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Predictive Modeling of Adolescent Cannabis Use From Multimodal Data

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PREDICTIVE MODELING OF ADOLESCENT CANNABIS USE FROM
MULTIMODAL DATA

A Thesis Presented

by

Philip Aaron Spechler

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Abstract

Predicting teenage drug use is key to understanding the etiology of substance abuse. However, classic predictive modeling procedures are prone to overfitting and fail to generalize to independent observations. To mitigate these concerns, cross-validated logistic regression with elastic-net regularization was used to predict cannabis use by age 16 from a large sample of fourteen year olds ($N=1,319$). High-dimensional data ($p = 2,413$) including parent and child psychometric data, child structural and functional MRI data, and genetic data (candidate single-nucleotide polymorphisms, “SNPs”) collected at age 14 were used to predict the initiation of cannabis use (minimum six occasions) by age 16. Analyses were conducted separately for males and females to uncover sex-specific predictive profiles. The performance of the predictive models were assessed using the area under the receiver-operating characteristic curve (“ROC AUC”). Final models returned high predictive performance (generalization mean ROC AUC_{males}=.71, mean ROC AUC_{females}=.81) and contained psychometric features common to both sexes. These common psychometric predictors included greater stressful life events, novelty-seeking personality traits of both the parent and child, and parental cannabis use. In contrast, males exhibited distinct functional neurobiological predictors related to a response-inhibition fMRI task, whereas females exhibited distinct neurobiological predictors related to a social processing fMRI task. Furthermore, the brain predictors exhibited sex-specific effects as the brain predictors of cannabis use for one sex failed to predict cannabis use for the opposite sex. These sex-specific brain predictors also exhibited drug-specific effects as they failed to predict binge-drinking by age 16 in an independent sample of youths. When collapsed across sex, a gene-specific analysis suggested that opioid receptor genetic variation also predicted cannabis use by age 16. Two SNPs on the gene coding for the primary mu-opioid receptor exhibited genetic risk effects, while one SNP on the gene coding for the primary delta-opioid receptor exhibited genetic protective effects. Taken together, these results demonstrate that adolescent cannabis use is reliably predicted in males and females from shared and unique biobehavioral features. These analyses also underscore the need for refined predictive modeling procedures as well as sex-specific inquiries into the etiology of substance abuse. The sex-specific risk-profiles uncovered from these analyses might inform potential etiological mechanisms contributing to substance abuse in adolescence as all predictors were measured prior to the onset of cannabis use.

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Chapter 1: Introduction and Literature Review

Inquiry into the nature of adolescence and drug use can be approached using animal models and human studies. In this introduction, we will review a sampling of the adolescent literature pioneered by many scientists who made great efforts in studying animals and humans during arguably the most fascinating yet challenging period of development. Here, we will review the biobehavioral characteristics of adolescents who use drugs, while considering important sex-differences. The empirical work reported in the second half of this thesis probes a major longitudinal study to uncover sex-specific predictive profiles of adolescents who initiated cannabis use by age 16. These profiles are comprised of psychometric, brain, and genetic features, all of which preceded their cannabis use. Therefore, this review will pay special attention to the relationship between cannabis use and psychosocial and neurobiological development.

1.1 Adolescent Psychosocial Development

Adolescence is a developmental period of considerable changes during which the individual navigates the transition between childhood dependence and adulthood independence. Throughout this period, the adolescent will experiment with novel behaviors, environments, and reinforcers in order to learn new skills necessary for independence. Youthful behavioral patterns from childhood that previously sustained the individual will be phased out and replaced by adult-like behaviors and personality traits developed during experimentation with independence.

During adolescence, the individual experiences a drive to emigrate from the natal family environment and begins to incorporate more peers into their social network. In a

study on adolescent social interactions, adolescents report communicating with their peers throughout 33% of their waking hours during a typical week, compared to 8% of their waking hours communicating with adults (Csikszentmihalyi, Larson, & Prescott, 1977). Hence, increased interactions with peers provide opportunities for the adolescent to experiment with and learn social skills necessary for adulthood. Furthermore, motivation to emigrate from the home and associate with peers is evolutionarily advantageous in order to find genetically dissimilar reproductive partners.

1.2 Risk-taking in Adolescence

When the adolescent spends more time within their social network of peers, various novel environments and social interactions will arise, some of which involving the potential for risk. In these settings, adolescents may be more susceptible to engage in risky behaviors in the presence of peers (Rose et al., 1996). One study identified more than half of adolescents report ever engaging in driving under the influence of drugs or alcohol, unsafe sexual activity, criminal activity, and/or substance use (Arnett, 1992), the latter of which will be surveyed extensively in this thesis. After participating in risk-taking behaviors, adolescents may enjoy social reinforcement from their peers (Kaplan, Johnson, & Bailey, 1987) along with a boost in self-esteem (Shedler & Block, 1990). Additionally, adolescents report they approach risk in order to satisfy curiosity and augment the sense of arousal, intensity, and complexity of novel experiences (Lipsett & Mitnick, 1991). Unfortunately, risk-taking activity can have dire impacts on adolescents as the majority of mortality during this period is comprised of homicide, suicide, and accidental death (Irwin Jr, 1989, 1993). While risk-taking appears to be a common

feature of adolescence, the ability to moderate the frequency and severity of risky activities is an important skill that must also be learned during this period.

A propensity for risk-taking behaviors is commonly operationalized in psychometric questionnaires aimed at measuring the construct of a novelty-seeking personality (Cloninger, 1999; Woicik et al., 2009). In studies of psychosocial development, novelty-seeking personality levels typically peak during adolescence (Maggs, Almeida, & Galambos, 1995; Moffitt, 1993). As such, an elevated novelty-seeking personality is highly predictive of engaging in substance use (Hale et al., 2003; McCormick et al., 1998; Sher & Trull, 1994), a topic we will consider extensively in this thesis. Adolescents also tend to discount delayed rewards in favor of immediate rewards (Steinberg, 2008) and display an insensitivity to both punishment and the aversive properties of some drugs (Cauffman et al., 2010; Doremus-Fitzwater, Varlinskaya, & Spear, 2010; Schramm-Sapyta, Morris, & Kuhn, 2006). Taken together, these behavioral characteristics make adolescents especially vulnerable to engage in risky behaviors like substance use.

1.3 Stress in Adolescence

Animal models of stress reactivity consistently report that adolescents exhibit augmented behavioral signs of stress compared to adult animals when subjected to physiological (Veenema, 2009; Wagner, 1993) and social stressors (Einon & Morgan, 1977; McGivern et al., 1996; Stone & Quartermain, 1997). Behavioral signs of stress are supported by elevated hormonal stress levels, with some studies reporting the highest levels of corticosterone in adolescent female rats compared to adolescent males and adult

female rats (Cirulli, Terranova, & Laviola, 1996; Gabriel, Roncancio, & Ruiz, 1992). Interestingly, surgical and pharmacological ablation of the stress response system has been shown to attenuate alcohol (Fahlke et al., 1994) and cocaine consumption (Goeders & Guerin, 1996), thus highlighting a key contribution of these systems in the development of drug consuming behaviors.

Studies on stress in humans mirror that of the animal studies and suggests the magnitude of stress perception is greater in adolescent females than adolescent males and adult females (Hampel & Petermann, 2006). Indeed, numerous studies have identified frequency of early life stressors (Barrett & Turner, 2006), as well as perceived level of stress (Baer et al., 1987; Deykin, Levy, & Wells, 1987; Johnson & Pandina, 1993; Tschann et al., 1994) as strong predictors of substance use in adolescence. In studies of early life stress, findings indicated that physical and/or sexual abuse during childhood is more frequent in females than males, but nonetheless strongly predicts substance abuse later in life for both sexes (Liebschutz et al., 2002). For males, severity of later drug use was inversely correlated with age at first abuse, such that the younger the age of physical and/or sexual abuse, the more severe substance abuse problems later in life. This dose response relationship was not evident in females, as any history of abuse during childhood strongly predicted substance use problems in adulthood (Liebschutz et al., 2002). In a related study on the association between post-traumatic stress disorder (PTSD) and later substance abuse, findings indicated that PTSD from sexual abuse predicted cocaine use in males, while PTSD from physical abuse predicted cocaine use in females (Hyman et al., 2005). Taken together, prior life stress might promote the generation of maladaptive coping strategies like substance use. Despite the association between stress and substance

abuse, it is challenging to infer causality for a multifaceted phenotype. Furthermore, while stress may precede drug use and lead an individual to consume drugs (DeWit, MacDonald, & Offord, 1999; Tschann et al., 1994; Wills, 1986), drugs and drug-seeking behaviors may potentiate perceptual and biological reactivity to stress (Cinciripini et al., 1989; Cobb & Van Thiel, 1982; D'Souza et al., 2004; Heesch et al., 1995).

Despite the relationship between stress and drug use in females, overall rates of drug use and addiction tend to be higher in men (Kuhn, 2015), possibly due to women being more sensitive to the stigma associated with use. In terms of treatment, women with substance abuse problems progress faster towards treatment than men (Hernandez-Avila, Rounsaville, & Kranzler, 2004). In general, these findings are also applicable to adolescents. Next, we will focus more specifically on rates of drug use and the features that correlate with drug use in adolescence

1.4 Substance Use in Adolescence

Alcohol is currently the most commonly used substance in adolescence. In 2015, nearly half of all 10th graders (ages 14-16) reported any lifetime use, and over a quarter reported ever being drunk (Johnston et al., 2011). Indeed, numerous studies of alcoholism have found that an increase in alcohol use during adolescence predicts problematic drinking (Armario et al., 1987; Barnes & Welte, 1986; Hawkins et al., 1997; Robins & Przybeck, 1985) as well as the use of other drugs (Yamaguchi & Kandel, 1984) in adulthood. This relationship might also be reciprocal, as the initiation of illicit drugs predicts alcohol abuse later in life (Robins & Przybeck, 1985). Problematic drinking in adolescence is concerning, as teens who abuse alcohol might exhibit behavioral signs of

dependence that mirrors adults (Pollock & Martin, 1999), but escalate more rapidly (Clark, Kirisci, & Tarter, 1998) despite a shorter duration of problematic drinking behaviors. Furthermore, alcohol dependent teens exhibit similar rates of relapse following treatment to that of adults (I. Grant et al., 2003). Importantly, the likelihood of becoming dependent on alcohol decreases by 14% with every year that alcohol initiation is postponed (B. F. Grant, Stinson, & Harford, 2001). Therefore, the ability to predict at-risk youth to target prevention strategies is of substantial value.

Regardless of sex, adolescents who complete puberty at an earlier age than their peers are more likely to initiate alcohol, however, early pubertal development is a better predictor of alcohol dependence in females (Costello, 2007). In a path analysis attempting to uncover the mediators of problematic drinking later in life, authors found that the effect of gender on problematic drinking was mediated by proactive parenting style, such that greater proactive parenting styles on female offspring reduced the likelihood of problematic drinking later in life (Hawkins et al., 1997). The role of parental influence on drug use will be considered again in a later section.

Beyond alcohol use, tobacco use is very common in adolescence, although rates have declined over time. Nearly 20% of 10th graders report ever smoking cigarettes in 2015, compared to nearly 40% in 2005 (Johnston et al., 2011). Nonetheless, tobacco is often used in tandem with other drugs. In one study of teens who entered substance abuse programs, nearly 85% report comorbid nicotine dependence (Stewart & Brown, 1995). Likewise, alcohol and tobacco use tend to be highly correlated with cannabis use, in addition to being highly correlated with each other (Moss, Chen, & Yi, 2014).

1.4.1 Parental Influence

Parents may indirectly contribute to the likelihood of their offspring using substances. Indeed, the risk of an offspring abusing or becoming dependent on alcohol is strongly increased when raised by parents with alcohol abuse or dependency problems (Lieb et al., 2002). Likewise, the risk of becoming a tobacco smoker is nearly doubled when raised by a parent who smokes (Patton et al., 1998), a finding that has been replicated and reportedly magnified by maternal smoking (Melchior et al., 2010). Similar patterns of parent-offspring drug transmission regarding cannabis use have also been identified (Duncan et al., 1995; Kerr, Tiberio, & Capaldi, 2015). While many factors may contribute to parent-offspring transmission of drug use, possible mechanisms include shared neurobiological predispositions, or parental mimicry.

Parenting style has also been correlated with adolescent behaviors. Typically, an inverse relationship between parental monitoring and drug use is present, such that decreased parental monitoring may facilitate drug use in adolescence. One study suggest this relationship is most pronounced when parental monitoring is decreased prior to or in early adolescence, as low levels of monitoring at age 11 was most strongly correlated with later drug use (Chilcoat & Anthony, 1996). A similar study corroborated these findings and specifically identified parental knowledge of their adolescent's location on Saturday nights as the strongest predictor of adolescent drug use levels (Ledoux et al., 2002). Given that adolescence is also marked by an increase in perceived parent-offspring conflict (Palermo, 2014), stressed relationships between parent and adolescent could have behavioral health implications.

1.5 Cannabis Use and Adolescence

Longitudinal studies of adolescent development suggest cannabis use is typically initiated between the ages of 16 to 18 (Chen & Kandel, 1995). In terms of the availability of the drug, 10th graders in 2015 reported that cannabis was just as easy to obtain as cigarettes (Johnston et al., 2011). According to a national survey, current rates of cannabis use among adolescents are high, with a quarter of all 10th graders, and over a third of all 12th graders in the US reporting trying cannabis at least once (2014). Chronic use also appears to be growing; in 2008, 5.5% of users aged 12 and older reported near daily use while in 2013 this rate had risen to 8.1% (2014). These increasing rates of use are consequential in that about 10% of those who try cannabis will become weekly users in adulthood (Hall & Pacula, 2003).

