2019

Biobehavioral Predictors Of Cannabis Use In Adolescence

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BIOBEHAVIORAL PREDICTORS OF CANNABIS USE IN ADOLESCENCE

A Dissertation Presented

by

Philip Aaron Spechler

to

The Faculty of the Graduate College

of

The University of Vermont

In Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
Specializing in Psychology

August, 2019

Defense Date: April 18, 2019
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ABSTRACT

Cannabis use initiated during adolescence may precipitate lasting consequences on the brain and behavioral health of the individual. However, research on the risk factors for cannabis use during adolescence has been largely cross-sectional in design. Despite the few prospective studies, even less is known about the neurobiological predictors. This dissertation improves on the extant literature by leveraging a large longitudinal study to uncover the predictors of cannabis use in adolescent samples collected prior to exposure. All data were drawn from the IMAGEN study and contained a large sample of adolescents studied at age 14 (N=2,224), and followed up at age 16 and 19. Participants were richly characterized using psychosocial questionnaires, structural and functional MRI, and genetic measurements. Two hypothesis-driven studies focused on amygdala reactivity and two data-driven studies across the feature domains were completed to characterize cannabis use in adolescence.

The first study was cross-sectional and identified bilateral amygdala hyperactivity to angry faces in a sample reporting cannabis use by age 14 (n=70). The second study determined this amygdala effect was predictive of cannabis use by studying a sample of cannabis-naïve participants at age 14 who then used cannabis by age 19 (n=525). A dose-response relationship was observed such that heavy cannabis users exhibited higher amygdala reactivity. Exploratory analyses suggested amygdala reactivity decreased from age 14 to 19 within the cannabis sample, although statistical significance was not found.

In the third study, data-driven machine learning analyses predicted cannabis initiation by age 16 separately for males (n=207) and females (n=158) using data from all feature domains. These analyses identified a sparse set of shared psychosocial predictors, whereas the identified brain predictors exhibited sex- and drug-specificity. Additional analyses predicted initiation by age 19 and identified a sparse set of psychosocial predictors for females only (n=145). The final study improved on drug-specificity by performing differential prediction analyses between matched samples of participants who initiated cannabis+binge drinking vs. binge drinking only by age 16 (males n=178; females n=148). A sparse subset of psychosocial predictors identified in the third study was reproduced, and novel brain predictors were identified. Those analyses were unique as they compared two machine learning algorithms, namely regularized logistic regression and random forest analyses.

These studies substantiated the use of both hypothesis- and data-driven prediction analyses applied to large longitudinal datasets. They also addressed common issues related to human addiction research by examining sex-differences and drug-specificity. Critically, these studies uncovered predictors of use in samples collected prior to cannabis-exposure. The identified predictors are therefore disentangled from consequences of use. Results from all studies inform etiological mechanisms influencing cannabis use in adolescence. These findings can also be used to stratify risk in vulnerable adolescents and inform targets for interventions designed to curb use.
CITATIONS

Material from this dissertation has been published in the following form:

**Chapter 2:**

**Chapter 4:**

**Appendix 2:**

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ACKNOWLEDGEMENTS

I was very fortunate to receive an extraordinary amount of support during these past five years from many colleagues, friends, and family. Starting with my dissertation committee, I owe a tremendous amount of gratitude to Hugh Garavan for guiding my research and shaping my skills as a scientist, Rob Althoff for supporting our project on the neural correlates of emotional and behavioral dysregulation, John Green for always being happy to serve on all my scientific committees, and lastly Jennifer Laurent and Matthew Albaugh for their encouragement throughout this process.

Throughout my grad school career, I was lucky to work on many fascinating projects with a group of friends and scientists. I am grateful to Bader Chaarani for teaching me new skills, being my guide in foreign countries, and for spending long hours with me in the lab staring at our computer code. Thanks go to Alex Ivanciu for keeping the lab and our studies running while always making us laugh. Additionally, I am grateful to Scott Mackey, Nick Allgaier, and Catherine Orr for being exemplary scientists, and all my fellow graduate students including Nick D’Alberto and Kelsey Hudson for always being supportive.

Finally, it is out of sheer luck that during my graduate student years I somehow managed to meet and get married to my wife, Andrea. This dissertation would not be possible without her endless amounts of support. And now my childhood dream of becoming the second Dr. Phil is finally coming to fruition.
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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Drug use is one of the most common and chronic behavioral health issues facing society, yet little is known about the neurobiological, genetic, and psychosocial predictors of use. Individuals who struggle with substance use disorders typically begin drug experimentation in adolescence. Therefore, it is important to identify predictive profiles during this period of development when individuals are most vulnerable to initiating drug use. Here, the literature specifically concerning adolescent cannabis use will be reviewed in light of the psychosocial and neurobiological changes that occur during adolescence. Special focus will be given to the animal and human studies that reported on the correlates and consequences of cannabis use in adolescence. In light of those studies, it is suggested that cannabis initiation during early adolescence is related to differences in the neurobiology and psychosocial functioning later in life.

