent results (de Wit et al., 2002; Acheson and de Wit, 2008; Hamidovic et al., 2008; de Wit, 2009; Pine et al., 2010), although these studies have not accounted for intrinsic variations in DA signaling that could interact with pharmacological effects. For example, we recently found that genetic variation in *COMT* modulates the effect of an acute DA manipulation on *Now* bias (Kelm and Boettiger, 2013). Recent data showing that *COMT* genotype interacts with cyclic estradiol (E+) changes to affect working memory (Jacobs and D'Esposito, 2011), which is also frontal DA-dependent, suggests that cyclic variation in E+ could also modulate *Now* bias.

Data showing less DD among females relative to males (Bobova et al., 2009; Peper et al., 2013) supports this idea, but no studies have investigated whether cyclic E+ fluctuations are associated with changes in *Now* bias. We hypothesized that if E+ modulates *Now* bias, naturally cycling females should demonstrate reduced *Now* bias from the menstrual phase (MP) to the follicular phase (FP). Moreover, such cycle effects should be modulated by *COMT* genotype. To test these ideas, we measured *Now* bias in a DD task among naturally cycling females (ages 18-40), during both the MP (cycle day 1-2; putative low E+), and the FP (cycle day 11-12; putative high E+). We also determined *COMT* genotype for each subject, and were able to measure free salivary E+ in each visit from a subset of participants.

METHODS

Sample Characteristics

Participants (n=91) were recruited from the University of North Carolina, Chapel Hill (UNC) and surrounding community. Participants were healthy females 18-40 years old reporting

no use of hormonal birth control and regular menstrual cycles of approximately 28 days. Participants also had no known past or present neurological or psychiatric diagnoses, no history of substance use disorders, and no current use of psychoactive medications or other psychoactive substances aside from moderate caffeine, nicotine or alcohol. All subjects were native English speakers and had at least a high-school education. Participants gave written informed consent, as approved by the UNC Office of Human Research Ethics. Subjects participated in two sessions, one on cycle days 1-2 (menstrual phase, MP) and another on cycle days 11-12 (follicular phase, FP), in a counterbalanced, within subject design. In addition to the behavioral testing (see "*Delay Discounting Task*"), during session 1 (n=45 MP, n=46 FP), we collected information on participants' age, years of education, trait impulsiveness (Barratt Impulsiveness Scale-11 (BIS) (Patton et al., 1995)), orientation toward the future (Future Time Perspective Inventory; FTPI (Wallace, 1956)), and locus of control (Rotter's Locus of Control Scale; LOC (Rotter, 1966)).

COMT Genotyping

COMT Val^{158}Met (rs4680) genotyping was performed on DNA extracted from saliva samples (DNA Genotek, Kanata, Ontario, Canada) using TaqMan technology (Life Technologies, Foster City, CA), as previously described (Boettiger et al., 2007; Smith and Boettiger, 2012).

Delay Discounting Task

During each testing session, participants completed a delay discounting (DD) task described in detail previously (Altamirano et al., 2011; Smith and Boettiger, 2012). Subjects were given task instructions, completed a short practice, and then completed 8 blocks of 42 trials

each during each test session. In each session, subjects made a series of choices between smaller, sooner ("*Now*") and larger, later ("*Later*") hypothetical monetary rewards. Each trial began with an instruction cue, followed by two options. In each trial, the *Later* option was one of five amounts ($2, $5, $10, $20, or $100) at one of five future delays (1 week, 2 weeks, 1 month, 3 months, or 6 months), and the *Now* option was an amount discounted by 70, 85, 90, or 95% from the *Later* amount, available "TODAY". The instruction cue was determined by trial type. There were four trial types: WANT, DON'T WANT, SOONER, and LARGER; the latter two conditions are considered together as control (CON) trials. Trial types were pseudorandomly ordered and weighted, with 50% W trials, and 16.7% each of the other trial types. Participants indicated their preferred option on WANT trials, their non-preferred option on the DON"T WANT trials, and the side with the sooner time or larger amount of money for SOONER and LARGER (CON) trials, respectively. The *Later* amount, delay time, percent discount, and left/right position were pseudorandomly selected for each trial. The reaction time (RT) for each response was also collected. Four subjects failed to follow task instructions, based on faster RTs in the WANT and/or DON'T WANT trials than in the CON trials for one or more sessions, and were excluded from all analyses, leaving n=87 participants. Of these, n=44 were first tested in the MP, and n=43 were first tested in the FP.

Our primary index of *Now* bias was the proportion of *Now* choices made in the WANT condition, the impulsive choice ratio (ICR). From our DON'T WANT trials, we determined the inferred ICR (*i*ICR) as a function of delay time and calculated the average of the absolute value of the difference between ICR and *i*ICR at each delay. This value provides a measure of response consistency, termed motor mismatch, with larger values indicating less controlled response

selection (Mitchell et al., 2007). Change in ICR from the MP to FP was calculated as a simple subtraction of the ICR in the FP session from ICR in the MP session.

Salivary Estradiol Quantification

Multiple saliva samples (3 collections over ~1 hour) were collected during each session from a subset of participants (n=34) via passive drool into 15mL tubes over a period of ~1 hour while they completed questionnaires and/or during task breaks. Samples were pooled and stored at -20°C until analysis. Upon thawing, saliva samples were centrifuged at 1500 × g for 15 minutes to separate mucous material and pellet out any potential contaminants. Cleared saliva was then decanted into a separate tube. We quantified salivary estradiol via an enzyme immunoassay kit (Salimetrics, State College, PA, USA). On each plate, pooled samples were tested in duplicate, with samples from both visits for each subject tested on the same plate. Optical densities were measured using a μQuant microplate spectrophotometer (BioTek, Winooski, VT), and, following kit procedure, were transformed into pg/ml E+ values based on plate-specific 4-parameter sigmoid minus curves derived from standard E+ samples. E+ concentrations for each sample were averaged across plates. Only individuals with higher average E+ in their FP sample than in their MP sample were classified as showing a positive change in E+ (ΔE+). The proportional change in E+ value used in our correlation analyses reflect the difference in E+ between the FP and MP samples divided by MP E+ ($\Delta E+=(FP^{E+} - MP^{E+})/ MP^{E+}$).

Statistical Analysis

As ICR data in this sample was not normally distributed ($D(174)=0.12$, $p<0.001$; Kolmogorov-Smirnov test), the Wilcoxon sign-ranked test was used for comparison of ICR across sessions. Paired *t*-tests and repeated measures ANOVAs were used to test for the effect of Cycle Phase on other DD task measures as well as on arc-sine root transformed ICR values. Group comparisons of ICR change were made using unpaired *t*-tests. Pearson's *r* or Spearman's Rho ρ were used for correlation analyses, as indicated.

RESULTS

Sample characteristics

Eighty-seven healthy females from the Chapel Hill/Durham area volunteered and were paid for their participation (Table 4.1). In the sample as a whole, we observed a positive correlation ($r_{(85)}=0.22$, $p=0.040$) between *Now* bias measured in session 1 and the non-planning subscale of the Barratt Impulsiveness Scale (BIS), a trait measure of impulsivity, which is consistent with some previous findings (Mitchell et al., 2005; de Wit et al., 2007).