1.5.1. Cognition

Cannabis use is frequently described as compromising cognitive abilities, at least under acute intoxication. Impairments in attention, memory, and processing speeds are commonly reported in the literature. A meta-analysis of the neurocognitive findings in *adult* cannabis users suggests impairments do not persist after prolonged abstinence (I. Grant et al., 2003). However, the extent to which these problems abate with abstinence for *adolescent* users remains unclear.

In studies of cognition in adolescent cannabis users, findings are relatively inconclusive, with more evidence pointing to cognitive deficits in teens reporting heavy or earlier onset of use. These cognitive deficits are usually with regard to sustained attention and working memory (Crane et al., 2015; Harvey et al., 2007). Research by

Tapert and colleagues examined cognitive development in relation to cannabis use in a longitudinal study of teens. Early reports found a decrease in composite attention scores with cannabis use (Tapert et al., 2002). These findings were corroborated later and suggest that chronic cannabis use throughout the course of adolescence is associated with a decrease in complex attention, slow processing speeds, and reduced verbal learning and sequencing skills (Jacobus & Tapert, 2014; Medina et al., 2007).

In terms of the lasting effects, Schwartz and colleagues report short-term memory impairments persist at least six-weeks after monitored abstinence (Schwartz et al., 1989). However, in a prospective study by Fried, Gary, & Watkins, researchers analyzed cognitive performance in current users, former users, and never-using controls, while accounting for performance levels prior to drug use. Investigators assessed cognitive ability across many domains (IQs, memory, processing speed, attention) using an extensive battery. Findings indicated that current cannabis users performed worse than the non-users across all domains. However, former users had performance levels similar to never-users despite initiating use earlier and consuming more of the drug than the current users. It will be valuable to see if these effects replicate in larger representative samples as this study contained n=16 former users who also had the highest socioeconomic status (SES) compared to the two comparison groups (Fried, Watkinson, & Gray, 2005). Taken together, it is challenging to compare findings across investigators as the use of different tasks and participant confounders precludes replication or invalidation.

Lastly, in relation to cognitive ability, Lane and colleagues assessed motivation in adolescent cannabis users using a reward task that allowed subjects to switch task

difficulties for smaller monetary reinforcement. Heavy users switched task difficulties at an earlier rate than their non-using peers, and earned a greater proportion of their earnings from the smaller reinforcement level. Interestingly, this proportion of earnings was correlated with the amount of cannabinoids present in participant urine samples on the day of testing (Lane et al., 2005). This finding was also displayed in adults tested under acute intoxication of smoking cannabis compared to placebo (Lane & Cherek, 2002). Thus, cannabis use is associated with impaired motivation and sensitivity to reward, at least under acute and lingering effects. Authors conclude that cannabis use might disrupt healthy motivational processes that coordinate favorable behavioral adaptations (Lane et al., 2005).

As exemplified by Fried, Gary, & Watkins, studies *failing* to find a correlation between cannabis use and cognitive impairment in adolescence are evident in the literature (Tait, Mackinnon, & Christensen, 2011; Takagi et al., 2011; Teichner et al., 2000). However, they are poorly represented and overshadowed by investigators publishing in support of the hypothesis that cannabis use compromises cognition. Nonetheless, functional MRI (fMRI) studies of cannabis use in adolescence, though sparse, typically do not find between group behavioral task differences but report brain differences. Therefore, cannabis use in adolescence might better be characterized by neuroimaging modalities.

1.5.2. Genes

To date, there have been two genome-wide association studies (GWAS) of cannabis use dependence. In the most recent GWAS study, researchers uncovered three

single nucleotide polymorphisms (SNPs) as having an association with cannabis use dependence (Sherva et al., 2016). One SNP was found on an antisense transcription region (*RP11-206M11.7*, rs143244591) whose function is unknown. The other SNPs were found on a gene coding for a protein that regulates extracellular calcium concentrations (*SLC35G1*, rs146091982), and a gene coding for a protein that regulates neuronal inflammation (*CSMD1*, rs77378271). Thus highlighting the potential of identifying predictors of cannabis use from brain and genetic features.

In the earlier GWAS study, none of the SNPs identified passed significance levels appropriate for GWAS studies ($p < 1.0 \times 10^{-8}$). Nonetheless, researchers reported a small collection of marginally significant SNPs on intron and non-genic regions on the genome, for which the authors were unable to provide a mechanistic interpretation for their contribution to cannabis use (Agrawal et al., 2011). However, the authors offered a review of candidate-gene analyses and suggest genes coding for neurotransmitter (e.g., cannabinoid, opioid, dopamine) receptors, and relevant neurotransmitter enzymes (e.g., fatty-acid amide hydrolase, FAAH) might be better suited to uncover genetic associations with cannabis use (Agrawal & Lynskey, 2009).

Considering these studies, a GWAS approach might not yet yield the most clear or robust findings. The SNPs uncovered in these two studies seem to be indirectly related to cannabinoid pharmacology or drug-seeking behaviors. Therefore, as suggested by the authors, a candidate-gene approach might yield more robust findings until more individuals with cannabis use disorders become available in GWAS samples (Agrawal & Lynskey, 2009).

1.6 Adolescent Brain Development

With the advent of *in-vivo* human neuroimaging, great strides have been made to paint a portrait of the human brain as it develops into the adult form. During this period, the brain undergoes a series of developments including global and focal volume changes, myelination, synaptic pruning, and receptor proliferation until the stable adult form is reached in early adulthood (Spear, 2000, 2013). Throughout this process, the neural architecture supporting cognitive, motor, and sensory functions reach maturity. The studies to be covered in this section illuminate these structural and functional developmental processes in light of the behavioral characteristics displayed in adolescence.

1.6.1 Structural Development

From gestation to late childhood, the human brain generates an excess of neurons and affiliated synaptic connections in order to supply individuals with an overabundance of neural resources (Huttenlocher & Dabholkar, 1997; Oppenheim, 1991). Synaptic pruning is a major aspect of adolescent neurodevelopment. Pruning results in considerable gray matter volume loss as a healthy means to promote functional neuronal efficiency. During this process, the important neural connections established from prior experience and learning are preserved, while redundant connections are terminated. Early inquiry into adolescent brain development using non-human primate models postulated that thousands of synapses per second are pruned during the peak of adolescent gray matter development, with some regions resulting in the loss of nearly half the connections formed prior to adolescence (Rakic, Bourgeois, & Goldman-Rakic, 1994).

In addition to synaptic pruning, neuronal myelination, the process by which neurons become coated in fatty projections from oligodendrocytes, also proliferates during adolescence. The myelin sheath expedites signal propagation during synaptic transmission thus allowing distal regions of the brain to communicate more rapidly. In particular, Paus and colleagues report that adolescent brain development is specifically characterized by a marked increase in the myelination of the corticospinal tracts supporting voluntary movements, and the frontotemporal tracts supporting language (Paus, 1999).

Pruning and myelination processes are especially active in the prefrontal cortex (PFC) (Gogtay et al., 2004; Whitford et al., 2007), medial temporal lobe structures, and are orchestrated in part by the endogenous cannabinoid system (Bossong & Niesink, 2010). Studies have shown that exogenous cannabinoids can interfere with the endogenous system (Hoffman et al., 2007; Mato & Pazos, 2004). Given the natural maturation occurring in the brain during adolescence and the propensity towards cannabis use, the consumption of exogenous cannabinoids during adolescence may disrupt typical neurodevelopment within the cognitive and emotional neural systems. Hence, it is crucial to investigate the impact of cannabis use on these brain systems. Research on this topic will be considered in later sections.

In light of these structural changes, males and females exhibit different neurodevelopmental features. Generally, the most commonly reported sex-difference is the finding that male brains are between 9-14% larger than female brains (Paus, 2010), with relatively inconsistent findings for focal volume differences by sex. Pioneering work by Giedd and colleagues have uncovered divergent neural developmental trajectories for

each sex (Giedd et al., 1999). Total brain volume and total gray matter volume (but not white matter) exhibit an inverted U shape. Volumes increase throughout childhood and then reaches an inflection point in early adolescence triggering the decline of brain volume, most likely driven by pruning processes. On average, females reach peak total brain volume at age 10.5 years while males reach peak total brain volume at age 14.5 years. Research from Lenroot and colleagues suggest that white matter volume increases steadily throughout the lifespan, but at a much faster rate in adolescence for males than females (Lenroot et al., 2007). In a related study of white matter development by Perrin and colleagues, authors postulate white matter sex-differences may be due to axonal diameter, rather than myelination, as testosterone upregulates expression of microtubules within the axon (Perrin et al., 2008).

1.6.2 Functional Development

While structural maturity can be tracked via volumetric measurements following developmental trajectories, it is challenging to assert functional maturity from neuroimaging studies. Some argue that augmented blood oxygenated level dependent (BOLD) signal intensity indicates functional immaturity as the neural resources are taxed to a greater extent, whereas others argue that increased activation is a sign of neural specialization. Perhaps more important than signal intensity, Durston and colleagues argue that spatial-extent of activity might be a better characteristic of functional maturity (Durston et al., 2006). Compared to immature diffuse activity, organized and predictable focal activation networks may indicate more efficient processing. These networks are established as a result of neuronal maturation and the strengthening of relevant

connections via long-term potentiation. As such, spatial-extent is also considered an important way to characterize functional between-group differences.

During childhood and throughout adolescence, brain activation networks develop into organized distributed processes favoring sparse “small world” network structures (meaning spatially distant regions of the brain are connected by a small number of connections). In a study of resting state network structures by Fair and colleagues, *a-priori* defined regions of interest supporting the default mode network were studied at two stages of development. Findings indicated that five of the thirteen *a-priori* regions modeled as network nodes exhibited weak interconnectivity at age 7, were then followed by a positive shift to strong interconnectivity by age 21. For instance, correlated activity between a node in the medial prefrontal cortex and a node in the lateral parietal cortex was weakly evident in children but later exhibit robust correlated activity in adults. This example of functional integration among seemingly distant cortical regions is a hallmark of functional development and reflects mature functional efficiency grounded in the changes to the structural architecture (Churchwell, Lopez-Larson, & Yurgelun-Todd, 2010; Giedd et al., 1999; Lenroot et al., 2007; Perrin et al., 2008).

1.6.3 Brain Development and Adolescent Behavior

Given the unique profile of adolescent behavior, investigators postulate that there might be neural correlates supporting these behavioral characteristics. For example, the increase in reward-seeking behaviors described earlier may be correlated with neurodevelopmental changes. The current framework of adolescent neurodevelopment in relation to substance use behaviors postulates that a divergent rate of maturation between

the subcortical and prefrontal regions of the brain might drive reward-seeking behaviors. Previous studies of resting-state functional connectivity between the ventral striatum and medial prefrontal cortex (mPFC) suggest connectivity differs by age. Children and adolescents exhibit positively correlated activity between the ventral striatum and mPFC regions, whereas adults exhibit a developmental switch to anti-correlated activity (Fareri et al., 2015). This effect is also evident in the connectivity between the amygdala and mPFC (Gee et al., 2013). As such, anti-correlated activity is interpreted as a sign of top-down cognitive control mechanisms whereby the prefrontal cortex down-regulates subcortical activity.

Specifically, the bottom-up mesocortical and mesostriatal projections of the ventral tegmental area achieve functional maturity prior to opposing top-down prefrontal projections. Animal models indicated mesocortical dopamine projections to the PFC reach high levels during adolescence (Kalsbeek et al., 1988; Leslie et al., 1991; Rosenberg & Lewis, 1994). Animal models have also identified the three major dopamine receptor subtypes, D₁, D₂, and D₄, reach peak concentrations in the striatum during adolescence, whereas the concentrations in cortical regions continued to rise throughout adulthood (Tarazi & Baldessarini, 2000). While more research is needed, functional MRI studies of reward processing have found heightened BOLD signals in the striatum and ventromedial prefrontal cortex (vmPFC) specific to adolescents during receipt of reward (Cohen et al., 2010; Leijenhorst et al., 2010). Interestingly, another study found a positive correlation between this signal and a self-report measure of risk-taking behaviors (Galvan et al., 2007). In considering these finding as functional

significance of a primed dopamine system in adolescence, the striatum may potentiate teenage reward-seeking behaviors.

1.6.4 Structural Brain Correlates of Adolescent Drug Use

In addition to studying the neural correlates of adolescent behavior, there has been a considerable amount of research on the neural correlates of drug use. In terms of structural MRI findings, an early neuroimaging study of adolescent cannabis users failed to find any gross anatomical differences between users and controls (Block et al., 2000), however, these findings were underpowered relative to the more recent reports.

The cerebellum and medial temporal lobe structures are especially interesting to study in the context of cannabis use, given the high amount of primary cannabinoid receptor (CB1) densities in these regions (Glass, Dragunow, & Faull, 1997), along with their contribution to cognitive and appetitive behavioral processes. Ashtari and colleagues identified bilateral hippocampal volume reductions in adolescent cannabis users compared to controls when scanned after 1 month of monitored abstinence. Furthermore, self-reported levels of use was inversely correlated with the right hippocampus, suggesting a dose response in volume reduction (Ashtari et al., 2011). Similarly, Yücel and colleagues also report gray matter volume (GMV) reductions in the bilateral amygdala (Yücel et al., 2008). Lastly, in a study of young adults, GMV was *increased* in the anterior cerebellum of heavy cannabis users, whereas a negative correlation between dependency scores and right amygdala GMV, and, weekly cannabis use and bilateral hippocampal GMV was identified (Cousijn et al., 2012). All authors

interpret their finding in the context of the CB1 densities in these medial temporal lobe structures.