Cannabis use has become a topical matter of public health in light of the risks associated with early initiation and chronic use. Recreational cannabis use for adults is now legalized in many different parts of the United States and abroad. Moreover, recent trends in adolescent use indicated that cannabis is now the second most popular drug used by adolescents (as indicated by any lifetime use), surpassing cigarettes in 2011 (Johnston et al., 2018). Despite these trends, the scientific evidence related to the biobehavioral predictors of cannabis use remains unclear. An important gap in the literature is the lack of longitudinal studies with multimodal assessments aimed at uncovering the predictors of cannabis use. The identification of these predictors will inform pathways to cannabis...
initiation, stratify risk in vulnerable adolescents, and supply targets for proactive interventions tailored to mitigate use and attenuate the consequences of use.

1.1 Adolescent Development

*Psychosocial Development*

The World Health Organization defines adolescence as the period of development between ages 10-19 ([www.who.int](http://www.who.int)), although some argued for an extension into age 24 (Sawyer et al., 2018). Adolescence is a developmental period characterized by substantial psychosocial and neurobiological changes (Spear, 2000a). Throughout this period, adolescents experiment with various new behaviors, environments, and reinforcers in order to learn new skills necessary for independence. Childhood behavioral patterns that previously sustained the individual are replaced by adult-like behaviors developed through experimentation with independence.

Adolescents also experience a drive to emigrate from the natal family environment, incorporate more peers into their social networks, and increase time spent outside of adult supervision (Spear, 2000a). Increased interactions with peers provide opportunities for the adolescent to experiment with and learn social skills necessary for adulthood. And from an evolutionary biology perspective, motivation to emigrate from the home to associate with peers is advantageous in facilitating the identification of reproductive partners.

Opportunities to engage in risky behaviors are likely during this phase of development when adolescents spend more time engaged with their social network of peers (Rose et al., 1996). Participating in similar risky behaviors yields powerful social
reinforcement for adolescents (Kaplan et al., 1987) and can boost self-esteem (Shedler and Block, 1990). Adolescents also reported they approach risky situations in order to satisfy curiosity and augment the sense of arousal, intensity, and complexity of novel experiences (Lipsett and Mitnick, 1991). While risk-taking behaviors are a common feature of adolescence, moderating the frequency and severity of these behaviors is an important skill that must also be learned during this period (Galvan et al., 2007).

Structural Neurodevelopment

Across early development and into adulthood, the brain undergoes a series of changes including global and focal volume changes, increased myelination, synaptic pruning, and receptor proliferation until the stable adult form is reached (Spear, 2000b, 2013). Throughout this process, the neural architecture supporting cognitive, affective, motor, and sensory functions reach maturity.

From gestation to late childhood, the brain generates an excess of neurons and synaptic connections to supply individuals with an overabundance of neural resources (Huttenlocher and Dabholkar, 1997; Oppenheim, 1991). Following this overabundance, synaptic 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 (Casey et al., 2008).

In addition to synaptic pruning, neuronal myelination also proliferates during adolescence. Paus and colleagues reported 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).

Work by Giedd and colleagues uncovered separate neural developmental trajectories by sex (Giedd et al., 1999). Total brain and total gray matter volume (but not white matter) exhibited an inverted U shape trajectory from childhood to adolescence. Volumes increased throughout childhood and approached an inflection point that triggered a decline in brain volume, most likely driven by pruning processes. On average, females reached peak total brain volume at age 11 years while males peaked at age 15. In terms of white matter volume, research by Lenroot and colleagues suggested white matter increased 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 postulated white matter sex-differences may be due to axonal diameter, rather than myelination, as testosterone up-regulates expression of axonal microtubules (Perrin et al., 2008).

Functional Neurodevelopment

Durston and colleagues argued that spatial-extent of brain activity characterizes functional maturity (Durston et al., 2006). Compared to immature diffuse activity, authors argued that focal activations indicated more efficient processing resulting from
the strengthening of relevant connections via long-term potentiation. As such, spatial-extent is an important way to characterize functional between-group differences.