Impulsive choice declines from early to mid-cycle

We predicted a decline in our measure of *Now* bias, the impulsive choice ratio (ICR; see Methods) from the MP to the FP, which was indeed observed. Across all participants, we found a significant effect of cycle phase on ICR with higher ICRs in the MP (Median=0.72) relative to the FP (Median=0.66) using either a Wilcoxon signed-rank test on the raw ICR values ($z=-2.77$, $p=0.006$, $r=-0.21$), or a paired samples *t*-test on arc-sine root transformed ICR data ($t_{86}=2.13$,

p=0.036; Fig. 4.1). Although we counter-balanced session order, we confirmed that this cycle effect remained significant after covarying for session order in a repeated measures ANOVA ($F_{(1,85)}$=4.65, p=0.034, η^2=0.049).

Table 4.1: Demographic data overall and by first session cycle phase.

	Demographics Across All Participants and Broken Down by First Session Cycle Phase		
	All (n = 87)	MP (n=44)	FP (n=43)
Age (yrs)	24 ± 5		
Education (yrs)	16 ± 2		
Psychometric Measures			
BIS Total Score	60.2 ± 11.7	60.6 ± 11.6	59.8 ± 12.0
BIS Attention	16.4 ± 4.1	16.6 ± 4.1	16.3 ± 4.2
BIS Motor	21.6 ± 4.2	22.0 ± 3.9	21.3 ± 4.6
BIS Planning	22.2 ± 5.4	22.1 ± 5.4	22.3 ± 5.5
FTPI			
Mean extension	6.9 ± 5.2	7.2 ± 6.0	6.7 ± 4.3
Max extension	23.7 ± 18.2	25.6 ± 20.7	21.7 ± 15.3
LOC	11.0 ± 4.0	11.1 ± 4.6	11.0 ± 3.3
Genetics			
COMT Val^{158}Met genotype (%)			
Met/Met (%)	19.5		
Met/Val (%)	44.8		
Val/Val (%)	35.6		

Demographic data. Values are reported as mean ± standard deviation. BIS, Barratt Impulsiveness Scale; FTPI, Future Time Perspective Inventory; LOC, Rotter's Locus of Control Scale; MP, Menstrual Phase; FP, Follicular Phase

This *Now/Later* task includes objective choice control (CON) trials; accuracy in these trials did not differ significantly between the MP and FP sessions (t_{86}=-0.66, p=0.51). The task also includes a control condition (DON'T WANT) in which participants are instructed to select the monetary reward option that they *do not* prefer. Comparing ICR in the WANT trials to *inferred* ICR in the DON'T WANT trials provides a measure of response consistency. We observed significant effect of cycle on response consistency, or motor mismatch (see *Methods*), with greater mismatch in the FP (0.13±0.08) relative to the MP (0.10±0.06; t_{86}=-2.63, p=0.010). Importantly, our observed cycle effect on *Now* bias remained significant after covarying for

changes in response consistency in a repeated measures ANOVA ($F_{(1,85)}$=6.02, p=0.016, η^2=0.066), indicating that a drop in response consistency cannot explain the decrease in *Now* bias at mid-cycle. We observed no cycle effects on reaction time (RT) in the objective choice (CON) trials (t_{86}=1.28, p=0.21), subjective choice (WANT) (t_{86}=-0.01, p=0.99), or DON'T WANT (t_{86}=-0.27, p=0.79) trials.

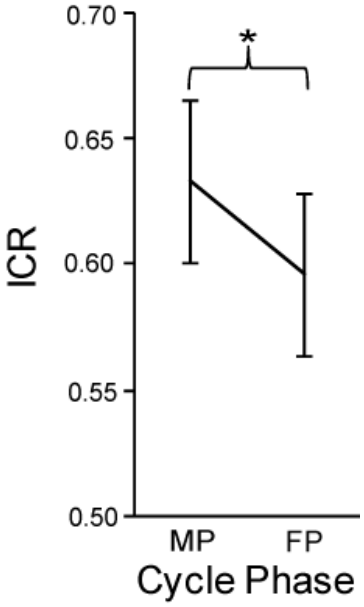

Figure 4.1: Immediate reward (Now) bias declines from early to mid-cycle. Plot depicts an index of *Now* bias, the impulsive choice ratio (ICR), measured during the menstrual phase (MP; cycle day 1-2) and follicular phase (FP; cycle day 11-12), within subjects. ICR's were significantly reduced at mid-cycle (FP) relative to the MP (*t_{86}=2.13, p=0.036). Values reflect mean ± SEM ICR.

The mid-cycle-drop in Now bias is associated with an E+ rise

While the decrease in *Now* bias from the MP to the FP is consistent with a hypothetically E+-mediated effect, hormonal variation across the cycle varies, even among women with regular cycles. To more directly assess the relationship between changes in E+ and changes in *Now* bias, we assayed salivary E+ from both sessions from a subset of participants (n=34). On average, E+ rose (0.50±0.23 pg/mL) from the MP to the FP ($t_{(33)}$=-2.20, p=0.035). However, of the 34, only

23 participants demonstrated a detectable increase in E+ from the MP to the FP (E+ rose 1.15 ± 0.89 pg/ml, $t_{(22)}$=-6.20, p<0.001; critically, these 23 participants also showed a significant decrease in *Now* bias (-0.07±0.03) from the MP to the FP ($t_{(22)}$=-2.18, p=0.041; Fig. 4.2). In contrast, in those without a detectable rise in E+ (n=11; E+ *decreased* by 0.85±1.07 pg/ml; $t_{(10)}$=2.62, p=0.026) *Now* bias tended to rise from the MP to FP (0.05±0.05; $t_{(10)}$=1.09, p=0.303; Fig. 4.2). The cycle-effect on *Now* bias differed significantly between the E+ rise and the no E+ rise participants (t_{32}=2.145, p=0.040; Fig. 4.2).

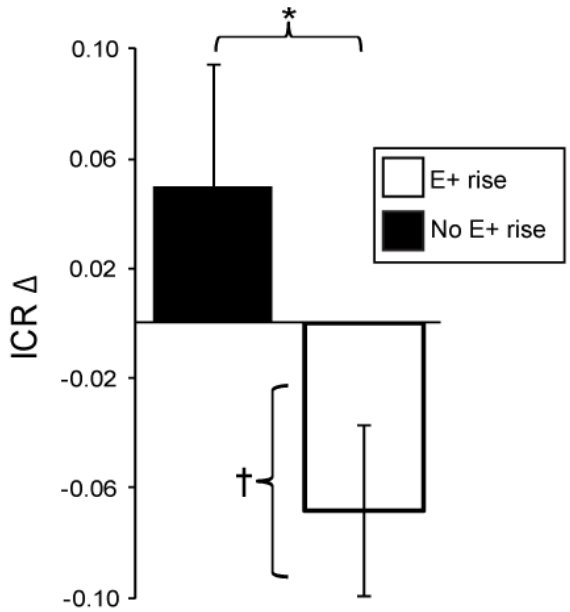

Figure 4.2: *Now* bias declines at mid-cycle when estradiol (E+) rises at mid cycle. Plot depicts change in ICR from early to mid-cycle in participants with a verified rise in E+ at mid cycle (E+ rise), and those without a detectable rise in E+ at mid-cycle (No E+ rise). ICR declines significantly at mid-cycle in the E+ rise group ($^{\dagger}t_{22}$=-2.18, p=0.041). The change in ICR from early to mid-cycle differed significantly between the E+ rise & no E+ rise groups (*t_{32}=2.145, p=0.040).