As these studies were cross-sectional by design, it is difficult to determine the causal relationship between cannabis use and structural changes in humans. However, a longitudinal study by Cheetham and colleagues suggests some volumetric effects might precede use. Researchers found gray matter reduction in the orbital frontal region at age 12 in those subjects who went on to use cannabis at age 16 (Cheetham et al., 2012). These findings are intriguing as work by Volkow & Fowler report hypoactivity in orbital frontal regions using fMRI and PET studies of individuals with drug dependence (Volkow & Fowler, 2000). Nonetheless, given the lack of subcortical findings in the Cheetham study, it might be the case that the medial temporal lobe structures are most sensitive to exogenous cannabinoids.

In light of these studies, the majority of these findings point to a correlation between volume reduction and cannabis use. Authors provide mechanistic interpretations using two different strategies. First, if volumetric differences precede drug use as suggested by Cheetham et al., this might signal evidence of accelerated development as gray matter pruning completed sooner than their peers. Alternatively, if volumetric effects are a consequence of use, then cannabinoids might have neurotoxic effects. Indeed, in a study of cultured hippocampal neurons, researchers observed shrinkage of cell bodies and nuclei, as well as DNA strand breaks induced by delta-9-tetrahydrocannabinol (THC). However, the authors state their findings may not replicate in human cells *in situ* (Chan et al., 1998).

1.6.5. Functional Brain Correlates of Adolescent Drug Use

Functional neuroimaging inquiries into the neural correlates of adolescent cannabis use are sparse. One study conducted by the author of this thesis probed for differences in reactivity to social threats between age 14 cannabis users vs. matched controls. Findings indicated the cannabis users exhibited greater BOLD signals from the bilateral amygdala during the viewing of angry faces and thus might characterize teen cannabis users as hyper-sensitive to signals of threat (Spechler et al., 2015).

In an interesting fMRI study of insular activity, Migliorini and colleagues pleasantly stimulated regions on the palm and forearm thought to send afferents to the insula. Investigators had *a-priori* interest in striatal and insular activity as previous literature suggests interoceptive dysregulation might promote the initiation and maintenance of drug-seeking behaviors (Naqvi & Bechara, 2010). In comparison to adolescents with alcohol and/or cannabis use disorders to matched controls, findings indicated that drug users exhibit greater activation in the left anterior insula and lentiform nucleus during soft touch stimulation. However, self-report measures of pleasantness were inversely correlated with anterior insula BOLD activity, such that lower ratings of pleasantness was coupled to higher insular BOLD activation, a finding that was positively correlated in controls. Authors interpret this disconnect between somatic self-report and neural activation as a sign of body prediction error, whereby the individual misdiagnoses internal states and makes maladaptive homeostatic behavioral modifications through drug consuming and addictive behaviors (Koob & Le Moal, 2008; Paulus, Tapert, & Schulteis, 2009).

Work by Tapert and colleagues studied response inhibition in a group of teens with and without cannabis use histories after 1 month of monitored abstinence. Despite not observing behavioral task differences, greater diffuse cortical activations during successful inhibition trials were evident in the cannabis using group. In additional studies by the same lab, findings follow similar patterns despite using spatial working memory, and verbal learning tasks. Teen cannabis users failed to exhibit differences on cognitive task performance after one month abstinence, and were instead better characterized by diffuse functional brain activations (Norman & Shallice, 1985; Padula, Schweinsburg, & Tapert, 2007). Similar effects have also been reported from groups outside of the Tapert lab (Abdullaev et al., 2010; Jager et al., 2010), including one study identifying greater diffuse activity during a verbal working memory task correlating with earlier age of cannabis initiation (Becker et al., 2010). The consistency of these effects all offer support in the interpretation that adolescent cannabis users may have compromised healthy synaptic pruning during development, thus resulting in inefficient processing capabilities. However, these interpretations might be flawed given the cross-sectional study design.

1.7 Predictive Modeling of Drug Use

Considering that the majority of work presented above were cross-sectional studies, it is imperative to conduct more research in order to disentangle cause from effect. Longitudinal studies are very useful in identifying predictive features and consequences of drug use, however, these studies are often challenging to complete, and the analyses are prone to error in methodology and interpretation. Nonetheless, the ability to predict later drug use would inform targeted clinical interventions designed to curb

initiation, and subsequently minimize or eliminate negative consequences of use. Therefore, predictive modeling of drug use needs to be conducted using a set of best practices in order to have a meaningful impact. In the remaining sections, we will consider predictive modeling procedures in relation to substance abuse research.

1.7.1. Predictive Modeling Procedures

As outlined by Whelan & Garavan, predictive modeling can be a challenging pursuit, especially in the field of psychiatric neuroimaging (Whelan & Garavan, 2014). When using neuroimaging to predict a phenotype, the number of independent variables (hundreds of regions of interest (ROIs) or thousands of voxels) often exceeds the number of sample observations, resulting in predictors $p \gg n$ observations. In this scenario, when estimating a typical multiple-regression model (i.e., ordinary least squares for linear regression; maximum likelihood for logistic regression), model estimation fails to converge on a single unique solution and results in a perfect fit to the data. In general, model fit statistics increase as the number of estimated parameters increases, and/or the number of subjects decreases. In these scenarios, the researcher might be driven to overoptimistic interpretations and beliefs concerning their results (Whelan & Garavan, 2014).

Importantly, the ultimate goal for predictive modeling aims to yield a model that will make accurate predictions about novel observations. However, a model estimated on a dataset where $p > n$ will overfit to the observations and consequently poorly predict the outcome of a novel or out-of-sample observation. To overcome these challenges, we will

consider two proposed remedies that can be used in parallel— cross-validation, and regularization.

Cross-validation is a procedure used to partition the original dataset into subsamples of observations. A model is then estimated on one subsample of the dataset (“training data”), and then evaluated using the observations in the subsample not used (“test data”) during model estimation. Thus, the researcher is able to evaluate how well their predictive model generalizes to out of sample observations. Generalizability may be quantified using typical model performance statistics, like R^2 for linear models or the area under the curve of the receiver-operating characteristic (AUC) from logistic models, returned from evaluating the model on the test data. For these two statistics, values closer to 1 reflect superior performance.

One specific form of cross-validation is k -fold cross-validation, where k = number of partitions (or, “folds) of the original dataset. Each fold contains an equal number of unique samples from the original dataset (i.e., When $k = 10$ and $N=100$, each k th fold will have $n=10$ observations). k -fold cross-validation then becomes an iterative process whereby a single fold is set aside as the test sample (“test fold”), and a model is estimated on the remaining $k-1$ folds. The model estimated on the $k-1$ folds is then evaluated on the set aside test fold, thereby insuring the independence of the final test sample. This process is repeated k times, resulting in k final models. In doing so, each observation is tested exactly once, and used in model estimation $k-1$ times.

Regularization is statistical technique used during model estimation that attempts to minimize the amount of overfit to the data. Similarly to model estimation using ordinary least squares, regularized regression techniques seek to minimize the error

between the predicted and observed outcome while also minimizing the magnitude of the regression coefficients. Prior to regularization, it is important that all independent variables are standardized. Here, two specific forms of regularization will be considered, LASSO (Least Absolute Shrinkage and Selector Operator, (Tibshirani, 1996)) regression, and Ridge regression (Hoerl & Kennard, 1970).

LASSO regression rejects complex models in favor of parsimonious models by minimizing the sum of the absolute values of the coefficients during model fit. In doing so, the LASSO estimator solves for the ℓ_1 -norm of the design matrix. During LASSO estimation, predictors that are weakly correlated with the outcome measure are assigned a regression coefficient equal to zero, effectively removing them from the final model. As such, the predictors that remain in the model are potentially more important to the outcome measure than the predictors set to zero. Therefore, LASSO regression is one option available to researchers when $p \gg n$ as the estimation procedure performs feature selection while fitting a model.

Ridge regression seeks to resolve problems arising from multicollinearity among predictor variables. The Ridge estimator minimizes the sum of the squared values of the regression coefficients during model fit. In doing so, the Ridge estimator solves for the ℓ_2 -norm of the design matrix. All predictors are estimated but tend to be assigned smaller coefficients to reduce their fit. As such, correlated predictor variables are given similar regression coefficients and allowed to coexist in the model. Therefore, Ridge regression might be especially valuable in modeling inherently correlated predictor variables common to neuroimaging and psychological research (e.g., neighboring or functionally co-activating brain data; alcohol and tobacco use levels).

In cases where $p \gg n$, and modeling correlated variables might be of theoretical interest, a hybrid approach balancing LASSO and Ridge regression, termed “elastic-net” regularization (Zou & Hastie, 2005), can be used. In elastic-net regularization, LASSO and Ridge are combined using a mixing parameter, α that balances the contribution of the LASSO to Ridge estimation methods. In addition to the α parameter, a second parameter, λ controls the magnitude of the shrinkage applied to the coefficients.

During elastic-net model estimation, the α and the λ values can be tuned within a cross-validation procedure in order to identify the optimal set of parameter values that minimize the test error returned from evaluating model fit on an independent sample of observations. These tuning parameters are always non-negative values, such that $0 \leq \alpha \leq 1$ and $0 \leq \lambda$. It can be shown that when α approaches 1, the LASSO estimator is favored. Likewise, when α approaches 0, the Ridge estimator is favored. Intermediate values of α provide an interpolation between the two estimation procedures. Thus, given N observation pairs (x_i, y_i) the elastic-net regularization problem for fitting regression coefficients, β to a linear model is solved from the following Equation 1.1.

Equation 1.1

$$\min_{\beta_0, \beta} \left(\frac{1}{2N} \sum_{i=1}^N (y_i - \beta_0 - x_i^T \beta)^2 + \lambda P_\alpha(\beta) \right)$$

where

Equation 1.2

$$P_\alpha(\beta) = \frac{(1 - \alpha)}{2} \|\beta\|_{\ell_2}^2 + \alpha \|\beta\|_{\ell_1} = \sum_{j=1}^p \left(\frac{(1 - \alpha)}{2} \beta_j^2 + \alpha |\beta_j| \right)$$

1.7.2. Predictive Modeling of Binge-Drinking by Age 16

In a study related to the empirical work described later in this thesis, Whelan and colleagues implemented many of the procedures described above to classify adolescent binge-drinkers at age 14, and, predict binge-drinking by age 16 from data collected age 14. In applying these methods to a wealth of neuroimaging, psychometric, and candidate SNP data, findings indicated a high degree of predictive modeling performance (classification AUC=0.90; prediction AUC=0.75). Specifically, the most reliable features that both classified and predicted adolescent binge-drinking were more frequent sexual life experiences and similar personality traits including elevated novelty-seeking, disorderly, and extravagant personalities, and diminished conscientiousness traits. The reliable brain features contributing to classification of age 14 binge drinking included reduced GMV of the vmPFC, and reduced BOLD activity in key subcortical regions including the left putamen and hippocampus during reward anticipation, and right hippocampus during reward outcome. The reliable brain features contributing to age 16 prediction involved reduced BOLD activity in pre- and post-central gyri during failed response inhibitions. Interestingly, classification was driven by blunted activity in regions serving appetitive processing during a reward task, while prediction was driven by blunted activity in a motor area during failed response inhibitions. Indeed, this study inspired the theory and methodological undertakings of the empirical work to be presented in the following chapter.

Chapter 2: Predicting Cannabis Use in Adolescent Males and Females from High-dimensional Biobehavioral Data

2.1 Introduction

Studies suggest cannabis use during adolescence is associated with a range of adversity in adulthood including cannabis use dependence (Hall & Degenhardt, 2009), polydrug use (Secades-Villa et al., 2015), cognitive deficits (Meier et al., 2012; Schuster et al., 2016), compromised physical (Kalant, 2004) and mental health (Degenhardt et al., 2013; Kedzior & Laeber, 2014; Malone, Hill, & Rubino, 2010), and diminished life attainment goals (Fergusson & Boden, 2008). These findings are supported by animal models linking cannabis exposure during adolescence with detrimental outcomes in adulthood (O'Shea, 2004; Quinn et al., 2008). However, in humans, it is notoriously difficult to assert a causal role for cannabis in subsequent outcomes. As drug users self-select into use, any negative outcomes arising from use could be related to a number of factors that are confounded with the choice to initiate and continue cannabis use (Jackson et al., 2016).

As indicated from the 2013 National Survey on Drug Use and Health, rates of cannabis use in adolescence are high as nearly 25% of all 10th graders now report trying the drug (2014). In parallel, from 2005 to 2010, rates of cannabis-related emergency room visits has increased 54% in males and 42% in females aged 15-17 years (2014). Moreover, beliefs concerning the risk of use are declining (Johnston et al., 2011) despite the considerable increase in drug potency compared to previous decades (ElSohly et al., 2016). These findings are a source of concern as *in vitro* models indicate that delta-9-tetrahydrocannabinol (THC), the key psychoactive compound of cannabis, could be more

toxic in adolescent than in adult tissue (Pope et al., 2003; Quinn et al., 2008; Renard et al., 2016; Rubino et al., 2015; Schneider, 2008), and studies in humans suggest that early initiation of cannabis is associated with deleterious outcomes compared to delayed onset users (Brook et al., 2011; Coffey & Patton, 2016).

Longitudinal studies of adolescent development suggest cannabis use is typically initiated between the ages of 16-18 (Chen & Kandel, 1995). Thus, adolescence might be a developmental phase during which initiation can be best predicted and, consequently, might yield occasions for preventative interventions. Indeed, characterizing the individuals who are most likely to initiate drug use can give insights into the risk factors for use and thereby inform etiological mechanisms. With recreational cannabis legalization efforts sweeping the United States and abroad, there is an added imperative to identify which adolescents are most at risk for initiation of use in order to curb any consequences of adolescent exposure.