In terms of the functional network structure, brain activation networks develop into organized distributed processes from adolescence into adulthood, and prioritize sparse “small world” network structures (i.e., 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 for the default mode network were studied at two stages of development (Fair et al., 2009). Findings indicated distant network nodes exhibited weak interconnectivity at age 7, but then shifted to strong interconnectivity at age 21. For example, authors reported modest correlated activity between the medial prefrontal cortex and the lateral parietal cortex at age 7. After functional development, these regions exhibited strong correlated activity. This example of functional integration among distant cortical regions is a hallmark of functional development and reflects mature functional efficiency grounded in the changes to the structural architecture (Churchwell et al., 2010; Giedd et al., 1999; Lenroot et al., 2007; Perrin et al., 2008).

*Brain Development and Adolescent Behavior*

The current framework of adolescent neurodevelopment in relation to drug use behaviors postulates that a divergent rate of maturation between the subcortical and prefrontal regions of the brain might drive reward-seeking behaviors (Casey et al., 2000; Galvan et al., 2006). In a study by Fareri and colleagues, children and adolescents exhibit
positively correlated activity between the ventral striatum and medial prefrontal 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 whereby the prefrontal cortex down-regulates subcortical activity.

A developmental functional MRI (fMRI) study by Galvan and colleagues indicated the bottom-up mesocortical and mesostriatal dopaminergic projections of the midbrain reached functional maturity prior to the opposing top-down prefrontal projections (Galvan et al., 2006). This finding is supported by animal models that indicated dopaminergic projections to the PFC reached high levels during adolescence (Kalsbeek et al., 1988; Leslie et al., 1991; Rosenberg and 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 and Baldessarini, 2000). While more research is needed, functional MRI studies of reward processing found heightened blood-oxygen level dependent (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). Furthermore, another study by Galvan and colleagues reported a positive correlation between ventral striatum activation and a self-reported measure of risk-taking behaviors (Galvan et al., 2007). In sum, these findings support the hypothesis that dopaminergic hyperactivity in adolescence may potentiate sensation-seeking behaviors.
1.2 Substance Use in Adolescence

Current Prevalence Rates of Alcohol, Cannabis, and Cigarette Use in Adolescence

Current prevalence rates of drug use in adolescence can be derived from the most recent report of the Monitoring the Future Study (“MTF”, Johnston et al., 2018). The MTF study surveyed US classroom students in grades 8 (age 13-14), 10 (age 15-16), and 12 (age 17-18) on their patterns of drug use. For the 2017 report, roughly 43,700 teenagers in 360 schools across the United States were studied. This nationally representative dataset provides context for the need to study drug use in adolescence.

Alcohol is currently (and historically) the most commonly used substance in adolescence. As of 2017, 23%, 42%, and 62% of teens in grade 8, 10, and 12 respectively reported any lifetime alcohol use. Conversely, binge drinking (defined by the MTF study as consuming five or more drinks) prevalence has reached it’s historic low and currently stands at roughly 9%, 25%, and 45% of grades 8, 10, and 12. Despite this historic low, it is troubling that nearly half of adolescents initiated at least one binge drinking episode before finishing 12th grade.

Following alcohol, cannabis is the second most commonly used drug (surpassing cigarettes in 2011) with any lifetime use reported in 14%, 31%, and 45% of grades 8, 10, and 12. These rates have been stable over the past 10 years (2007 – 2017), and demonstrate an improvement relative to the past 20 years when rates peaked at 23%, 42%, and 50% in 1997. Nonetheless, rates of use are consequential in that about 10% of teens who try cannabis will become weekly users in adulthood (Hall and Pacula, 2003).
In terms of the availability of the drug, roughly 65% of 10th graders in 2017 reported that cannabis was “fairly easy” or “very easy” to obtain. For comparison, roughly 63% said the same for cigarettes, indicating that cannabis is remarkably within reach for these adolescents. It is also important to note that cannabis risk perception is at a historic low, with only 40% of 10th graders who perceived “great risk” in regular cannabis use, and only 20% who perceived “great risk” for occasional use (Johnston et al., 2018).

The MTF study also provided estimates on the differences in prevalence between years 2016 and 2017. For binge drinking, there was no significant difference in use rates from 2016—2017, providing very early evidence for the stabilization of the historically low levels of adolescent binge drinking. For cannabis, a significant 1.3% increase in use was observed from 2016—2017, but only when considering all grades combined. No significant differences were observed for each grade in isolation, although trends indicated subtle increases (Johnston et al., 2018). Thus, the significant increase across the grades is a small effect that only reached significance with a large sample.