Changes in Now bias from the MP to FP are inversely related to changes in E+

As we observed substantial variation in E+ changes from the MP to the FP, we tested whether the magnitude of E+ change between sessions was correlated with individual change in *Now* bias. To avoid concerns about violating parametric assumptions, we calculated the Spearman's rho (ρ) between the proportional change in E+ from the MP to the FP and ICR

change from the MP to the FP and conducted a robust regression analysis procedure using bootstrapping. We found a significant negative correlation between E+ change and ICR change ($\rho_{(32)}$=-0.39; 95% CI: -0.67, -0.06, p=0.023; Fig. 4.3, solid line), suggesting a role for E+ in mediating the observed cycle effects on *Now* bias.

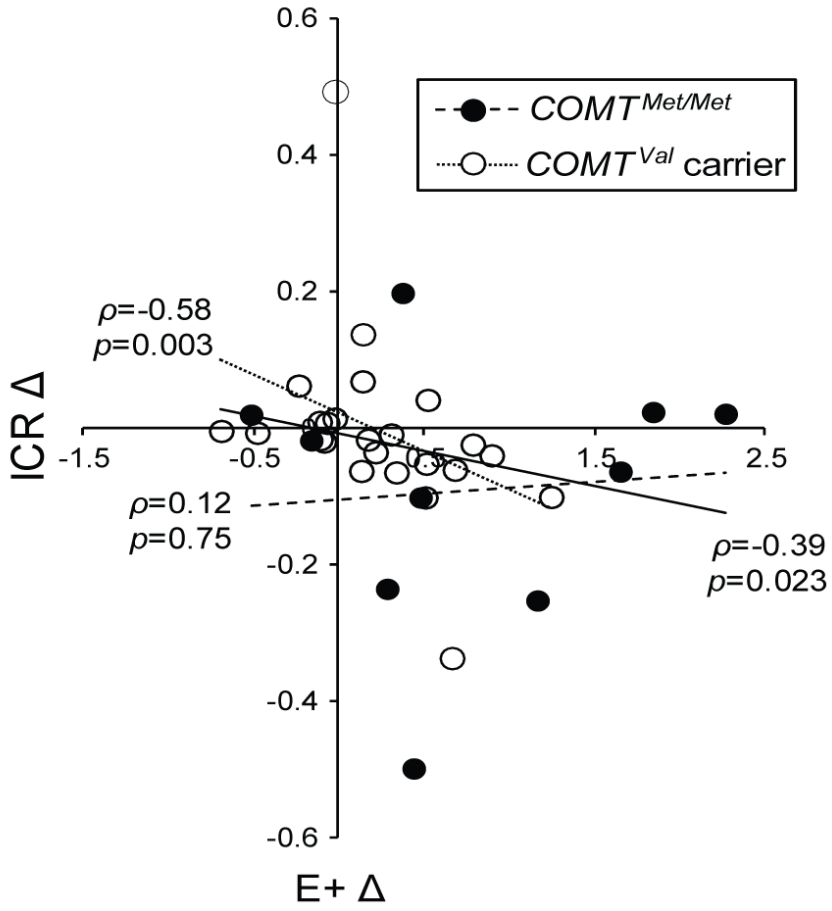

Figure 4.3: Change in ICR from early to mid-cycle is proportional to the change in E+ and driven by low putatively lower tonic frontal dopamine *COMT*^{Val} carriers. In the sample as a whole, ICR decreases correlate with E+ increases (ρ=-0.39, p=0.023, solid line). This effect is driven by *COMT*^{Val} allele carriers (open circles; ρ=-0.58, p=0.003, dotted line). Changes in E+ and ICR from MP to FP are not correlated in individuals with putatively high tonic frontal dopamine (*COMT*$^{Met/Met}$ (filled circles), ρ=0.12, p=0.75, dashed line).

E+ in session 1 correlates with trait impulsivity

Based on our findings that session 1 ICR positively correlated with BIS non-planning scores, and that ICR fluctuated from the MP to the FP in tandem with E+ changes, we tested whether E+ levels in session 1 correlated with BIS non-planning scores. We found that indeed each additional pg/ml of E+ measured in session 1 was associated with 0.79 points lower BIS non-planning scores ($r_{(32)}$=-0.42, 95% CI: -0.63, -0.20, p=0.014). A similar relationship between session 1 E+ levels and total BIS scores ($r_{(32)}$=-0.36, p=0.036) was also observed.

COMT genotype modulates E+ effects on Now bias

As the effects of E+ changes from the MP to FP on working memory, which is also frontal DA-dependent, are modulated by *COMT Val*^{158}*Met* genotype (Jacobs and D'Esposito, 2011), we tested whether the observed relationship between E+ elevation and reduced *Now* bias is also modulated by *COMT* genotype. Of the participants from whom we had E+ measures, the distribution of *COMT* genotypes was: 10 Met/Met, 9 Met/Val, 15 Val/Val. Unlike the full sample, within the Met/Met group, we observed a statistically insignificant increase in ICR with greater E+ rises ($\rho_{(8)}$=0.12, p=0.75; 95% CI: -0.68, 0.70; Fig. 3, dashed line). However, in the Met/Val group, we observed a trend toward greater mid-cycle declines in ICR with greater E+ rises (ρ(7)=-0.63, p=0.067; 95% CI: -1.0, 0.24); Val homozygotes showed a similar negative relationship (ρ(13)=-0.29, p=0.302; 95% CI: -0.73, 0.34). Based on this similarity between the latter two groups, we considered Val allele carriers together and found significantly greater midcycle declines in ICR with greater midcycle E+ rises among Val carriers (ρ(22)=-0.58, p=0.003; 95% CI: -0.78, -0.22; Fig. 3, dotted line), indicating that Val carriers drove the effect

observed in the sample as whole. Note that average E+ in the MP ($F_{(2,31)}$=0.91, p=0.412) and FP ($F_{(2,31)}$=0.05, p=0.952) visits did not differ significantly by *COMT* genotype. However, there was a trend toward *COMT* genotype-dependent effects on the difference in measured E+ between the MP and FP visits ($F_{(2,31)}$=3.05, p=0.062), reflecting the fact that we observed the greatest rise in E+ among $COMT^{Met/Met}$ individuals (0.78±0.91, standardized values), with substantially smaller average E+ rises in $COMT^{Val/Met}$ (0.31±0.41) and $COMT^{Val/Val}$ (0.18±0.47) individuals. The difference in E+ rise between the $COMT^{Met/Met}$ and $COMT^{Val/Val}$ groups approached statistical significance ($t_{(12.28)}$=-1.97, p=0.072). Thus, the apparently greater sensitivity of *Now* bias to changes in E+ among $COMT^{Val}$ carriers cannot be attributed to differences in baseline E+ levels or to a greater degree of E+ change among the $COMT^{Val}$ carriers, who together had smaller increases in E+ from the MP to the FP than $COMT^{Met/Met}$ individuals (0.22±0.45; $t_{(10.86)}$=1.87, p=0.088).

Based on our previous observation in a mixed sample of males and females that age interacts with *COMT* genotype to predict *Now* bias (Smith and Boettiger, 2012), we also conducted an exploratory examination of the correlation between E+ change and ICR change within *COMT* Val carriers separately within our previously defined age groups (ages 18-21 vs. 22-40). We observed a substantially larger correlation among 22-40 year old $COMT^{Val}$ carriers ($\rho_{(9)}$=-0.77, p=0.005; 95% CI: -0.95,-0.26), who, according to our inverted-U model (Smith and Boettiger, 2012), should have the lowest tonic PFC DA, relative to 18-21 year old Val carriers ($\rho_{(11)}$=-0.604, p=0.029; 95% CI: -0.93, -0.09). Within the group with the highest predicted tonic PFC DA (18-21 year old Met/Met individuals), increasing E+ was associated with *increased* ICR at mid-cycle; although the effect was not statistically significant ($\rho_{(2)}$=0.400, p=0.60). The relationship between E+ change and ICR change was relatively flat in 22-40 year old Met/Met

group ($\rho_{(4)}$=-0.086, p=0.872). While these observations are consistent with our hypothesized results, these are highly preliminary findings, and it is particularly important to note that the tests within the Met/Met age groups are grossly underpowered to detect even large effects.