Here, we seek to uncover risk profiles that predict the initiation of cannabis use in adolescence. Participants (N=1,389; see table 2.1.1 for demographics) from the IMAGEN study (Schumann et al., 2010) were characterized across three domains— psychometric, genetic (single nucleotide polymorphisms, SNPs), and brain data (see table 2.1.2 for summary). Data were submitted to a retrospective longitudinal analysis such that the outcome measure, cannabis use by age 16, was predicted from data across all three domains measured at age 14 prior to any cannabis exposure. Cannabis use by age 16 was determined from a self-reported drug use questionnaire. Participants who endorsed using cannabis greater than or equal to six times by age 16 were assigned to the outcome group, as these individuals have used cannabis beyond experimentation levels. All participants

reported no cannabis use at age 14 and those who also endorsed no cannabis use by age 16 were assigned to the comparison group.

2.1.1. Participant Demographics

Measure	Groups					
	Males			Females		
	Cannabis Use by Age 16 (n=110)	Comparison Group (n=538)	<i>p</i>	Cannabis Use by Age 16 (n=63)	Comparison Group (n=678)	<i>p</i>
Age (<i>M,SD</i>)	14.49, 0.50	14.52, 0.39	.511	14.50, 0.53	14.54, 0.42	.518
Handedness (L,R)	11, 99	66, 472	.503	7, 56	60, 618	.549
PDS (<i>M,SD</i>)	2.63, 0.48	2.54, 0.55	.114	3.23, 0.35	3.17, 0.44	.266
Perceptual IQ (<i>M,SD</i>)	108.30, 13.89	108.18, 14.56	.938	106.56, 13.31	107.77, 13.23	.487
Verbal IQ (<i>M,SD</i>)	115.45, 11.97	112.07, 13.14	.013	111.75, 11.86	109.22, 13.80	.159
SES (<i>M,SD</i>)	18.92, 3.92	17.88, 3.82	.010	17.60, 4.0	17.88, 3.68	.567

Table 2.1.1: PDS: Puberty Development Scale (Petersen et al., 1988). ESPAD: European School Survey Project on Alcohol and Drugs (Hibell et al., 1997). SES: Socioeconomic Status.

2.1.2 Summary of Data

Domain	Measures	Data points
<i>Psychometric</i>	<ul style="list-style-type: none"> • Demographics • Cognitive assessments • Personality assessment • Life-events questionnaires • Baseline cigarette & alcohol use • Parent personality and drug use 	<ul style="list-style-type: none"> • 80 measures
<i>Genetic</i>	<ul style="list-style-type: none"> • A-priori SNPs <ul style="list-style-type: none"> • Cannabinoid Receptor • Catecholamine Receptors • Opioid Receptors 	<ul style="list-style-type: none"> • 108 SNPs
<i>Structural Neuroimaging</i>	<ul style="list-style-type: none"> • Total GMV • Gray-Matter Volume ROIs 	<ul style="list-style-type: none"> • 1 total GMV • 278 GMV ROIs
<i>Functional Neuroimaging</i>	<ul style="list-style-type: none"> • Reward Processing Task <ul style="list-style-type: none"> • (2 Contrasts) • Stop Signal Task <ul style="list-style-type: none"> • (2 Contrasts) • Face Processing Task <ul style="list-style-type: none"> • (3 Contrasts) 	<ul style="list-style-type: none"> • 1946 ROIs • 278 per contrast
Predictors per subject		2413

Table 2.1.2: Summary of data used in predictive modeling. A related analysis including psychopathology measures was conducted but did not improve predictive performance. See supplemental text for details regarding each measure. Site was modeled in the analysis and yielded Paris (data not shown) as a significant predictor due to the higher base rate of age 16 cannabis use for both sexes.

Given the challenges of predictive modeling in psychiatric neuroimaging (Whelan & Garavan, 2014), we utilize practices from the field of machine learning to ensure our predictive modeling efforts guard against overfitting, generalize to out-of-sample observations, and select the most predictive variables from a large feature space ($p = 2,413$). Thus, 10-fold cross-validated logistic regression with nested elastic-net regularization (Zou & Hastie, 2005) was implemented. Predictive model performance

was assessed using the area under the receiver-operating characteristic curve (“ROC AUC”) returned when evaluating the estimated training models on the set-aside test-folds during the cross-validation scheme. As neuroimaging and psychometric profiles of adolescents exhibit clear sex differences, these analyses were executed separately for each sex. Hence, we report sex-specific predictive models of the initiation of cannabis use in adolescence.

2.2 Predictive Modeling Performance

The male-specific analysis returned a mean ROC AUC=0.71 ($\sigma = .02$, $p < 1.92 \times 10^{-12}$; see figure 2.2.1), while the female-specific analysis returned a mean ROC AUC=0.81 ($\sigma = .02$, $p < 1.99 \times 10^{-16}$; see figure 2.2.1). The superior prediction for females is notable given that they were fewer in number and lighter in use compared to males (Tables 2.1.1 & A1.4). To assess the impact that the differences in sample size and use severity might have on modeling, an analysis conducted on a smaller number of males, matched on sample size and use levels to the female cohort, was shown to worsen the prediction for males (mean Δ AUC= -.08 relative to full male sample). Thus, the superior prediction of cannabis use in the female sample indicates that females exhibit a more distinct profile at age 14 relative to their non-using peers.

2.2.1 ROC Curves by Sex

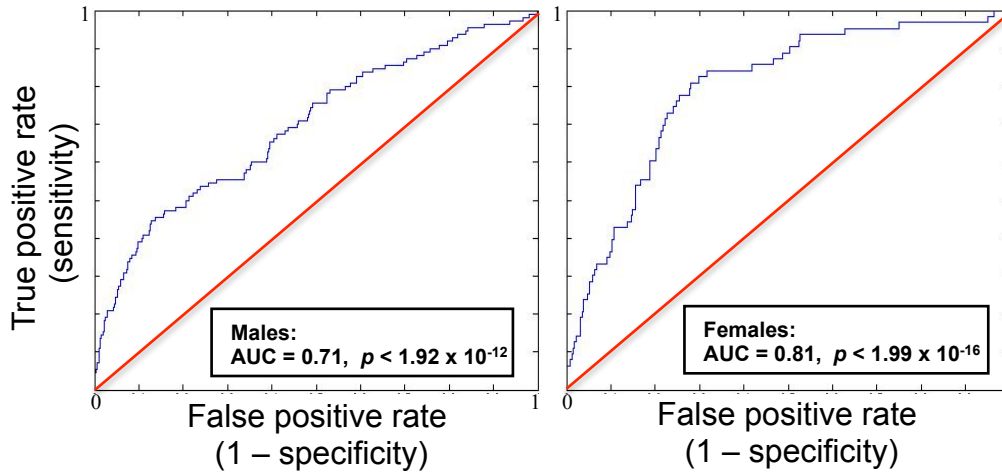


Figure 2.2.1: Visualizing the performance of the predictive model on independent observations. Representative of the mean AUC across 100 runs for each sex.

2.3 Predictive Features

The analyses identified seven psychometric predictors of cannabis use by age 16 common to both sexes including greater lifetime alcohol and cigarette use by age 14, parental lifetime cannabis use, greater novelty-seeking personality and the disorderliness personality subscale (Cloninger, 1999), and, more frequent sexual and distressful life events (See figure 2.3.1 & 2.3.2). These seven shared psychometric predictors replicate previous findings, as alcohol and nicotine use are well-established predictors of cannabis use (Hall & Pacula, 2003; Siegel et al., 2014), as are stressful (Sinha, 2008) and sexual (Cornelius et al., 2007) life events, parental transmission of drug use (Brook et al., 2001; Kandel, Kessler, & Margulies, 1978; Kosty et al., 2015), and novelty-seeking personality and the disorderliness subscale (Hale et al., 2003; McCormick et al., 1998; Sher & Trull,

1994). Taken together, this psychometric profile suggests that behaviorally disinhibited lifestyles precedes cannabis use as these teens exhibit more risky and novelty-seeking behaviors by age 14 compared to their non-using peers.

2.3.1 Shared Psychometric Predictors of Cannabis Use by Age 16: Drug Use Measures

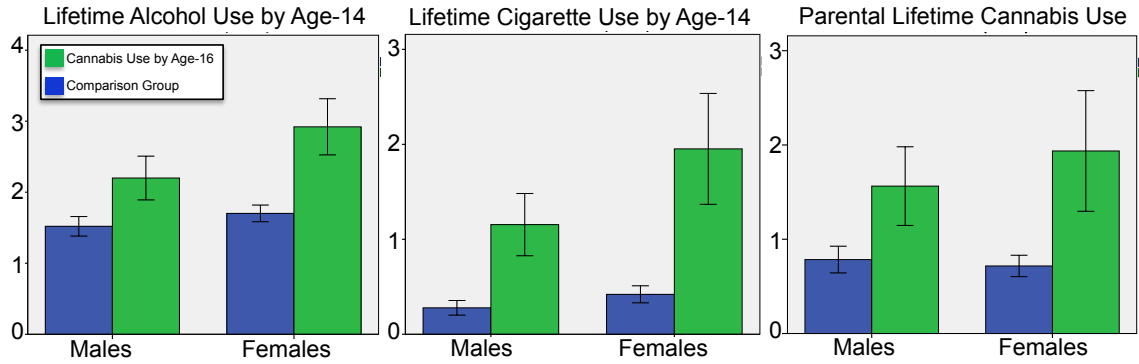


Figure 2.3.1: All measures from the ESPAD (Hibell et al., 1997). Drug use measured on an ordinal scale (0=0, 1=1-2x, 2=3-5x, 3=6-9x). Green bars represent participants (or the parents of the participants) using cannabis at age 16, blue bars represent non-using participants (or parents). Error bars represent the standard error of the mean.

2.3.2 Shared Psychometric Predictors of Cannabis Use by Age 16: Life Events & Personality

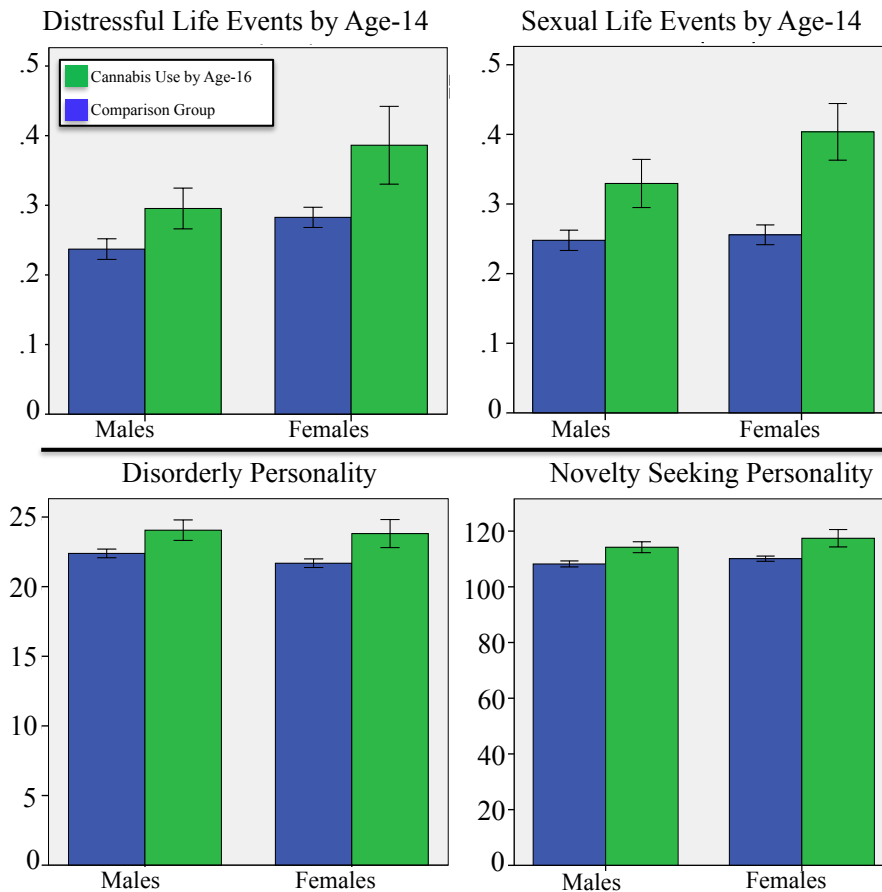


Figure 2.3.2: Top row: from the Life Events Questionnaire (LEQ, (Newcomb et al., 1981)) and represents the mean frequency of endorsing a life event pertaining to either distressful or sexual experiences. Data measured on a binary scale (1=Yes). Bottom row: from the Temperament and Character Inventory (TCI, (Cloninger, 1999)), and represent the sum score of items related to disorderly or novelty-seeking personality traits. Data measured on an ordinal scale (5=definitely true, 1=definitely false). Green bars represent subjects using cannabis at age 16, blue bars represent non-using subjects. Error bars represent the standard error of the mean.

Male-specific psychometric predictors of cannabis use by age 16 included less-negative feelings towards deviant behaviors, and greater parental novelty-seeking personality and the excitability personality subscale (Cloninger, 1999). Less-negative feelings towards deviant behaviors in the males may signal a slight predisposition

towards delinquency and conduct problems in these adolescents. While previous studies have identified a correlation between problematic conduct and cannabis use (Crowley et al., 1998; Robins & McEvoy, 1990), our findings suggest that a predisposition towards problematic conduct might be a target of proactive intervention. Moreover, although personality traits are highly heritable, the novelty-seeking personality traits of both the parent and son independently predicted cannabis use by age 16 (*post-hoc* partial-correlation between the outcome measure and child novelty-seeking personality $r_{645} = .17, p < .001$, after first accounting for parent novelty-seeking personality, $r_{648} = .12, p < .005$).

Female-specific psychometric predictors of cannabis use by age 16 included greater extravagant personality subscale (Cloninger, 1999) in both the parent and daughter. As was the case with males, the heightened extravagant personality subscale of both the parent and daughter made separate contributions to the prediction (*post-hoc* partial-correlation between the outcome measure and child extravagant personality $r_{738} = .15, p < .001$, after first accounting for parent extravagant personality $r_{741} = .08, p < .05$). Additionally, greater parental impulsive personality subscale (Cloninger, 1999) was identified as a predictor.