Finally, cigarette use is common in adolescence, although rates have declined over time. Current prevalence of lifetime cigarette use stands at 9%, 16%, and 26% for grades 8, 10 and 12. These numbers are a major improvement relative to 1997 when rates were 47%, 60%, and 65% (Johnston et al., 2018). Within the context of cannabis use, alcohol and tobacco use are highly correlated with cannabis, in addition to being highly correlated with each other (Moss et al., 2014a). Given this association, some researchers posit the lack of an anticipated increase in cannabis use was driven in part by the decline in cigarette use (Miech et al., 2017).
Psychosocial Outcomes Associated with Drug Use in Adolescence

Developmental patterns of cannabis use have indicated that early initiation is correlated with higher levels of cannabis dependence (DSM-IV) in adulthood (Hall & Degenhardt, 2009; Moss, Chen & Yi, 2009). Correlations have also been identified between adolescent cannabis use and diminished socio-economic attainment (Fergusson & Boen, 2008), and although this effect is presumably bi-directional (low-SES predicts cannabis use), carefully controlled models have suggested a causal mechanism for cannabis use (Melchior et al., 2017). Many of the psychosocial consequences of cannabis use may be attenuated by delaying cannabis use until later in life (Lisdahl et al., 2013). Therefore, the ability to predict and inform prevention strategies for cannabis use is of substantial value to minimize psychosocial consequences later in life.

The literature linking cannabis use to mental health outcomes is inconclusive. An earlier study reported that roughly 10% of males and 22% of females who used cannabis in adolescence experienced depression and anxiety as adults (Patton, 2002). However, a recent meta-analysis by Gobbi and colleagues offered an updated perspective on the associations between adolescent cannabis use conferring risk for depression, anxiety, and suicidality. Authors reported that adolescent use was associated with a 1.4 increase in the odds for developing depression, 1.2 increase for anxiety (although not statistically significant), 1.5 increase for suicidal ideation, and finally a 3.5 increase for a suicide attempt (Gobbi et al., 2019). These findings should be interpreted within in the context of the relatively high prevalence of lifetime cannabis use in adolescence (45% of 12th
graders). And although those odds ratios are modest, a different meta-analysis reported stronger odds for both depression and suicide attempt when considering more frequent use (Silins et al., 2014). Therefore, preventing cannabis use is hypothesized to partially lower the incidence of these disorders and experiences.

Lastly, the relationship between adolescent cannabis use and schizophrenia or psychotic like disorders has been an active area of research. The Swedish Conscript study (Andréasson et al., 1987), and Dunedin, NZ study (Arseneault et al., 2002) were instrumental in catalyzing interest in these associations. Those studies each supplied evidence that higher frequency of use (Andréasson et al., 1987) and earlier age of onset (Arseneault et al., 2002) predicted the likelihood of developing psychosis. Since those reports, more recent papers adjusted their risk models for confounding variables like other drug use, family histories, and prodromal symptomology, and concluded the risk for these disorders is still valid (Gage et al., 2016; Mustonen et al., 2018). Future studies are needed to examine the extent to which interventions or decreasing prevalence rates of cannabis use correlate with psychotic like disorder diagnoses.

1.3 Predictors and Correlates of Cannabis Use in Adolescence

Psychosocial Predictors

Substance use in adolescence is predicted by a set personality traits and environmental factors. To start, novelty-seeking personality levels typically peak during adolescence (Maggs et al., 1995; Moffitt, 1993), and are also highly predictive of substance use (Conrod et al., 2000; Hale et al., 2003; Malmberg et al., 2012). Adolescents
also tend to discount delayed rewards in favor of immediate rewards (Steinberg, 2008) and display insensitivities to the aversive properties of some drugs (Cauffman et al., 2010; Doremus-Fitzwater et al., 2010; Schramm-Sapyta et al., 2006). Taken together, these behavioral characteristics make adolescents vulnerable to engage drug use.

For environmental factors, numerous studies have identified frequency of early life stress (Barrett and Turner, 2006), as well as perceived level of stress (Baer et al., 1987; Deykin et al., 1987; Johnson and Pandina, 1993; Tschann et al., 1994) as strong predictors of drug use in adolescence. More specifically, studies indicated that physical and/or sexual abuse during childhood is more frequent in females than males, but nonetheless strongly predicted drug use 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).