DISCUSSION

Here, we demonstrate that *Now* bias decreases significantly from the MP to the FP. Participants with a verified rise in E+ from the MP to the FP visit showed significantly greater decreases in *Now* bias at midcycle than those without, with *Now* bias decreasing significantly in the E+ rise group but not in the no E+ rise group. The change in *Now* bias from the MP to the FP inversely correlated with the change in E+, and this effect was driven by individuals with putatively lower frontal DA based on *COMT* genotype. These data suggest that the rise in E+ during the peak fertility window modulates *Now* bias through interactions with tonic frontal DA.

Role of Estrogen in Frontal Function

Existing data link E+ and frontal-dependent function in animals, presumably via E+-induced enhancement of DA signaling (Xiao and Becker, 1994; Pasqualini et al., 1995; Shansky et al., 2004). E+ increases frontal dopaminergic innervation in primates (Kritzer and Kohama, 1999), increases spine number on frontal pyramidal cells (Tang et al., 2004), and affects frontal-dependent task performance (Tinkler and Voytko, 2005; Hao et al., 2007). Direct evidence for the relationship between E+ and frontal DA in humans are lacking, although PET studies suggest sex-specific differences in DA D2 receptor levels (Kaasinen et al., 2001) and in amphetamine-induced DA release in the brain (Riccardi et al., 2006; Riccardi et al., 2011), including frontal effects, although, no published studies comparing the sexes have used radiotracers suited to

detect frontal D2 binding. Furthermore, there is no published human PET data regarding the effect of cyclic E+ changes on DA signaling. However, behavioral and fMRI data from humans indicate that E+ modulates frontal-dependent cognitive processes (Keenan et al., 2001), including working memory span (Rosenberg and Park, 2002), inhibitory control (Colzato et al., 2010), and *n*-back task performance (Jacobs and D'Esposito, 2011; Joseph et al., 2012). In the case of *n*-back performance, E+ effects interact with frontal DA tone, as indexed by *COMT* genotype (Jacobs and D'Esposito, 2011). Given evidence of heightened responses to affective stimuli in brain regions linked to arousal during low E+ points in the menstrual cycle (Goldstein et al., 2005), the heightened *Now* bias we observed in the MP could theoretically reflect both heightened responsiveness to more proximate rewards and a decrement in frontal function when E+ is low.

Dopamine as a potential mediator of the cycle effect on decision-making

The present data support the idea that E+ modulates *Now* bias via the DA system, especially frontal DA. This conclusion derives from the data described in the previous section coupled with our finding that the correlation between E+ changes and *Now* bias changes from the MP to the FP varies with *COMT* $Val^{158}Met$ genotype. The Val158Met polymorphism substitutes the ancestral valine (Val) for a methionine (Met) at position 158 in the COMT enzyme, resulting in 4-fold reduced enzyme activity (Chen et al., 2004). Reduced tonic frontal DA is observed in people with the *COMT* $158^{Val/Val}$ genotype relative to $COMT^{Met}$ allele carriers (Wu et al., 2012). According to a model wherein *Now* bias varies with frontal DA according to an inverted-U function (Altamirano et al., 2011; Smith and Boettiger, 2012; Kelm and Boettiger, 2013), these data suggest that E+ elevations alter *Now* bias via interaction with frontal DA levels. This idea

remains to be empirically tested, but frontal-dependent tasks are generally sensitive to frontal DA according to a quadratic function (Sawaguchi and Goldman-Rakic, 1991; Vijayraghavan et al., 2007; Cools and D'Esposito, 2011).

Role of Other Hormones in Decision Making

Other steroid hormones are implicated in the modulation of *Now* bias. For example, salivary testosterone correlates with DD among healthy male students (Takahashi et al., 2006). Cortisol is also implicated in DD behavior, based on the initial finding that salivary cortisol negatively correlates with *Now* bias (Takahashi, 2004). However, a later study by the same group found that sex moderates the effect of salivary cortisol on *Now* bias, with a negative relationship among males, and a positive relationship among females (Takahashi et al., 2010). It is worth noting that stress (associated with elevated cortisol) impacts frontal function in an inverted-U manner (Arnsten, 2009) and that E+ exacerbates stress-induced working memory impairment in animal models (Shansky et al., 2004; Shansky et al., 2009). To date, no studies have investigated progesterone effects on DD. Moreover, no study has yet examined multiple steroids, and this study is the first to control for cycle phase in females. Future work investigating the individual contributions of different steroid hormones is needed for a complete picture of the hormonal modulation of *Now* bias. Perhaps most importantly, to date, no hormone challenge studies have been conducted, which are required to determine causal roles in modulating *Now* bias.

Other potential mediators of the cycle effect on decision-making

Although our finding of *COMT*-dependent E+ effects on *Now* bias suggests a role for DA in mediating the observed cycle effect on *Now* bias, other neurotransmitter systems may also be

involved. In particular, progesterone (PROG) or its neuroactive steroid (NAS) derivatives, such as allopregnanolone (ALLO), which also rise across the cycle, may contribute. Moreover, ALLO appears to modulate cognitive function in humans (Marx et al., 2009), possibly through its positive modulation of $GABA_A$ receptors (Majewska et al., 1986). Neuroimaging studies have demonstrated that PROG increases and E+ decreases from FP to the luteal phase (LP) result in changes in activity in a host of PFC, limbic, and striatal regions (Dreher et al., 2007; van Wingen et al., 2008; Ossewaarde et al., 2011; Mareckova et al., 2012). Like PROG and ALLO, GABA levels also rise from the FP to LP (Epperson et al., 2002). Thus, PROG, ALLO, or GABA may alter the function of neural circuitry implicated in *Now* bias and requires further study. Imprecision in our sampling method means that we cannot rule out a role for cyclic changes in PROG, ALLO, or GABA in reducing *Now* bias at mid-cycle. Increased GABAergic signaling could theoretically improve cognitive control, leading to reduced *Now* bias. Indeed, PROG administration can improve cognitive control and reduce smoking urges (Sofuoglu et al., 2011). However, in women, ALLO impairs episodic memory (Kask et al., 2008), which shares common neural substrates with episodic prospection, a mental action that decreases *Now* bias via increased connectivity between the medial temporal lobe, amygdala, and dorsal anterior cingulate (Peters and Buchel, 2010; Benoit et al., 2011), a functional connection weakened by increases in PROG (van Wingen et al., 2008). Perhaps most pertinent, enhancing $GABA_A$ signaling reportedly has no effect on *Now* bias (Reynolds et al., 2004; Acheson et al., 2006). Thus, although the reduced *Now* bias we observed at mid-cycle could theoretically reflect some contribution of PROG, ALLO or other NAS, or GABA, no direct data are available, and the circumstantial evidence does not favor that interpretation.