In considering the parental influence of cannabis use in their offspring, parents with behaviorally disinhibited personality features, coupled with a history of cannabis use, were found to confer risk of use to their children. Recent work by Kerr and colleagues corroborate these findings as they report that adolescents have increased risk of cannabis use when raised by parents who use cannabis and have diminished monitoring of their children (Kerr et al., 2015).

In the male sample, three brain activation measures pertaining to the motor response inhibition (“stop signal”) task were found to reliably predict cannabis use by age 16. Relative to the comparison group, lower activation during successful response inhibitions in the left paravermis and the posterior-lateral part of the left hemisphere of the cerebellum, and, lower activation during failed response inhibition in the right inferior temporal gyrus, all predicted cannabis use by age 16 in males (see figure 2.3.3 & 2.3.5). Animal models using neuronal tracers suggests that the lateral cerebellum is involved in motor preparation and inhibition via projections to cortical motor and inhibitory regions through the thalamus (Middleton & Strick, 2001). These regions have also been previously identified as participating in a network underlying motor inhibitory control (Stevens et al., 2007). Thus, hypoactivity in these cerebellar regions underlying inhibitory control suggests that diminished recruitment of cerebellar motor resources might have behavioral consequences for adolescent males.

As these findings were evident prior to cannabis use initiation, a compromised motor inhibitory control system at age 14 might constitute a neurobiological vulnerability and promote the initiation of drug-consuming behaviors by age 16 in males. Moreover, the right inferior temporal region is considered part of the visual processing stream and has been previously associated with greater activity during stop failures (Hu & Li, 2012). Therefore, hypoactivity in this region correlating with failed response inhibitions might suggest cannabis use by age 16 in males is predicted by impaired processing of visual stop signal cues in their environment.

2.3.3 Male Brain Predictors

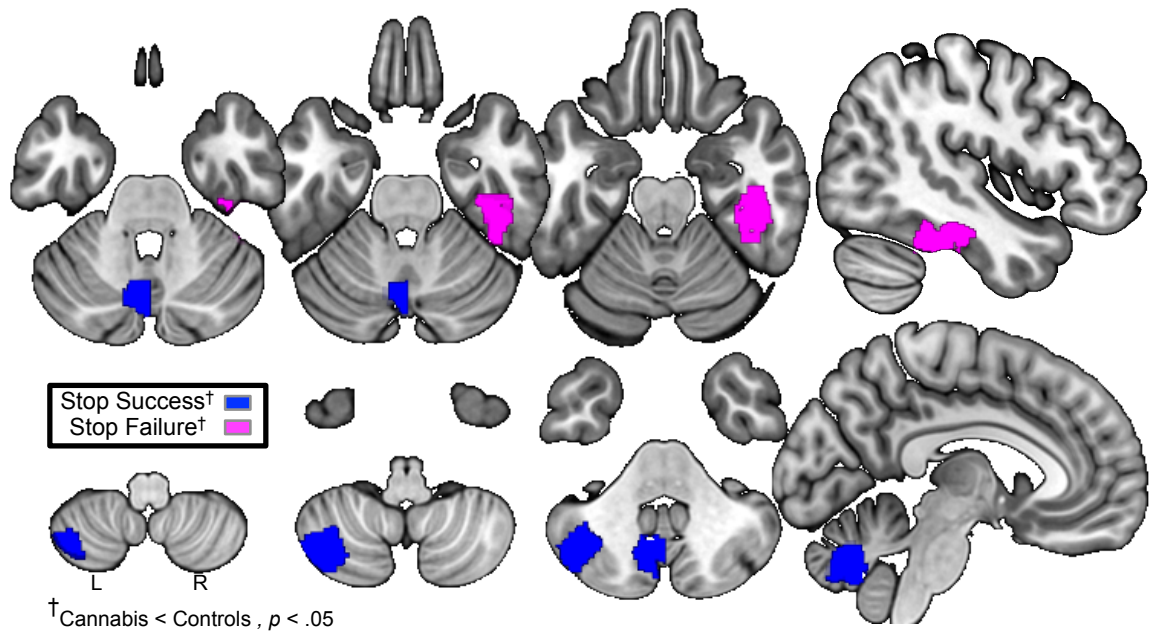


Figure 2.3.3: Lower activation in three regions predicts cannabis use by age 16 in males. *Stop Success* relates to successful inhibition trials minus implicit baseline during the stop signal task. Regions of interest (ROI) in left cerebellum include the left paravermis, and posterior-lateral part of the left hemisphere. *Stop failure* relates to failed inhibition trials minus implicit baseline during the stop-signal task. ROI in right inferior temporal gyrus.

†2-sample *t*-tests on participants endorsing cannabis use by age 16 vs. their non-using controls confirms significant differences in activations.

In the female sample, four brain measures were found to reliably predict cannabis use by age 16. Relative to the comparison group, higher activation during failed response inhibition in the right pre-supplementary motor area (pre-SMA) predicted female use. In this same region, larger gray matter volume relative to the comparison group also predicted cannabis use. As cortical myelination proliferates during adolescence, especially in motor areas requiring expedited signal propagation (Paus, 1999), these structural-functional findings suggest a functional consequence of delayed cortical

maturation. This structural finding is notable for the female sample as cortical maturation occurs earlier in adolescent females compared to their male peers (Giedd, 2004). As such, a larger gray matter volume might influence the observed higher activity of the motor system, which in turn, jeopardizes inhibitory control.

Dissimilar to males, the females exhibited activation differences when processing social stimuli. Specifically, when viewing neutral faces, cannabis users by age 16 had higher activation in the right anterior inferior temporal lobe and lower activation in the right lingual gyrus of the visual cortex (see figure 2.3.4 & 2.3.5). As these two regions have previously been established in the processing of face stimuli (Britton et al., 2006; Loughead et al., 2008), these findings highlight female sensitivity to social stimuli and implicate these processes in a predisposition towards cannabis use. In light of these findings, face processing studies suggest neutral faces can be misperceived as threatening, especially in individuals with social anxiety disorder (Cooney et al., 2006; Yoon & Zinbarg, 2008). Given the higher prevalence of social anxiety in females (Schneier, 1992) and the correlation between social anxiety and prevalence of cannabis use in females (Buckner et al., 2006, 2007) the results point to a female-specific pathway towards cannabis use.

2.3.4 Female Brain Predictors

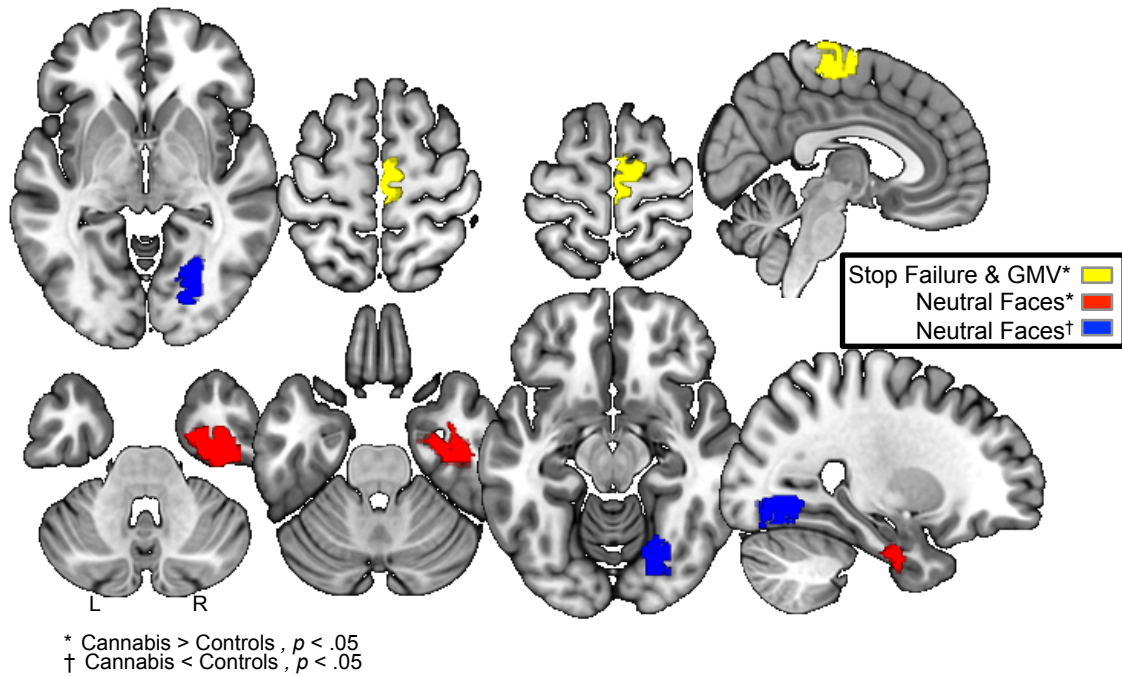


Figure 2.3.4: Activations and structural differences in three regions predicts cannabis use by age 16 in females. *Stop failure* relates to failed inhibition trials minus implicit baseline during the stop-signal task. Region of interest (ROI) in right pre-supplementary motor area. *GMV* measures gray-matter volume. ROI in right pre-supplementary motor area (identical ROI with stop failure). *Neutral Faces* relates to passive viewing of neutral faces minus control images during the face task. ROIs in right anterior inferior temporal gyrus and lingual gyrus (visual cortex).

*† 2-sample *t*-tests on participants endorsing cannabis use by age 16 vs. their non-using controls confirms significant differences in activations and GMV.

2.3.5 Correlations Between Identified Predictors and Outcome Measure

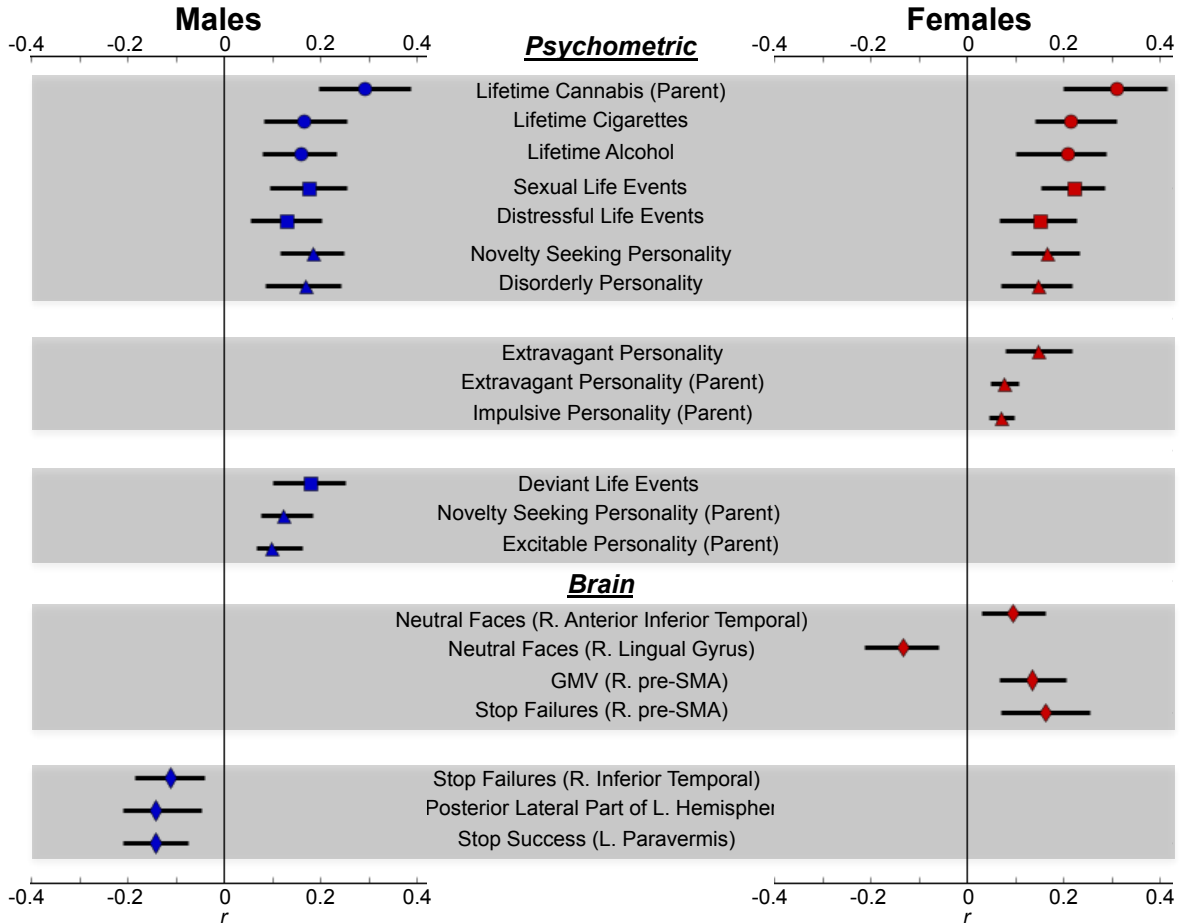


Figure 2.3.5: Pearson's point-biserial correlation (r) between predictor and outcome. Error bars represent 95% confidence intervals generated from 5000 bootstrap samples. Circles = Drug use (ESPAD, (Hibell et al., 1997)). Squares = Life Events (LEQ, (Newcomb et al., 1981)). Deviant life events in males relates to reported positive feelings towards deviant behaviors. Sex & distressful life events relates to the lifetime frequency. Triangles = personality (disorderly, extravagant, and impulsive personality are subscales of the TCI novelty-seeking personality scale, (Cloninger, 1999)). Diamonds = Neuroimaging data

2.4 Sex- and Drug-specificity of Brain Features

Given the observed sex differences and the known sexual dimorphisms in brain structure and function, we assessed the sex-specificity of the brain findings. To do so, we tested how well the brain measures identified to predict cannabis use for one sex

performed for the other sex. To assess drug-specificity, these predictors were then used to model binge drinking by age 16 (defined as a minimum of three lifetime binge drinking episodes by age 16) in a separate sample of 14 year olds (Whelan et al., 2014). Overlapping participants identified as cannabis users *and* binge drinkers by age 16 were excluded from the binge drinking sample (N=436; n=89 of whom transitioned to binge drinking by age 16). Sex- and drug-specificity model fit was assessed using a chi-square goodness of fit statistic and the delta-Akaike information criterion (Δ AIC) of model selection (Akaike, 1974). Model fit statistics were generated by comparing the relative change in model fit between a model estimated using only the base rate of cannabis use (or binge drinking) and a model estimated using only the features identified from the preceding prediction analyses.