Taken together, prior life stress might promote the generation of maladaptive coping strategies that include substance use. And while stress more generally precipitates drug use (DeWit et al., 1999; Sinha, 2008; Tschann et al., 1994; Wills, 1986) the exact mechanism driving this association are actively being studied (Milivojevic and Sinha, 2018). Drugs and drug-seeking behaviors may potentiate perceptual and biological reactivity to stress (Cinciripini et al., 1989; Cobb and Van Thiel, 1982; D'Souza et al., 2004; Heesch et al., 1995) and drive self-medication behaviors. Additionally, stress may
impair capacity for impulse control, which is hypothesized to contribute to the maintenance of substance use disorders (Sinha, 2009). Therefore, various forms of life stress are key predictors to be studied as well as how they might influence the neurobiology to confer risk for use.

**Parental and Peer Influences**

Correlations between adolescent cannabis use and lifetime cannabis use of the parent has been identified (Duncan et al., 1995; Kerr et al., 2015). A recent paper by Sokol and colleagues reported that any maternal lifetime cannabis use shifted the age of initiation two years earlier in their adolescent offspring relative to adolescents from parents without cannabis use histories (Sokol et al., 2018). Furthermore, O’Loughlin and colleagues reported adolescents with one parent endorsing any lifetime use were roughly twice as likely to use cannabis, while adolescents with two parents endorsing any lifetime use were eight times more likely to use cannabis, relative to their peers whose parents do not report lifetime use (O’Loughlin et al., 2018). Hence, the influence of parental lifetime cannabis use on the initiation of use for their child is remarkable. While many factors may partially contribute to parent-offspring transmission of drug use, possible mechanisms include shared genetic and neurobiological predispositions.

Peer influences are also strong indicators of cannabis use. At a very basic level, an adolescent is more likely to use cannabis if they are involved in a network of peers who use cannabis. This finding has been consistent throughout the literature (Ali et al., 2011; Duncan et al., 2005; Kuntsche and Jordan, 2006). Mechanisms contributing to this effect
are hypothesized to be related to the individual differences in the reinforcing properties of both the drug and the social context (Caouette and Feldstein Ewing, 2017). For example, some adolescents may not find pleasure in drug use but enjoy the social reinforcement achieved by engaging in cannabis use behaviors with their peers. In contrast, some adolescents may not enjoy social situations but they are necessary as a means to obtain drugs. Evidence for both these scenarios are reported in the literature. Lee and colleagues studied motives for cannabis use in high school graduates and reported that 26% endorsed social enhancement and bonding as their primary motive for use (Lee et al., 2007). Buckner and colleagues designed an instrument to measure the likelihood of cannabis use in different social situations and reported that adolescents with social anxieties avoided social situations where cannabis is unavailable (Buckner et al., 2012). Therefore, it is important to consider relationships with peers and social contexts as contributing factors for cannabis use in adolescence.

Cognitive Associations with Cannabis Use

In studies on cognition in adolescent cannabis users, findings are relatively inconclusive, with more evidence pointing to cognitive deficits in teens with heavy or earlier onset of use. 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 suggested chronic cannabis use throughout adolescence is
associated with a decrease in complex attention, slow processing speeds, and reduced verbal learning and sequencing skills (Jacobus and Tapert, 2014; Medina et al., 2007a).

In a longitudinal study by Hanson and colleagues, researchers assessed verbal learning, working memory, visual attention, and time estimation at three time points across one month of monitored abstinence in cannabis using teens compared to controls. Across all measures, cannabis users showed worse performance than controls, however, users showed a recovery effect following three-weeks monitored abstinence for all measures except attention. These findings provided evidence that cannabis related insult only to attention might persist into adulthood, or, at least require protracted periods of abstinence to recover (Hanson et al., 2010). These findings have been corroborated by Fontes and colleagues (Fontes et al., 2011), and Gruber and colleagues qualified this finding and reported attentional deficits negatively correlated with age of initiation (Gruber et al., 2012). Therefore, cannabis might partially impair attentional capacities in individuals who initiate cannabis earlier than their peers.

As reflected above, an ongoing area of research is the extent to which adolescent cognitive differences persist into adulthood. In early studies, Schwartz and colleagues reported short-term memory impairments persist at least six-weeks after monitored abstinence (Schwartz et al., 1989). However, in a prospective study by Fried and colleagues, researchers analyzed cognitive performance in current users, former users, and never-using controls, while accounting for performance levels prior to drug use. Investigators measured IQ, memory, processing speed, and 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 using more cannabis than the current users. Although that study was confounded as former users also had the highest socioeconomic status (SES) compared to the two comparison groups (Fried et al., 2005).