Study Limitations

Some limitations of the current study hinder our ability to draw strong conclusions regarding the role of specific hormones in the observed cycle effect on *Now* bias. First, we did not assay PROG or ALLO levels in our subjects, so roles for these hormones in our observed behavioral effects cannot be ruled out. Second, E+ samples were only available from a subset of participants. Concern on this point is somewhat mitigated by the fact that the subsample from whom we were able to measure E+ did not differ from the subsample from whom these samples were available. Considering the two subsets as independent samples, this fact could be seen instead as a replication of our basic behavioral finding across subsamples, strengthening that finding. Third, our method relied on self-report of cycle day, and did not document cycle duration or hormonal markers of ovulation across the cycle, which may have substantially reduced variance, particularly in the FP. We likely failed to catch the E+ peak in many participants, which could substantially diminish effect size. Indeed, among the participants for whom we quantified E+, many showed little or no E+ rise from the FP to the MP visit, and the effect size was substantially greater in the participants for whom we verified a rise in E+ in the FP. More precise monitoring of hormone levels within individuals and targeting behavioral measurements to individually determined cycle phases are advised for future research. Finally, here we took advantage of the natural "experiment" whereby hormone levels change cyclically. While the results suggest a role for E+ in modulating *Now* bias, direct manipulation of E+ is required to establish a causal role.

Finally, while our finding that E+ interacts with a marker of frontal DA tone is consistent with some existing literature, our interpretation of those findings remains speculative. In particular, we have no direct evidence that E+ is modulating frontal DA, nor that frontal DA

modulates *Now* bias according to a U-shaped function. Future PET studies of DA signaling are needed to test our model. Regardless, our model suggests that the effects of dopaminergic medications may be optimized by taking E+ levels and *COMT* genotype into account. Given the large number of conditions treated with such medications, such personalization could have broad therapeutic impact.

CHAPTER 5: GENERAL DISCUSSION

SUMMARY OF RESEARCH FINDINGS

Support for inverted-U model relating Now bias to putative PFC DA signaling

Over the course of this dissertation work, we have investigated the neurocognitive basis of immediate reward selection (*Now*) bias and the relevance of this behavior as an intermediate phenotype for alcohol use disorders. Specifically, we add to the literature that frontal DA as indexed by variation in the *COMT* gene is related to *Now* bias. We reconciled the divergent *COMT* findings of Boettiger et al. (2007) and Paloyelis et al. (2010) by hypothesizing an inverted-U function for the effect of frontal DA on *Now* bias. Given that DA signaling declines with age (Mukherjee et al., 2002; Wahlstrom et al., 2010) and the COMT enzyme activity itself is modulated by aging (Tunbridge et al., 2007), we show that the high-activity *COMT* Met/Met polymorphism in late adolescents (ages 18-21) keeps these individuals' high age-related DA levels controlled via enhanced PFC DA clearance. Thus, ICR (our index of heightened *Now* bias) is low in 18-21 Met/Met relative to low-activity *COMT* Val/Val individuals. The same *COMT* Met/Met polymorphism reduces PFC DA in adults (22-40) to a proposed suboptimal level (as age-related tonic DA is lower in adults to begin with), leading to higher ICR in these individuals (Figure 2.1). This led to a proposed inverted-U function explaining the relationship between tonic PFC DA and *Now* bias (Figure 2.3).

This proposed inverted-U function for the role of PFC DA on ICR was tested in the context of naturally-varying dopaminergic signaling across the menstrual cycle in female

participants in Chapter 4. We found that as estradiol (and hypothesized DA signaling) rose, ICR tended to decline *and* that this relationship was driven by *COMT* Val allele carriers with proposed lower PFC DA levels (Figure 4.3). Thus, increases in DA-related signaling associated with rising estradiol reduced ICR in participants whose proposed tonic PFC DA levels were at the left of the inverted-U function. These sub-optimally dosed individuals had the most to gain from increased DA-related signaling. Furthermore, considering the age-related differences in *COMT*-related modulation of ICR from Chapter 2, we subdivided our *COMT* groups into 18-21 and 22-40 year olds and found that the negative relationship between increasing estradiol and decreasing ICR was strongest in 22-40 year old Val carriers with proposed lowest tonic PFC DA (far left of the inverted-U function). Conversely, in 18-21 year old *COMT* Met/Met individuals, we found a non-significant but *positive* relationship between increasing estradiol and ICR (i.e., ICR tended to get higher). These individuals would be hypothesized to have the highest PFC DA levels (far right of the inverted-U function) and perhaps the boost in DA signaling associated with increased estradiol pushed them too far to the right on the inverted-U function where their PFC DA was overdosed. While age-related *COMT* groups in our estradiol study were small and we were underpowered to determine if age x *COMT* effects may have been present in our estradiol study, these data point to the utility of our inverted-U function in explaining dopaminergic-modulation of *Now* bias.

Increased Now bias as an intermediate phenotype for AUD risk

The importance of understanding the neurobiological basis of *Now* bias was highlighted in Chapter 3 where we show that elevated *Now* bias may serve as a useful intermediate phenotype for AUD risk in adults (26-40). Support for increased *Now* bias as an intermediate phenotype for substance use disorders, including AUDs, has recently been reviewed (MacKillop,

2013). A meta-analysis has found *Now* bias tends to be stronger in those meeting DSM criteria for AUDs while subclinical problem alcohol use is associated with modestly elevated *Now* bias (MacKillop et al., 2011). However, only one of the five studies used in the meta-analysis was based on a behavioral DD task consisting of a small college-age sample of light and heavy social drinkers (Vuchinich and Simpson, 1998). Here, we extend those findings by demonstrating problematic drinking (as defined by the Alcohol Use Disorders Identification Test) adults (26-40 years old) without a self-reported AUD display elevated *Now* bias compared to light/moderate drinkers when controlling for *COMT* x age effects observed in Chapter 2 (Figure 3.3). The degree of *Now* bias in these problem drinkers was nearly equivalent to that of abstinent alcoholics tested previously (Mitchell et al., 2005; Boettiger et al., 2007). Thus, elevated *Now* bias is present in those engaging in risky drinking behaviors before the potential development of an AUD. Additionally, we demonstrate adult light/moderate drinkers with at least one first degree relative (parent or sibling) with an AUD or possible AUD display heightened *Now* bias relative to those with no family history of AUDs (Figure 3.4). This behavior cannot be explained by the individuals' own drinking behavior as that analysis focused on non-heavy drinkers. While this result will need replication due to our relatively small sample size, it is to our knowledge the first data lending support to this key intermediate phenotype criterion. Thus, our work provides additional evidence that increased *Now* bias is a useful intermediate phenotype for AUD risk.

Age-related differences in Now bias: Explanation for inconsistent elevated Now bias in individuals with a family history of AUDs?

In the current work, we found *Now* bias to be elevated in 18-24 year old "emerging adults" regardless of their drinking behavior (Figure 3.3). This age effect occludes the ability to

use elevated *Now* bias as an intermediate phenotype for AUD risk in this population. Also, this data suggests the possibility that decreased *Now* bias may be associated with increased PFC structural and functional maturity as this is one of the last structures to complete maturation in humans with full development not complete until the early-to-mid twenties (Giedd, 2004; Lenroot and Giedd, 2006). We believe our age-dependent *Now* bias effect, if generalized to other studies of *Now* bias behavior, could explain the lack of consistent effects of family history of AUDs on discounting behavior. Most of these family history studies focused on individuals under the age of 26 (Crean et al., 2002; Herting et al., 2010) and found very modest to no effect of family history status on discounting behavior. A study by Petry et al. (2002) which found effects of family history on discounting in females had an average age around 26; thus, this study potentially contained enough adult participants (using the definition of 26-40 proposed in dissertation Chapter 3) to observe an effect of family history. The fact that Petry et al. observed family history effects in females only (Petry et al., 2002) supports the idea that developmental "maturity" may be needed to see family history effects as female brains mature earlier than males (Lenroot et al., 2007; Lenroot and Giedd, 2010). Thus, future studies of *Now* bias behavior should consider age-related effects on this behavior in addition to other variables of interest. Careful control of age distributions across groups of interest (problem drinkers versus non-problem) will be needed to remove the potential confound of age from future studies.