As anticipated, *post-hoc* logistic regressions confirmed that the male-specific brain predictors of cannabis use returned strong model fits relative to the base rate model when estimated on the entire male sample ($\chi^2_{3,N=648} = 28.6, p < .001$; base rate model Δ AIC = 22.6 relative to the model with predictors). However, when estimated on the entire female sample, the model using the male-specific brain predictors is worse than the base rate model in predicting cannabis use ($\chi^2_{3,N=741} = 2.3, p > .05$; model with predictors Δ AIC = 3.7 relative to the base rate model). These regions were also found to be specific in their prediction of cannabis as they returned poor model fits in a logistic regression predicting binge drinking in males ($\chi^2_{3,N=206} = 2.0, p > .05$; model with predictors Δ AIC = 4.0 relative to the base rate model).

Post-hoc logistic regression confirmed that the female-specific brain predictors of cannabis use returned strong model fits for the female sample ($\chi^2_{4,N=741} = 48.5, p < .001$;

base rate model $\Delta AIC = 40.5$ relative to the model with predictors). However, when estimated on the entire male sample, the model using the female-specific brain predictors failed to predict cannabis use in males ($\chi^2_{4,N=648} = 2.2, p > .05$; model with predictors $\Delta AIC = 5.8$ relative to the base rate model). Likewise, these regions were found to be specific in their prediction of cannabis as they returned poor model fits when used to predict binge drinking in females ($\chi^2_{4,N=230} = 0.45, p > .05$; model with predictors $\Delta AIC = 7.5$ relative to the base rate model). See table 2.4.1 for *post-hoc* regression summaries.

2.4.1 *Post-hoc* Regression Model Summaries

Cannabis Predictive Features	Test Sample		Model Fit	
	Sex-Specificity	Drug-Specificity	χ^2, p	ΔAIC^*
<i>Male & Female Shared Psychometric Features</i>		Males & Females: Binge Drinking	74.6, $p < .01$	32.05 (base rate model –model with predictors)
<i>Male Brain Features</i>	Females: Cannabis Use		2.3, $p > .05$	3.7 (model with predictors –base rate model)
		Males: Binge Drinking	2.0, $p > .05$	4.0 (model with predictors –base rate model)
<i>Female Brain Features</i>	Males: Cannabis Use		2.2, $p > .05$	5.8 (model with predictors –base rate model)
		Females: Binge Drinking	0.45, $p > .05$	7.5 (model with predictors –base rate model)
<i>Male & Female Shared Genetic Features</i>	Males & Females: Cannabis Use		14.8, $p < .01$	8.8 (nuisance model –model with predictors)
		Males & Females: Binge Drinking	7.8, $p = .05$	1.8 (nuisance model –model with predictors)

Table 2.4.1: Features identified from each cannabis predictive modeling scenario were used to probe sex- and drug-specific effects. Male & Female shared psychometric and genetic predictors of cannabis use by age 16 also predicted binge drinking by age 16. Male brain predictors and female brain predictors of cannabis use by age 16 failed to predict cannabis use in the opposite sex, or, binge drinking by age 16 in the same sex. * ΔAIC always in reference to the better fitting model. $\Delta AIC = AIC_{model_i} - AIC_{min}$ and reflects the relative increase in information gained from the AIC_{min} (better) model. Values ≥ 2 favor the AIC_{min} model.

2.5 Genetic Predictors

The sex-specific analyses did not identify any predictive SNPs, therefore we collapsed across sex and reran the analysis with only the genetic predictors (plus nuisance covariates). This analysis returned a mean ROC AUC=0.60 ($p < 3.0 \times 10^{-4}$; see figure 2.5.1), however, the genetic multidimensional scaling factors plus demographic covariates inflated model performance and the predictive genes were less consistently selected across the generated models. With that in consideration, a lower threshold (see supplemental information) identified two SNPs on genes coding for the μ_1 -opioid receptor and one on a gene coding for the δ_1 -opioid receptor. The minor alleles of the two μ_1 -opioid receptor SNPs (rs511420, rs1074287) were found to impart genetic risk as a greater number of the age 16 cannabis users had the minor allele, whereas the minor allele of the δ_1 -opioid receptor SNP (rs2236857) was found to impart a protective effect as its frequency was higher in the comparison group. See table 2.5.2 for the three SNP statistics including their correlation with the outcome measure.

2.5.1 ROC Curve for Gene-specific Analysis

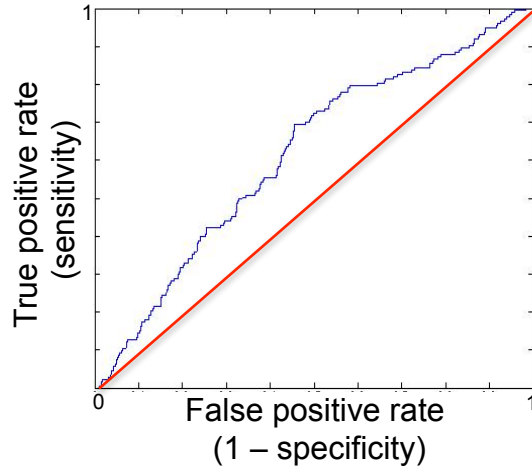


Figure 2.5.1: Visualizing the performance of the predictive model on independent observations. Representative of the mean AUC across 100 runs.

2.5.2 Statistics and Frequencies for Cannabis Predictive SNPs

Locus	Gene	HW P value	MAF	Major: Minor Alleles	Imputation Quality (R ²)	Association with age 16 Cannabis Use		Genotype (% H _{minor} : HT : H _{major})		Minor Allele Effect
						<i>r</i>	<i>p</i>	Cannabis Use by age 16	Comparison Group	
rs511420	OPRM1	.87	0.097	T:C	0.999	-.052	.051	2:19:79	1:15:84	Risk
rs1074287	OPRM1	.90	0.256	A:G	0.991	-.058	.030	10:35:55	7:30:63	Risk
rs2236857	OPRD1	.89	0.266	T:C	1 (Genotyped)	.053	.048	4:30:66	5:37:57	Protection

Table 2.5.2: Measures of Hardy-Weinberg Equilibrium (HW), Minor Allele Frequency (MAF). Association with cannabis use by age 16 calculated using Spearman's rank correlation between SNP and the outcome measure. H_{minor}: Homozygote minor (high-risk genotype), HT: heterozygote (intermediate-risk genotype), H_{major}: Homozygote major (low-risk genotype).

When including these three SNPs in a *post-hoc* hierarchical logistic regression predicting cannabis use, the model exhibits strong fit to the full sample after first modeling the nuisance covariates ($\Delta\chi^2_{3,N=1389} = 14.8, p < .005$; nuisance covariates model $\Delta\text{AIC} = 8.8$ relative to the model with nuisance covariates and SNPs). Likewise, including these three SNPs returned marginally adequate fits to the full sample of binge drinkers after first modeling the nuisance covariates ($\Delta\chi^2_{3,N=436} = 7.8, p = .05$; nuisance covariates model $\Delta\text{AIC} = 1.8$ relative to the model with nuisance covariates and SNPs). Thus, these SNPs might weakly generalize to predict binge drinking by age 16, however, the model fits effect sizes are far greater in their prediction of the cannabis using sample.

The cannabinoid and opioid neurotransmitter systems have been found to co-localize in the striatum (Rodriguez, Mackie, & Pickel, 2001) and exhibit reciprocal signaling (Robledo et al., 2008). However, the biobehavioral effects orchestrated by these systems remain unclear in humans. Animal models suggests the μ -opioid (but not δ) receptor is specifically involved in reinforcement properties as μ -opioid receptor knockout mice failed to exhibit THC-induced conditioned place preference compared to δ -knockout and wild-type mice (Ghozland et al., 2002). Hence, our findings that cannabis users by age 16 had a greater number of risk alleles for the μ -, but not δ -receptor SNPs might signal alterations to their neurobiological processing of rewards. Additionally, SNP rs511420 has previously been identified to interact with stressful life events to promote depressive symptoms in African-American adolescents (Swann et al., 2014). Therefore, adolescents who experience life stress and possess the high-risk allele are at an elevated likelihood to experience depressive symptomology and potentially self-medicate with cannabis for the phasic anxiolytic and euphoric effects of the drug.

2.6 Domain-Specific Effects

To investigate the contribution of each domain in the prediction of adolescent cannabis use, *post-hoc* hierarchical regressions were conducted to measure the relative change in model fit after the inclusion of each domain-specific set of predictors. In the male sample, the psychometric predictors were entered first and significantly improved model fit relative to the base rate model ($\chi^2_{10,N=648} = 105.31, p < .001$; base rate model $\Delta\text{AIC} = 85.31$). The brain predictors were then included and significantly improved model fit relative to the psychometric predictors model ($\Delta\chi^2_{3,N=648} = 19.7, p < .01$; psychometric model $\Delta\text{AIC} = 13.71$). Finally, the three SNPs were added but did not improve model fit ($\Delta\chi^2_{3,N=648} = 4.73, p > .05$; model with SNPs $\Delta\text{AIC} = 1.27$ relative to psychometrics and brain model).

Likewise, for the female sample, the psychometric predictors were entered first and significantly improved model fit relative to the base rate model ($\chi^2_{10,N=741} = 97.33, p < .001$; base rate $\Delta\text{AIC} = 77.3$). Next, the brain predictors were included and significantly improved model fit relative to the psychometric predictors model ($\Delta\chi^2_{4,N=741} = 43.24, p < .01$; psychometric model $\Delta\text{AIC} = 35.24$). Lastly, the three SNPs were included but did not significantly improve model fit ($\Delta\chi^2_{3,N=741} = 6.65, p > .05$; psychometrics and brain model $\Delta\text{AIC} = 0.65$). For both sexes, the results of the domain-specific hierarchical regressions held irrespective of the order in which each domain were entered. Thus, while psychometric data alone can be used to significantly predict cannabis use by age 16, models containing both psychometric and sex-specific brain features return superior fits, highlighting their utility of capturing individual differences in the neurobiology predicting adolescent cannabis use.

2.7 Conclusions

These analyses have identified individual differences at age 14 that predict cannabis use two years later. Despite having information on thousands of multi-modal variables per individual, prediction with high generalizability was achieved with fewer than fifteen psychometric and brain measures. Some of these individual differences were specific to predicting cannabis use in each sex while others predicted both cannabis and binge drinking across the sexes. The generality of the psychometric variables gives some insights into what might be general risk factors associated with teenage substance use initiation. Moreover, results revealing that the brain predictors differ between the sexes underscore the importance of attending to sex-differences in addiction research. Indeed, the distinction in functional tasks observed for each sex highlights the sex-specific psychological processes (inhibitory control in males; social processing in females) potentially driving the initiation of cannabis use in adolescence.

Given the increased availability of cannabis that is likely to accompany ongoing public policy efforts towards recreational legalization, a potential concern is an increase in the rates of teenage consumption. Understanding the individual differences that predict use offers insights into the etiological mechanisms underlying that use, and provides knowledge that could guide interventions aimed at reducing any potential negative consequences on the brain and behavioral health of adolescents.