Lastly, 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 non-using peers, and earned a greater proportion of earnings from the smaller reward level. Proportion of earnings correlated with the amount of cannabinoids present in urine samples on the day of testing (Lane et al., 2005). This finding was also exhibited by adults tested under acute intoxication of smoked cannabis compared to placebo (Lane and Cherek, 2002). Thus, cannabis use is associated with impaired motivation and sensitivity to reward, at least under acute and lingering effects. Authors concluded cannabis use might disrupt healthy motivational processes that coordinate favorable behavioral adaptations (Lane et al., 2005).

It is worth noting there were studies that did not find differences in cognitive abilities related to cannabis use in adolescence (Tait et al., 2011; Takagi et al., 2011; Teichner et al., 2000). However, the prevalence of papers that reported cannabis use compromises cognition outnumbers those reports. Scott and colleagues reviewed the literature on adolescent cannabis use and cognition (Scott et al., 2018). Authors reported modest impairments on learning, working memory, delayed memory, inhibition, and attention in adolescent cannabis users (Scott et al., 2018). Moreover, authors concluded
that those differences generally abated following a minimum of three days monitored abstinence (Scott et al., 2018). This conclusion was also reported in an earlier meta-analysis adult cannabis use and cognition (Grant et al., 2003). Together, these findings indicated that cannabis cessation interventions would likely produce beneficial effects on cognitive ability in adolescents who initiated cannabis use.

**Cannabinoid Psychopharmacology & Insights from Animal Models**

The primary cannabinoid (CB1) receptor is a metabotropic G<sub>i</sub>-protein coupled receptor located throughout the brain, with high concentrations in the cerebellum, hippocampus (especially the CA1/CA3 and dentate gyrus), basal ganglia and amygdala (Glass et al., 1997; Herkenham et al., 1991; Katona et al., 2001). Within these regions, the CB1 receptor is found on presynaptic terminals of both GABAergic and glutamatergic interneurons and participates in neuromodulatory functioning. The endogenous ligands for these receptors are anandamide and 2-arachidonoylglycerol, while the primary exogenous cannabinoid responsible for psychoactive effects is delta-9-tetrahydrocannabinol (THC) (Gaoni and Mechoulam, 1964; Pertwee, 2008). When the CB1 receptor is bound by these ligands, neurotransmission is attenuated through the inhibition of cyclic adenosine monophosphate (cAMP) and subsequent signal transduction pathways, including the inhibition of pre-synaptic Ca<sup>2+</sup> channels to regulate vesicular release of neurotransmitters (Wilson et al., 2001).

Animal models have provided valuable evidence that linked adolescent cannabinoid exposure with differences in the neurobiology. Starting with CB1 receptors,
Dalton & Zavitsanou exposed adolescent rats to a CB1-receptor agonist at different doses and frequencies of infusion (one time use, light use, chronic use). Results indicated a dose-response decrease in CB1 receptor densities by dose and frequency of use relative to control animals. Lower CB1 receptor densities were reported across the brain with dramatic effects in the substantia nigra and CA1 subfield of the hippocampus (Dalton and Zavitsanou, 2010). Those results were in line with a previous study that showed similar effects on the amygdala, ventral tegmental area, and nucleus accumbens (Rubino et al., 2008). Related lines of inquiry indicated that THC might be neurotoxic to both cortical (Downer et al., 2001) and hippocampal neurons (Lawston et al., 2000; Quinn et al., 2008b) as measured by differences in tissue characterizations and metabolic changes. Together, these findings support the hypothesis that exposure to exogenous cannabinoids during adolescence disrupts typical neurodevelopment and might be partially related to phenotypic differences in those youths.

Animal studies have also reported on the functional neurobiological and behavioral differences following exposure to cannabinoids in adolescence. Renard and colleagues identified impaired long term potentiation in pretreated rats relative to controls by probing network activity between the hippocampus (CA1/subiculum) and prefrontal (prelimbic) cortex (Renard et al., 2016). Those findings elucidated previous studies that showed deficits in domains dependent on hippocampal-PFC circuits including object-recognition (O’Shea, 2004) and spatial learning (Cha et al., 2006).

Lastly, animal models have supported the link between adolescent use and mood disorders in adulthood. Rubino and colleagues studied rats pretreated with THC during
adolescence and identified more depressive-like behaviors in adulthood as indexed by a forced swim test and a sucrose preference test (Rubino et al., 2008). Those findings are in line with work by Bambico and colleagues who reproduced those two findings and also reported evidence for elevated anxiety-like behaviors as indexed by a novelty-suppressed feeding test in pretreated rats (Bambico et al., 2010). It is important to stress that all of these studies suggested adolescent, but not adult, exposure to cannabinoids precipitated structural, functional, and behavioral differences. These results underscore the need to predict cannabis use in human samples to minimize the likelihood of adolescents developing these brain and behavioral differences.