Role of PFC Dopamine in Now Bias – Direct measures needed

Throughout Chapters 2 and 4, we put forward the hypothesis that PFC dopamine (DA) is a key modulator of *Now* bias behavior. We emphasize here that we have inferred the $Val^{158}Met$ single nucleotide polymorphism (SNP) in the *COMT* gene is an index of PFC DA tone. There is

evidence that this *COMT* SNP is associated with tonic differences in PFC DA as assessed by positron emission tomography (PET) (Wu et al., 2012). We also emphasize that we believe that PFC DA level is associated with *Now* bias according to an inverted-U function where intermediate levels of DA are associated with the lowest *Now* bias. A recent DA depletion study has found that *COMT* SNP predicts the degree to which the depletion of DA affects *Now* bias according to an inverted-U function: those with lower DA tone's (*COMT* Val/Val) *Now* bias increased with DA depletion while the high tonic DA group (*COMT* Met carrier) saw a reduction in *Now* bias with decreased DA (Kelm and Boettiger, 2013). While these data support our hypothesis, the specific role of PFC DA and the presence of an inverted-U relationship between it and *Now* bias remain to be tested directly.

Assessing the role of PFC dopamine in Now bias with PET

Conducting positron emission (PET) studies using ligands sensitive to DA receptors in the PFC, particularly [(18)F]Fallypride (Mukherjee et al., 1995; Mukherjee et al., 1999; Elsinga et al., 2006; Riccardi et al., 2008), would allow us to measure the relationship between *Now* bias and D2/3 receptor density across the brain. Fallypride is unique in its ability to image both striatal D2/3 receptor as well as extrastriatal receptor binding. Thus, using fallypride PET, we could collect DA D2/3 receptor density maps across the age, *COMT* genotype, and menstrual cycle phase to test whether our observed group differences in *Now* bias across these measures is related to PFC DA tone specifically. Previous work with this particular tracer have shown age-related declines in D2/3 binding (Mukherjee et al., 2002), supportive of the notion that age modulates DA signaling.. Interestingly, another study has found that patients with alcohol dependence exhibit reduced D2/3 binding in extrastriatal sites including the hippocampus, insula,

and temporal cortex relative to age-matched controls and that age-related loss of D2/3 receptors was stronger in alcohol dependent individuals (Rominger et al., 2012). Thus, there is evidence for both age and alcohol use effects on the DA system. Relating these changes in the DA system as assessed by fallypride to *Now* bias behavior is the next logical step in testing our inverted-U model.

Potential role of striatal dopamine in Now bias

Striatal dopamine is potentially another factor to consider in our analysis of *Now* bias behavior as frontostriatal loops are critical in reward-based decisions and actions (Haber and Knutson, 2010). Using 6-[18F]-fluoro-L-*m*-tyrosine (FMT) PET to measure DA synthesis capacity in various striatal subregions (Jordan et al., 1997), we have preliminary data suggesting that those individuals (n=15, 8 male; age 27.7±2.3), with low FMT in the putamen have higher *Now* bias than those with higher levels of FMT, even when covarying for *COMT* genotype (Figure 5.1). Thus, DA tone in the putamen may be a critical mediator of *Now* bias (ICR). Using the same fallypride tracer mentioned previously, we could assess the role of D2/3 DA receptor density across the striatum on *Now* bias and begin to build a model of which frontal and striatal regions are critical in dopaminergic-modulation of *Now* bias.

In addition to PET methods for quantifying components of DA signaling across the brain, we can study the impact of genetic variations in striatal DA regulators such as the dopamine transporter (DAT) on *Now* bias. DAT is the main means of DA clearance in the striatum and a variation in the DAT gene is present in a variable number of tandem repeat (VNTR) elements in the 3' untranslated region of the gene (Vandenbergh et al., 1992). This particular VNTR consists of two primary variants, a 9 and 10 repeat (Doucette-Stamm et al., 1995). A recent meta-analysis

has shown that the 9 repeat DAT VNTR is associated with greater DAT expression (Faraone et al., 2013) and, thus, lower DA tone in the striatum. We have recently genotyped a large group of individuals, for whom we have existing *Now* bias and *COMT* genotype data, for the DAT VNTR. Testing effects of DAT VNTR (DAT 9 repeat carrier versus DAT 10/10 repeat individuals) and *COMT* genotype in 22-40 and 18-21 year olds, we observed a near-significant effect of *COMT* genotype on ICR in adults, $F_{(2,113)}$=2.97, p=0.056, confirming previous findings (Boettiger et al., 2007) and those reported in Chapter 2 (*COMT* Val/Val ICR > Val/Met > Met/Met). In 18-21 year olds, however, we observed a trend toward the DAT 10/10 repeat individuals displaying high ICR relative to the 9 repeat carriers ($F_{(1,99)}$=3.82, p=0.053) while the *COMT* effect in this age group was not present ($F_{(2,99)}$=0.68, p=0.51; Figure 5.2). Thus, striatal DA tone as assessed via the DAT VNTR may be a more important mediator of *Now* bias in late adolescents than *COMT*. Further investigation of the relative role of striatal and frontal DA in *Now* bias across individuals and how age may modulate this relationship is needed.

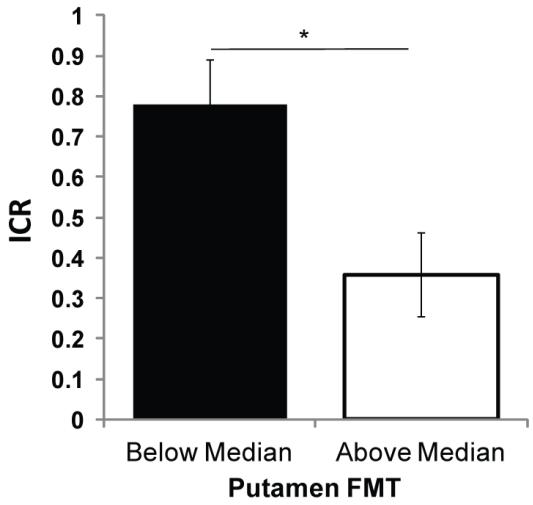

Figure 5.1: Low levels of DA synthesis capacity in bilateral putamen are associated with high ICR when controlling for COMT genotype. In a sample of 15 participants with FMT PET imaging data, a median split of those with high (n=8) and low (n=7) FMT in the bilateral putamen found a significant effect of putamen FMT level on ICR when covarying for Val158Met COMT genotype, $F_{(1,12)}$=5.27, p=0.041.