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Appendix 1: Supplemental Information

A1.1 Schematic of Analytic Method

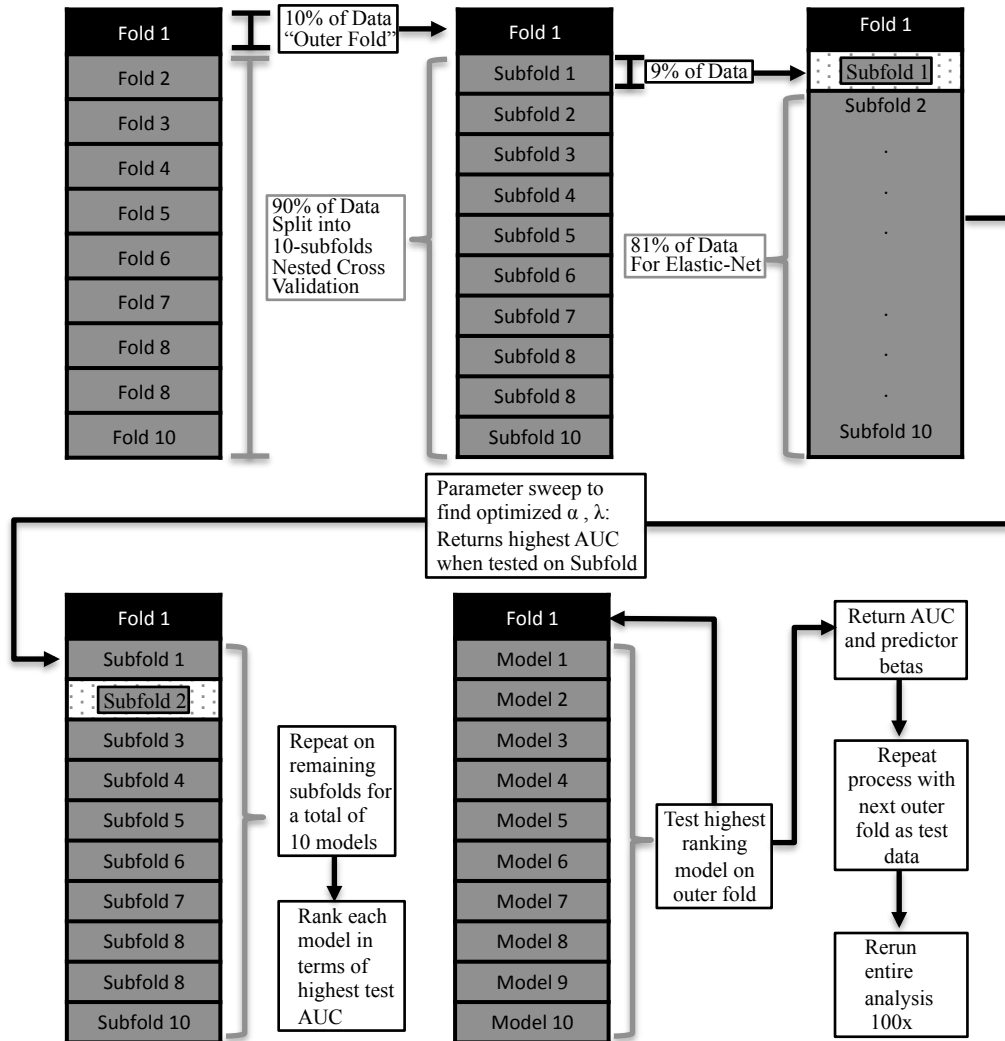


Figure A1.1: Schematic of Analytic Method. First, data are divided into $k(10)$ outer-folds. $k-1$ outer-folds are then divided into $k(10)$ nested-folds. Elastic-net regularized logistic regression applied to $k-1$ nested-folds, during which the α , λ parameters are tuned by finding the optimal pair returning the highest AUC when it's model is tested on the $k-1$ nested-fold. The iterative process is completed for the $k(10)$ nested-folds, generating 10 final nested-fold. The 10 nested models are ranked by their AUC returned when tested on each respective $k-1$ nested test fold. The highest-ranking model is then tested on the outer fold, and used to generate the reported test AUC. This entire process is repeated k -times.

A1.2 Analysis of Head Motion

Sex	Task	Mean Framewise Displacement: Age 16 Users vs. Comparison Group
Males	Faces	$t_{624} = 0.55, p > .05$
	MID	$t_{594} = 0.78, p > .05$
	Stop Signal	$t_{580} = -1.5, p > .05$
Females	Faces	$t_{712} = -1.48, p > .05$
	MID	$t_{685} = 0.43, p > .05$
	Stop Signal	$t_{678} = -0.18, p > .05$

Table A1.2: Framewise displacement was calculated from the six-directional head motion parameters estimated during image realignment. 2-sample *t*-tests on the participants endorsing cannabis at age 16 vs. their non-using peers failed to detect significant differences in head motion (mean FD) for any of the tasks for either sex.

A1.3 Binge Drinking Sample Demographics

Measure	Groups		<i>p</i>
	Binge Drinkers by age 16 (n=89)	Comparison Group (n=347)	
Age (<i>M,SD</i>)	14.54, 0.45	14.47, 0.39	.133
Sex (Males, Females)	48, 41	158, 189	.190
Handedness (L,R)	10, 79	40, 307	.999
PDS (<i>M,SD</i>)	3.55, 0.69	3.50, 0.80	.573
Perceptual IQ (<i>M,SD</i>)	106.30, 13.30	107.65, 14.26	.419
Verbal IQ (<i>M,SD</i>)	110.87, 13.98	109.41, 13.58	.396
SES (<i>M,SD</i>)	17.62, 3.92	18.00, 3.68	.399

Table A1.3: A sample of binge-drinkers by age 16 and never-binge-drinkers comparison group. Group differences assessed using two-group *t*-tests on the continuous measures, and chi-square tests on the categorical measures. PDS: Puberty Development Scale (Petersen et al., 1988). SES: Socioeconomic Status.

A1.4 Cannabis Use Levels

Lifetime Cannabis Use	Males (n)	Females (n)
6-9 joints	26	19
10-19 joints	24	20
20-39 joints	23	9
40+ joints	37	15
<i>Total Users</i>	<i>110</i>	<i>63</i>
<i>Comparison Group</i>	<i>538</i>	<i>678</i>
Sum	678	741

Table A1.4: Levels of cannabis use at age 16 by sex. Comparison group contained individuals who report using no cannabis at age 14 or 16.

A1.2 Materials and Methods

Supplemental Materials

Materials and Methods

Overview of IMAGEN protocols

Full details of the procedures employed by the IMAGEN study (Schumann et al., 2010), including details on ethics, recruitment, standardized instructions for administration of the psychometric and cognitive behavioral measures, and for blood collection and storage are available to view in the Standard Operating Procedures for the IMAGEN project (http://www.imagen-europe.com/en/Publications_and_SOP.php).

Participants

Data were acquired from 14-year-old adolescents recruited according to the standard IMAGEN operating procedures cited above. After complete description of the study to the participants and their parents/guardians, written informed consent was obtained. Individuals who provided assent completed an extensive battery of neuropsychological, clinical, personality and drug use assessments online and at the testing centers. Additional assessments were conducted at age 16. Participants were excluded if they had contraindications for MRI (metal or electronic implants and claustrophobia) or problematic medical history (diabetes, tumors, heart defects, etc.), neurological conditions (epilepsy, head trauma, neurodevelopmental disorders, etc.) or low IQ.

Participants were included in the analysis based on the self-report drug use questionnaire (ESPAD, described below). All participants in the analysis were cannabis naïve at age 14 (N=1389, of whom n=173 transitioned to use by age 16; see table 2.1.1 for demographics). Age 16 cannabis use was defined as using the drug at least six times by age 16. This threshold was used in order to identify youths who have initiated cannabis use beyond experimentation levels.

Data

All data (see table 2.1.2) in the analysis were collected at age 14 and used to predict the onset of cannabis use by age 16. Questionnaires surveying child *and* parent personality, stressful life events, drug use, and cognitive performance comprised the psychometric data.

Genetic data were comprised of 108 *a-priori* SNPs of interest coding for neurotransmitter receptors (cannabinoid, opioid and catecholamines), related enzymes (FAAH), eight SNPs previously associated with cannabis dependence (Hartman et al., 2009; Hopfer et al., 2006; Hurd et al., 2014), and one genetic risk-score (Cornelis, 2009) computed from those 8 SNPs.

Brain data comprised of functional and structural neuroimaging measures parcellated into 278 regions of interest (ROIs) (Shen et al., 2013). Measures include total and regional gray matter volumes and brain activation associated with response inhibition (assaying cognitive control systems), reward anticipation and receipt (assaying brain reward systems) and viewing neutral and angry faces (assaying social processing brain systems). At each gray matter volume ROI, the ratio of regional to total gray matter volume was calculated for each subject in order to account for anatomical variability across subjects.

Structural MRI

High-resolution anatomical magnetic resonance images were acquired, including a 3D T1-weighted magnetization prepared gradient echo sequence based on the ADNI protocol (<http://www.loni.ucla.edu/ADNI/Cores/index.shtml>). Structural MRI processing included data segmentation and normalization to the Montreal Neurological Institute template using the SPM optimized normalization routine. Gray matter images were modulated, thus facilitating comparisons of volumetric, rather than tissue concentration, differences (Ashburner & Friston, 2000).

Functional MRI

Full details of the magnetic resonance imaging (MRI) acquisition protocols and quality checks have been described previously, including an extensive period of standardization across MRI scanners (Schumann et al., 2010). MRI Acquisition Scanning was performed at the eight IMAGEN assessment sites (London, Nottingham, Dublin, Mannheim, Dresden, Berlin, Hamburg, and Paris) with 3T whole body MRI systems made by several manufacturers (Siemens: 4 sites, Philips: 2 sites, General Electric: 1 site, and Bruker: 1 site). To ensure a comparison of MRI data acquired on these different scanners, we implemented image acquisition techniques using a set of parameters compatible with all scanners that were held constant across sites, for example, those directly affecting image contrast or fMRI preprocessing. Site was dummy-coded for use in the machine learning procedure.

Standardized hardware for visual and auditory stimulus presentation (NordicNeurolabs, Bergen Norway, <http://www.nordicneurolab.com>) was used at all sites. BOLD functional images were acquired with a gradient-echo echo planar imaging (EPI) sequence using a

relatively short echo-time to optimize imaging of subcortical areas (details of sequence parameters for structural and functional imaging, and the neuroimaging tasks are given in Ref. 1). Briefly, the functional imaging processing was as follows: Time series data were first corrected for slice-timing, then corrected for movement, non-linearly warped onto MNI space using a custom EPI template, and Gaussian-smoothed at 5mm-full width half maximum. Nuisance variables were also added to the design matrix: estimated movement was added in the form of 12 additional regressors (3 translations, 3 rotations, 3 translations shifted 1 TR before and 3 translations shifted 1 TR later). Each individual fMRI time series underwent automatic spike detection, using a mean-squared based metric to identify unexpected values temporally and spatially slice per slice. Time-points with artifacts (if any) of each sequence were regressed out of each participant's data by adding a corresponding number of regressors with value 1 at the time- point of the artifact and 0 elsewhere to the design matrix.

Functional Tasks Descriptions

Stop Signal Task (SST). The SST required volunteers to respond to regularly presented visual go stimuli (arrows pointing left or right) but to withhold their motor response when the go stimulus was followed unpredictably by a stop-signal (an arrow pointing upwards). Stopping difficulty was manipulated across trials by varying the delay between the onset of the go arrow and the stop arrow (stop-signal delay, SSD) using a previously described tracking algorithm (Rubia et al., 2005). A block contained 400 go trials and 80 variable delay stop trials with between 3 and 7 go trials between two stop trials. Stimulus duration in go trials was 1000 ms and in stop trials varied (0– 900ms in 50 ms steps) in accordance

with the tracking algorithm (initial delay = 250 ms). We calculated contrast images for successful inhibitions (“stop success”) and unsuccessful inhibitions (“stop fail”), both vs. an implicit baseline.

Monetary Incentive Delay. The Monetary Incentive Delay (MID) task (adapted from a task described previously (Knutson et al., 2001)) required participants to respond to a briefly presented target by pressing either a left-hand or right-hand button as quickly as possible to indicate whether the target appeared on the left or the right side of the monitor display. If the participants responded while the target was on the screen, they scored points but if they responded before the target appeared or after the offset of the target they received no points. A cue preceded the onset of each trial, reliably indicating the position of the target and the number of points awarded for a successful response. A triangle indicated no points (No Win), a circle with one line 2 points (Small Win) and a circle with three lines 10 points (Large Win). Twenty-two trials of each type were presented in a pseudo-random order. The duration of the target was adjusted adaptively so that 66% of the trials produced a correct response. The participants were informed that at the end of the session they would receive one candy (M&M) for every five points won. We calculated contrast images for the anticipation period of Large Win minus No Win, and the outcome period for Large Win minus No Win.

Face Task. The Face task involved passive viewing of video clips that displayed ambiguous (emotionally “neutral”) or angry face expressions or control (nonbiological motion) stimuli (Grosbras, 2005). Each trial consisted of short (2 to 5 s) black-and-white video clips depicting either a face in movement or the control stimulus. The control

stimuli consisted of black-and-white concentric circles of various contrasts, expanding and contracting at various speeds, roughly matching the contrast and motion characteristics of the face clips. The stimuli were presented through goggles (Nordic Neurolabs, Bergen, Norway) in the scanner and subtended a visual angle of 10° by 7° . The video clips were arranged into 18-s blocks; each block included seven to eight video clips. Five blocks of each biological-motion condition (neutral and angry faces), and nine blocks of the control condition (circles) were intermixed and presented to the participant in a 6-minute run. We calculated contrast images from angry faces minus control stimuli, neutral faces minus control stimuli, and angry faces minus neutral faces. After the scanning session, participants completed a recognition task in which they were presented with three of the faces previously presented in the scanning session and two novel faces.

Personality

NEO. Broad dimensions of personality were assessed using the 60-item Neuroticism-Extraversion-Openness Five-Factor Inventory (NEO-FFI), which returns measures on the dimensions of Extraversion, Agreeableness, Conscientiousness, Neuroticism, and Openness to Experience as described in the Five-Factor Model of personality (Costa Jr. & McCrae, 1995). The Extraversion factor assesses preference for seeking and engaging in social interactions and may be linked to sensitivity to rewarding environmental cues (Watson & Clark, 1992). The Agreeableness factor assesses empathy and an individual's tendency towards compassion and co-operation rather than self-interest. Conscientiousness provides a measure of the degree to which a participant exercises self-discipline and expresses a preference for planned, rather than spontaneous, behavior. The

Neuroticism factor captures emotional liability and a tendency to experience lowered mood and elevated anxiety. Openness to Experience measures intellectual curiosity and creativity; lower scores on ‘openness’ are associated with a reduced tolerance for change and a preference for familiarity over novelty.

Substance Use Risk Profile Scale. The Substance Use Risk Profile Scale (SURPS; (Woicik et al., 2009)) assesses personality traits that confer risk for substance misuse and psychopathology. This scale measures four distinct and independent personality dimensions; anxiety sensitivity, hopelessness, sensation seeking, and impulsivity. The anxiety sensitivity dimension is characterized by the fear of symptoms of physical arousal. The hopelessness dimension is identified as a risk factor for the development of depression and characterized by dismal feelings. The sensation seeking dimension is characterized by the desire for intense and novel experiences. The impulsivity dimension involves difficulties in the regulation (controlling) of behavioral responses.