*Genetic Associations with Cannabis Use*

In a recent GWAS study using a very large sample (N=184,765), researchers identified eight single nucleotide polymorphisms (SNPs) that explained 11% of the variance of lifetime cannabis use. The top three SNPs were on a gene coding for CADM2, a cell adhesion molecule that is expressed widely in the brain. Authors reported this gene was previously affiliated with alcohol consumption and risk taking behaviors (Pasman et al., 2018).

There were two GWAS for cannabis use dependence (DSM-IV). The first study identified three SNPs having an association with cannabis use dependence. One SNP was found on an antisense transcription region (*RP11-206M11.7*, rs143244591) whose function was 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; Sherva et al., 2016). In the earlier GWAS study, none of their SNPs passed significance levels appropriate for GWAS studies ($p < 1.0 \times 10^{-8}$; Agrawal et al., 2011). However, authors suggested a candidate-gene approach using neurotransmitter receptor genes (e.g., cannabinoid, opioid, dopamine), and relevant enzymes (e.g., fatty-acid amide hydrolase, FAAH) might better uncover associations with cannabis use (Agrawal and Lynskey, 2009). Therefore, a GWAS approach might not yield the most clear or robust findings despite their potential for identifying novel biological predictors of cannabis use.

**Structural Brain Correlates of Adolescent Drug Use**

Recently, Orr and colleagues compared a sample of very light cannabis users at age 14 to a closely matched sample of controls and reported greater gray matter volumes (GMV) across many subcortical regions like the amygdala, hippocampus, and striatum with extent into the surrounding cortical regions (Orr et al., 2019). However, there is mixed evidence in the literature. Ashtari and colleagues identified bilateral hippocampal volume reductions in adolescent cannabis users compared to controls when scanned after 1 month of monitored abstinence. Self-reported levels of use were also inversely correlated with the right hippocampus, suggesting a dose response in volume reduction (Ashtari et al., 2011). Similarly, Yücel and colleagues reported GMV reductions in the bilateral amygdala in adolescent users compared to controls (Yucel et al., 2008). In a study of young adults, GMV was greater in the anterior cerebellum of heavy cannabis users compared to controls. However, a negative correlation between dependency scores
and right amygdala volumes, and negative correlation between weekly cannabis use and bilateral hippocampal GMV was identified (Cousijn et al., 2012).

Generally, those studies provided converging evidence on medial temporal lobe structures. All authors interpreted their finding in the context of the CB1 densities in those regions and concluded cannabis use in adolescence might be toxic to those tissues. Furthermore, those studies were inline with the animal models that reported neurotoxic effects of exogenous cannabinoids on the hippocampus mentioned above.

**Structural Brain Predictors of Cannabis Use**

As the above studies were cross-sectional, they were unable to infer a causal relationship between cannabis use and structural differences. Next, the few prospective studies will be surveyed. Starting with a study by Cheetham and colleagues, researchers studied an adolescent sample pre- and post-cannabis use. Findings indicated less GMV in the orbital frontal cortex (OFC) at age 12 predicted cannabis use by age 16 (Cheetham et al., 2012a). These findings are in line with work by Volkow & Fowler who reported hypoactivity in the OFC using fMRI and positron emission tomography (PET) characterized individuals with drug dependence (Volkow and Fowler, 2000). These two studies suggest a propensity for drug use is partially predicted by differences in structure and function of the OFC, a region putatively involved in relevant functions like reinforcer evaluation (Noonan et al., 2012) and behavioral regulation (Bryden and Roesch, 2015).

Jacobus and colleagues investigated cortical thickness before (age 13) and after (age 19) initiating alcohol-only, alcohol+cannabis, vs. drug-naïve controls. Less cortical
thickness across many prefrontal regions, including the left precentral and superior frontal gyri, predicted the future alcohol+cannabis group relative to alcohol-only, and less thickness in right middle frontal gyrus predicted the alcohol+cannabis group relative to controls (Jacobus et al., 2016). This study also contained repeated measures on some neurocognitive and mental health screeners. Results indicated lower performance on working memory tasks, and, higher externalizing scores at baseline predicted the alcohol+cannabis initiating group relative to the others. These results suggested a preexisting working memory deficit is continued (if not exacerbated) through prolonged cannabis exposure in adolescence. These findings also indicate that a combination of behavioral and neuroimaging measures yields a predictive profile of cannabis use in adolescence.