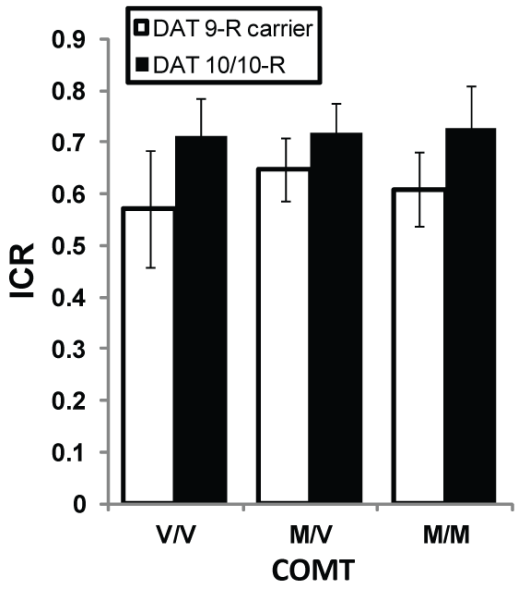

Figure 5.2: In late adolescents, high ICR is associated with the DAT 10/10 repeat allele, not *COMT* genotype. In individuals 18-21 years old, a near significant effect of DAT VNTR genotype on ICR is present, $F_{(1,99)}$=3.82, p=0.053) while the *COMT* effect in this age group is not present ($F_{(2,99)}$=0.68, p=0.51). 9-R carrier, 9/9 or 9/10 repeat DAT VNTR; 10/10-R, 10/10 repeat DAT VNTR

Differences in task-related activity and Now bias

Magnetic resonance imaging technology can take advantage of the different magnetic properties of oxygenated and deoxygenated hemoglobin to measure neural activity in humans via blood oxygenation level-dependent (BOLD) imaging through a process known as functional magnetic resonance imaging (fMRI) (Ogawa et al., 1990; Bandettini et al., 1992). This MRI technique is considered functional in that the BOLD signal is collected while human participants perform a particular task in the MRI machine and the experimenter can then compare this response to estimates of an expected BOLD response to stimuli of interest (those associated with when individuals are asked to make a WANT versus objective SOONER/LARGER response in our delay discounting task, for instance). These comparisons can be used to generate statistical parametric maps of task-related BOLD activity that fits a particular cognitive pattern of interest (using the general linear model approach) which can then be compared across individuals in an

approach titled statistical parametric mapping (SPM) (Friston et al., 1995a; Friston et al., 1995c; Friston et al., 1995b).

Using a simple regression approach in SPM, Boettiger et al. (2007) identified an area in the right lateral orbital frontal cortex (OFC) whose active during DD task WANT choice selection was negatively correlated with *Now* bias. Additionally, a group of areas including the left superior frontal gyrus of the PFC and right supramarginal gyrus of the parietal lobe displayed positive WANT activity correlations with *Now* bias such that these areas were more active in individuals with greater *Now* bias. The PFC and parietal area findings were interpreted as reflecting inefficient processing in these areas in high *Now* bias individuals (Boettiger et al., 2007). We hypothesized these areas might also be differentially engaged in heavy drinking adults and emerging adults found to display elevated *Now* bias in Chapter 3. We tested whether these age and alcohol use groups showing elevated *Now* bias in our pilot behavioral study (Figure 3.3) displayed common BOLD activity patterns during WANT choice selection. We conducted simple between group t-tests of activity differences specific for WANT choice selection in heavy versus light drinking adults and light drinking emerging adults versus light drinking adults. In these comparisons, light drinking adults with previously observed low *Now* bias served as the comparison group against two groups who previously displaying elevated *Now* bias. These initial analyses could provide insight on areas whose activity differentiates individuals with high *Now* bias. Furthermore, findings here would identify areas whose activity changes with age in light drinkers (light drinking emerging adult versus adult comparison) as well as areas engaged differentially in heavy versus light drinking adults. Overlap between these two activity comparisons may also suggest areas in the brain which are sensitive to aging and alcohol effects which could be further analyzed to better understand the age by AUDIT-c interaction on *Now*

bias we observed in Figure 3.3. Preliminary results from these comparisons found a region in right dorsal PFC (dPFC) (middle frontal gyrus, Brodmann area 9; MNI coordinates 42, 30, 34) that was more active in both emerging adult light drinkers and adult heavy drinkers as compared to light drinking adults (Figure 5.3).

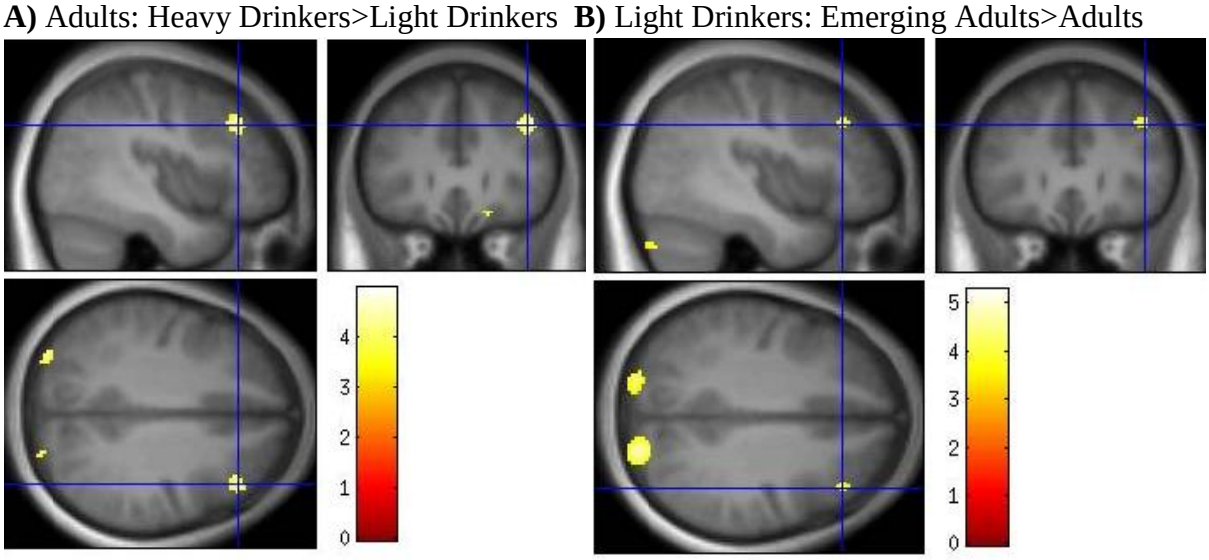

Figure 5.3: Neural activity is elevated in the right dorsal PFC in groups displaying high ICR, Emerging Adults and Heavy Drinking Adults, relative to light drinking adults. Figure reflects statistical parametric maps for T-tests comparing WANT versus CONTROL fMRI BOLD response activity differences between: A) heavy (n=13, 8 male) and light drinking (n=12, 6 male) adults B) emerging adult (n=14, 7 male) and adult light drinkers. A region at MNI coordinates 42, 30, 34 (crosshairs) showed elevated activity across both comparisons. Color bar reflects *t* test values for each group comparison. $p<0.001$, uncorrected, 20 voxel extent threshold

Comparing hyperactivity in PFC in groups with elevated Now *bias to previous findings*

The preliminary t-test analyses above represent our initial investigation of neural differences associated with elevated *Now* bias in an ongoing fMRI study of DD behavior in light drinking emerging adults, light drinking adults, and heavy drinking adults (see Figure 3.3 for AUDIT-c and age criteria used in recruitment). More complex analyses controlling for factors that may modulate *Now* bias (*COMT* genotype, cycle day, etc…) are planned. We found a

common area in PFC whose activity was greater in groups previously identified to have elevated *Now* bias. Increased activity in left dPFC has been shown to be positively correlated with *Now* bias and vary with COMT genotype using the same DD task employed here (Boettiger et al., 2007). Thus, there is convergence for dPFC being an important mediator of *Now* bias. We speculate that hyperactivity in this structure could be associated with inefficient processing during the DD task in individuals with elevated *Now* bias. Thus, the prefrontal control system may not function optimally in those individuals with elevated *Now* bias due to age or drinking behavior.