Temperament and Character Inventory. The novelty seeking scale of the Temperament and Character Inventory – Revised (TCI-R; (Cloninger, 1999)) was administered. The novelty seeking scale is composed of four subscales (excitability, impulsiveness, extravagance, and disorderliness). The excitability subscale contrasts with ‘stoic rigidity’ and reflects sensation-seeking and novelty-seeking behaviors. The impulsiveness subscale describes behavior on a dimension from impulsivity to reflection and captures elements of emotional reactivity, and unreflective, careless behavior. The extravagance subscale assesses overspending behavior and poor planning and is believed to reflect a tendency to approach reward cues. The disorderliness subscale reflects

disorganized, uncontrolled, and anti-normative behavior. Novelty seeking personality is assessed as the sum across all four subscales.

Cognition

Wechsler Intelligence Scale for Children. Participants completed a version of the Wechsler Intelligence Scale for Children WISC-IV(Wechsler, 2003), of which we included the following subscales. Perceptual Reasoning, consisting of Block Design (arranging bi-colored blocks to duplicate a printed image) and Matrix Reasoning (in which a series of colored matrices are presented and the child is asked to select the consistent pattern from a range of options). Verbal Comprehension consisting of Similarities (two similar but different objects or concepts are presented and the child is asked to explain how they are alike or different) and Vocabulary (a picture is presented or a word is spoken aloud by the experimenter and the child is asked to provide the name of the depicted object or to define the word).

Delay discounting. The Monetary-Choice Questionnaire (MCQ; (Kirby, Petry, & Bickel, 1999)) was administered to provide a measure of preference for immediate lower over delayed higher monetary rewards. The MCQ is a 27-item task in which the participant chooses between a smaller, immediate monetary reward and a larger, delayed monetary reward (e.g. €25 today or €60 in 14 days), with varying discrepancies and delays between the rewards. The task indexes impulsivity by providing a measure of the degree to which future rewards are diminished or discounted. The protocol is scored by calculating where the participant's answers place them in comparison to reference discounting curves,

where placement amid steeper curves indicates higher levels of impulsivity.

CANTAB. Participants completed five of the CANTAB tests.

The Affective Go/No-go task comprised of alternating blocks in which participants were presented with positively or negatively valenced target words embedded in a stream of neutral distracter words. Participants were instructed to respond to targets with a button press. Measures included in the analyses were the total number of omissions to positive and negative targets, and the average response latency to positive and negative target words.

In the Pattern Recognition Memory task participants were required to remember 12 abstract patterns; the percentage of patterns correctly recognized on a two alternative forced choice task completed immediately after encoding was included in the analyses.

The Spatial Working Memory Task required participants to “search” for a token hidden by one of a number of boxes on the monitor by selecting the boxes in sequence. Once the token is uncovered, participants must search again with the condition that the token will not be hidden in the same location more than once. The number of times participants returned to search a box that had already contained the token was entered into the analyses as an error measure. We also included a strategy score (ranging from 1-37, with lower scores indicating a more strategic approach), which reflects how often a search sequence was initiated from a novel position.

The Rapid Visual Information Processing task comprised of a stream of digits presented at 1.67Hz and participants were required to monitor the stream for target sequence of three digits. We included a signal detection measure of sensitivity to the target sequence

in the analyses.

The Cambridge Guessing Task (CGT) was a modified version of the Cambridge Gambling Task, renamed in order to make it appropriate to administer to adolescents. On each trial of the CGT the participant was presented with 10 boxes, some of which are blue, some of which are red, and must “guess” which color box conceals a hidden yellow token. Participants start the task with 100 points and lose or acquire points by wagering on their guess. The options the participant can choose to wager are determined by the program as a proportion of their total number of points, presented in either increasing or decreasing amounts. The analyses included measures of the time taken to select the option on which to bet, an average of the proportion of the total number of points wagered on each trial, the proportion of trials on which the more likely outcome was selected (quality of decision making), an average of the proportion wagered on trials when the participant selected the more likely result (rational bets), and an index of delay aversion reflected in making higher bets when the amount to bet is presented in descending order rather than in ascending order.

Behavioral data from functional imaging tasks. Behavioral data from the Monetary Incentive Delay (reward) task were as follows: the number of Big Win trials on which the target was not hit, the number of Big Win trials on which the target was hit, the number of Small Win trials on which the target was not hit, the number of Small Win trials on which the target was hit, the number of No Win trials on which the target was not hit, and the number of No Win trials on which the target was hit. Behavioral data from the Faces (emotional reactivity) task included the number of targets and the number of foils correctly categorized. Participants were not informed prior to the scanning session about

the subsequent recall task. Behavioral data from the stop signal task was incomplete due to technical errors, therefore this data was omitted from the modeling procedures, however, the stop signal task had an adaptive performance algorithm to account for individual differences in reaction time.

History

Life-Events Questionnaire. The Life-Events Questionnaire (LEQ) is an adaptation of the Stressful Life-Event Questionnaire (Newcomb et al., 1981), which uses 39 items to measure the lifetime occurrence (frequency) and the perceived desirability of stressful events covering the following domains: Family/Parents, Accident/Illness, Sexuality, Autonomy, Deviance, Relocation, and Distress. The life-events valence labels measured on an ordinal scale from -2 to +2 as follows: -2='Very Unhappy', -1='Unhappy', 0='Neutral', +1='Happy', +2='Very Happy'.

Gestational cigarette and alcohol exposure. The Pregnancy and Birth Questionnaire (PBQ, adapted from (Pausova et al., 2007)) assesses exposure of the child to potentially harmful conditions and substances such as maternal alcohol, cigarette, and cannabis use before and during pregnancy. The questionnaire was completed by each participant's parent or guardian and parental cigarette and alcohol use during pregnancy were recorded, then recoded as binary variables.

Alcohol Misuse. Michigan Alcohol Screening Test questions (MAST; (Selzer, 1971)), such as 'have you ever been in a hospital because of drinking', was used to assess alcohol misuse. Parental alcohol misuse was assessed using the Parent-Alcohol Use Disorders

Identification Test (AUDIT; (Saunders et al., 1993)).

Puberty Development Scale. The Puberty Development Scale (PDS; (Petersen et al., 1988)) was used to assess the pubertal status of each participant. This scale provides an eight-item self-report measure of physical development based on the Tanner stages with separate forms for males and females. For this scale, there are five categories of pubertal status: 1=prepubertal, 2=beginning pubertal, 3=midpubertal, 4=advanced pubertal, 5=postpubertal. Participants answered questions about their growth in stature and pubic hair, as well as menarche in females and voice changes in males.

Socioeconomic Status. The socioeconomic status score was comprised of the sum of the following variables: Mother's Education Score, Father's Education Score, Family Stress Unemployment Score, Financial Difficulties Score, Home Inadequacy Score, Neighborhood Score, Financial Crisis Score, Mother Employed Score, Father Employed Score.

Substance misuse measures. The European School Survey Project on Alcohol and Drugs (ESPAD; (Hibell et al., 1997)) was administered using the software program Psytools (London, UK) which is a computerized assessment platform. Psytools presented questionnaire items and response alternatives on a computer screen. Jump rules were implemented where applicable to skip irrelevant questions (e.g., drinking-related questions in self-reported non-drinkers) for the sake of brevity. As the Psytools program was run at the participant's home without direct supervision by the research team, the reliability of the data were checked in a two-stage procedure. Before every task,

adolescents were asked to report on the current testing context including questions about their attentional focus and the confidentiality of the setting. Automated flags highlighted potentially problematic testing situations and were followed-up by research assistants face-to-face with the volunteer in a confidential setting. Final reliability ratings were assigned which led to exclusion of the data. Exclusion criteria for substance use measures included an indication that the participant was in a hurry, somebody was watching, or an indication to have known or taken the sham drug Relevin. Inconsistency between baseline (age 14 years) and follow up (age 16 years) for all drugs was also an exclusion criterion (e.g., scoring 1 for cannabis at age 14 years, but 0 at age 16 years). Drug-usage levels were collected on an ordinal scale, 0=0, 1=1-2x, 2=3-5x, 3=6-9x, 4=10-19x, 5=20-39x, 6=40x+.

Genotyping

DNA purification and genotyping was performed by the Centre National de Génotypage in Paris. DNA was extracted from whole blood samples preserved in ethylene-diamine-tetra-acetic acid vacutainer tubes (BD, Becton, Dickinson and Company, Oxford, United Kingdom) using Gentra Puregene Blood Kit (QIAGEN, Valencia, California) according to the manufacturer's instructions. Genotype information was collected at 582,892 markers using the Illumina HumanHap610 and HumanHap660 Genotyping BeadChips (San Diego, California). The SNPs with call rates of <95%, minor allele frequency < 1%, deviation from the Hardy-Weinberg equilibrium ("HWE", $p \leq 1 \times 10^{-6}$), and non-autosomal SNPs were excluded.

Markers data imputation and quality control for ambiguous SNPs, low MAF, missingness

and HWE were done with MACH (Li et al., 2010), following the ENIGMA2 guidelines. The 1000 Genomes project reference set of markers (<http://www.1000genomes.org/data>) was used for the imputation after decreasing the markers from ~41 million to ~13 million relevant genetic variants observed more than once in the European populations

Genetic Analysis Four multidimensional scaling (MDS) components were calculated using a metric model in PLINK v1.9 (<http://pngu.mgh.harvard.edu/~purcell/plink/>). MDS was then included as a covariate to account for population stratification as part of the cross-validation logistic regression, where the genotypes were coded following an additive model (as 0, 1, and 2 for the number of risk alleles).

Imputation of Missing Data

Missing data for all three domains were replaced (where possible) by imputation. Continuous variables were replaced with the 95% trimmed mean derived according to the participant's site and sex taken from the whole IMAGEN database (N=2,462). Ordinal data were similarly replaced with the mode of that variable for the participant's site and sex.

Analytic Methods

Use of a cross-validation scheme allows for the tuning of the elastic-net regularization parameters for optimal feature selection and provides an assessment of model overfitting. The area under the curve (AUC) of the receiver-operating characteristic (ROC) was calculated for each run based on the model's ability to predict age 16 cannabis use in the set-aside test-folds, thereby quantifying the test error of the final predictive model.

Elastic Net regularization and feature selection. Regression with Elastic Net regularization is an example of a sparse regression method, which imposes a hybrid of both L1- and L2-norm penalties (i.e., penalties on the absolute (L1-norm) and squared values of the β weights (L2-norm)). This allows relevant but correlated coefficients to coexist in a sparse model fit, by doing automatic variable selection and continuous shrinkage simultaneously, and selects or rejects groups of correlated variables. Least absolute shrinkage and selection operator (LASSO; (Tibshirani, 1996)) and ridge regression are special separate cases of regularized regression. All independent variables were standardized prior to model estimation. These analyses were implemented using the “glmnet” function in MATLAB (Natick, MA).

The analysis was executed 100 times to incorporate the subtle differences in results incurred due to the random assignment of participants to folds. As a 10-fold cross validation scheme was implemented 100 times per analysis, results were thresholded to identify only the predictors present in a minimum 6 out of 10 final-models (from $k=10$), across a minimum of 80 runs.

To investigate sex differences, analyses were conducted on a male-only sample (N=648; age 16 cannabis-users n=110) and a female-only sample (N=741; age 16 cannabis-users n=63). For each sex, the analysis returned a reduced design matrix of significant predictors [$p \times 10$ (β s-per-fold)] x 100 runs. Null-hypothesis significance testing on the AUC was conducted using a Mann-Whitney U-test (Mason & Graham, 2002).

Gene-only Analysis

Given our interest in uncovering potential genetic predictors of adolescent cannabis use, a gene-specific analysis was conducted by collapsing across sex and included only genetic data and covariates (age, sex, handedness, puberty status, and four MDS scores) as independent variables. A less conservative threshold was applied to identify SNP predictors. As such, SNPs selected in four or more final models across a minimum of 50 runs was used. Despite this lenient threshold, post-hoc hierarchical regression analyses confirmed that including these 3 SNPs returned strong model fit statistics relative to a model estimated using only the eight nuisance covariates (see main text).

Head Motion

As head motion has been shown to confound structural and functional MRI findings (Pardoe, Kucharsky Hiess, & Kuzniecky, 2016), *a-priori* 2-sample *t*-tests confirmed that head motion (mean framewise displacement, “FD”) within each task did not differ between age 16 users and controls for either sex. The framewise displacement (FD) for each participant for each fMRI task was calculated using the six displacement parameters estimated during image realignment preprocessing procedures (see table A1.2 for statistics).

Permutation Analyses

To check for spurious findings and to confirm the independence of the 10-fold model training and testing procedure, random permutation analyses were conducted. Each sex-specific k-fold analysis was repeated 100 times while randomly assigning group membership to each participant while keeping original group sample sizes consistent.

Essentially, these analyses tested for significant findings on a sample-generated null model. These permutation models performed no better than chance (Males: mean AUC = 0.53, $\sigma = .02$, $p > .05$; Females: mean AUC = 0.51, $\sigma = .03$, $p > .05$). In addition to these models failing to predict randomized outcomes, the predictors selected for each final model did not mirror the predictors selected from the true analyses.

Post-hoc Multiple Regression Modeling

The predictors of cannabis use identified from the preceding sex-specific k-fold analyses were selected for use as the independent variables in a multiple logistic regression model assessing sex- and drug-specificity. Sex-specificity was assessed by including the predictors of male cannabis use in a multiple logistic regression model estimated on the entire female sample (and *vice versa*). Drug-specificity was assessed in a similar fashion while using a related but independent sample of binge drinking adolescents. In all *post-hoc* scenarios, independent variables were standardized to z-scores by sex before model estimation. Thereafter, coefficients and model fit statistics were freely estimated from their respective samples. The “model with predictors” consisted of a multiple-regression model containing the predictors identified from the respective preceding sex-specific k-fold analyses. Each model with predictors was compared to its sample-specific base rate model, which corresponds to a threshold at which the exact baseline rate would be classified as a cannabis user or binge drinker by age 16.