Following a competing neural systems account of discounting behavior (Bickel et al., 2007), we hypothesized that those with increased *Now* bias might show differences in fMRI BOLD activity in PFC and striatum while responding to the WANT choice (reflecting engagement of a more impulsive brain system during the task) (McClure et al., 2004; McClure et al., 2007; Jimura et al., 2013). Differences in task design (Jimura et al., 2013 and McClure et al., 2004; 2007) and reinforcer type (Jimura et al., 2013 and McClure et al. 2007 consisted of juice reinforcement), could explain why we have yet to identify differential striatal activity shared by two groups with elevated *Now* bias (light drinking emerging adults and heavy drinking adults).

While our increased dPFC activity across two groups with elevated *Now* bias is promising, a more circuit-level approach to investing differences in fMRI BOLD activity in our current dataset is needed. For example, a recent study investigating developmental changes in discounting behavior indentified a corticostriatal circuit that mediated behavioral change (Christakou et al., 2011). Utilizing approaches such as psychophysiological interaction (Friston et al., 1997) or dynamic casual modeling (Lee et al., 2006) to ask how, for example, PFC activity modulates striatal function during *Now/Later* choice could be informative. Furthermore, and

particularly relevant to the work presented here, administration of the COMT-inhibitor, tolcapone, has been found to reduce *Now* bias and alter corticostriatal activity (Kayser et al., 2012). Thus, investigation of brain connectivity differences in the fMRI BOLD data we collect both during our DD task and rest (so called, intrinsic functional connectivity (Raichle et al., 2001; Fox et al., 2005)) to assess neural network differences across groups or variables we think may modulate *Now* bias behavior (Gates and Molenaar, 2012; Smith, 2012) is planned in the future. While these approaches are relatively new and must deal with the high dimensional fMRI data we collect, they promise to greatly us in identifying neural circuits underlying group differences in *Now* bias behavior in a way the general linear model approach applied in SPM cannot (i.e., Figure 5.3). The current and planned neuroimaging analyses (using both fMRI and PET) have the goal of providing information on neural mechanisms and targets for *Now* bias reduction, including potential modulation of *Now* bias via transcranial magnetic stimulation (TMS) of dPFC (Figner et al., 2010; Cho et al., 2012) or pharmacological interventions that may target areas sensitive to DA modulation.

Implications of our findings for treatments to reduce Now bias

Our results here are suggestive that PFC DA modulates *Now* bias and may be one potential avenue for reducing this behavior. Reducing *Now* bias may be particularly useful in AUD treatment as we and others (see (MacKillop, 2013) for review) have demonstrated the behavior serves as a useful intermediate phenotype for AUDs and could partially explain why individuals with AUDs continue to use alcohol (valuing *Now*) despite knowing the long-term consequences of use (discounting *Later*). Previous studies investigating the role of dopaminergic agents on delay discounting behavior have been mixed (de Wit et al., 2002; Hamidovic et al., 2008). Increases in DA signaling across the brain after *d*-amphetamine was effective at reducing

Now bias (de Wit et al., 2002) while the DA D2/3 (highest affinity for D3) receptor agonist pramipexole in a sample of similarly aged healthy participants saw no effect on *Now* bias. Furthermore, the use of buproprion (a non-tricyclic antidepressant drug thought to elevate DA signaling by acting primarily as a DAT blocker (Terry and Katz, 1997)) did not alter *Now* bias in a group of healthy smokers and nonsmokers (Acheson and de Wit, 2008). Finally, a recent study by Pine et al. (2010) found that L-DOPA (a key precursor in DA synthesis) altered *Now* bias while the DA D2 receptor antagonist haloperidol had not effect (Pine et al., 2010). Thus, the literature is suggestive that DA signaling is important in modulating *Now* bias but not through global effects on D2/3 DA receptor signaling.

More recent studies of *Now* bias modulation suggest treatments may be more effective if targeted specifically at the COMT enzyme itself via tolcapone (Kayser et al., 2012) or if dopaminergic interventions are considered in the context of *COMT* genotype (Kelm and Boettiger, 2013). We believe the work we present in this dissertation adds support to the importance of taking tonic PFC DA levels and an inverted-U model into account when conducting any pharmacological intervention. Thus, screening participants for COMT genotype, considering their age, and female estradiol status may all be necessary for more effective individualized treatment. Specific interventions such as DA antagonists may be required for individuals whose *Now* bias is elevated due to excess PFC DA while DA agonists may be the optimal means of treatment in individuals with insufficient PFC DA. It is possible that more selective compounds targeting the D1 receptors critical in PFC goal representation and function (Goldman-Rakic et al., 2000; Vijayraghavan et al., 2007) would be more effective in modulating *Now* bias. Unfortunately, such compounds have yet to be approved for use in human populations.

Conclusions

The ultimate goal of our research program is to better understand the neurobiological basis of *Now* bias to inform attempts to modulate it. We have shown here that *Now* bias is elevated during emerging adulthood (ages 18-24) as well as in adults (aged 26-40) with problematic drinking behaviors or with first degree family members with an AUD (Chapter 3). Thus, this behavior has the properties of an intermediate phenotype for AUD risk as outlined previously (see Introduction; (MacKillop, 2013)). We also provide evidence that variations in the *COMT* gene, a key regulator of PFC DA and function, is associated with *Now* bias according to an inverted-U function (Chapter 2; (Smith and Boettiger, 2012)). This inverted-U relationship has been observed in relation to DA's role in modulating working memory at the level of the dorsolateral PFC (dlPFC) in particular (Mattay et al., 2003; Vijayraghavan et al., 2007; Cools and D'Esposito, 2011). To further support the critical role of DA signaling in the PFC in modulating *Now* bias, we observed *COMT*-dependent effects of estradiol on *Now* bias following a similar inverted-U function (Chapter 4) as has been seen in a working memory task known to engage the dlPFC (Jacobs and D'Esposito, 2011).

These data suggest that PFC DA may be critical in modulating *Now* bias but direct investigation for the role of DA in this behavior using PET techniques (see above) is needed to validate our hypothesis. This approach would also allow for the site of action of DA modulation of *Now* bias to be determined and whether complex fronto-striatal circuits which are both modulated by DA are involved. Regardless of the site of action and exact neural mechanism of our observed effects, our data suggest considering *COMT* genotype, age, and estradiol levels (as a result of cycle phase or various birth control medications) may be critical to determine where on our hypothesized inverted-U model individuals with elevated *Now* bias lie. With this

information, we propose that treatments to reduce *Now* bias may be more effective by personalizing interventions (DA agonist versus antagonist) across individuals with potential differences in PFC DA (insufficient or excess levels) that may both manifest themselves as elevated *Now* bias. Once an individual's PFC DA has been returned to an intermediate level, we expect *Now* bias to be reduced. Reducing *Now* bias may be critical in the context of substance abuse by giving individuals with AUDs or elevated AUD risk the ability to look past the immediate reward of drinking *Now* in favor of the long-term health and lifestyle benefits of not drinking to excess.

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