In light of these two studies, findings generally indicated lower gray matter volumes in prefrontal regions preceded cannabis use in adolescence. The interpretations regarding these effects are challenging, although they might signal precocious development as normative adolescent neurodevelopment is characterized by volume reductions. Exhibiting lower volumes relative to their peers at the same age might indicate an accelerated neurodevelopment, which in turn, facilitates maladaptive psychosocial development involving cannabis use.

*Functional Brain Correlates of Adolescent Cannabis Use*

Cannabis use in adolescence has been largely understudied using fMRI. Work by Tapert and colleagues studied response inhibition in a group of adolescents with and
without cannabis use histories after 1 month of monitored abstinence. Despite not observing behavioral task differences, higher and more diffuse cortical activations during successful inhibition trials were observed in many prefrontal regions including bilateral superior and middle frontal gyri in the cannabis using group relative to controls (Tapert et al., 2007a). These findings indicated cannabis use might have partially induced lasting functional differences as indexed by the lack of functional efficiency relative to their non-using peers.

Similar studies also indicated that adolescent cannabis users did not differ on cognitive task performance measures relative to controls following one-month of abstinence. Instead, they were characterized by more diffuse brain activations throughout parietal regions on a spatial working memory task (Padula et al., 2007; Schweinsburg et al., 2008). Similar studies reported diffuse prefrontal activations during working memory (Jager et al., 2010), and attention tasks (Abdullaev et al., 2010). Lastly, one study identified more diffuse activity during a verbal working memory task correlated with an earlier age of cannabis initiation (Becker et al., 2010). The consistency of these effects all supports the interpretation that adolescent cannabis users may have compromised healthy functional development as characterized by diffuse patterns of functional activations. However, these studies did not evaluate their samples prior to cannabis initiation, so causal interpretations are speculative.

*Studies on Affective Processing*
Cannabis use elicits positively and negatively valenced mood altering properties for the user, yet few investigators have used fMRI to study emotional processing in these samples. Green and colleagues reviewed studies on the self-reported subjective experiences related to acute cannabis use. Relaxation was the most common experience reported by 91% of cannabis users. Negatively valenced experiences were also reported as 40% of users reported anxiety and 27% reported depression (Green et al., 2003). Together with the animal and human findings related to the emotional correlates and consequences of cannabis use, these effects motivate a need to study cannabis use from an affective neuroscience perspective. Furthermore, there is a gap in the literature for studies using fMRI to illuminate differences in affective processing in adolescents. However, the adult literature offers some insights.

The amygdala is a key brain region involved in visual threat detection systems (Fox et al., 2015; Pessoa and Adolphs, 2010) and is commonly implicated during fMRI studies of social affective processing (Adolphs, 2010). Amygdala reactivity has only been studied in light of cannabis use for adults. A pharmacological fMRI study by Phan and colleagues reported that acute administration of THC was associated with lower amygdala reactivity during angry face processing relative to placebo (Phan et al., 2008). Outside of the acute period, Gruber and colleagues corroborated this affect by reporting that adults with chronic cannabis use displayed lower amygdala reactivity to angry faces following a minimum 12-hour cannabis abstinence relative to non-users (Gruber et al., 2009).
In adolescents, one study by Heitzeg and colleagues probed for differences using an emotional word processing task. In that study, researchers compared adolescents with heavy cannabis use histories to a sample of very light cannabis using controls. Results indicated that heavy users demonstrated lower bilateral amygdala activations when processing both positively and negatively valenced words (Heitzeg et al., 2015). Together with the adult studies, these reports indicated that cannabis use is correlated with differences in affective processing, with converging evidence on amygdala reactivity. Lower amygdala reactivity generally followed acute and protracted use histories. It will be important to assess if emotional processing differences preceded use, as it is hypothesized that higher emotional reactivity partially influences individuals to use cannabis for the relaxing (anxiolytic) effects.

Functional Brain Predictors of Drug Use

There is a lack of prospective studies that examined functional brain activations prior to cannabis use in adolescence. After searching the literature, a single report by Tervo-Clemmens and colleagues was identified (Tervo-Clemmens et al., 2018). In that study, researchers scanned children at age 12 and 15 using a working memory paradigm. Results indicated that higher frontoparietal activations and lower visual cortex activations during high task load in a sample of cannabis-naïve children predicted cannabis use by age 15. A dose-response effect was also found in the cuneus, such that lower activations predicted heavier use. Additionally, lower spatial planning scores from a cognitive test battery (CANTAB) also predicted use. These results add to the literature despite being
limited by a small outcome group (n=22 future cannabis users), therefore, replication and larger studies are needed.

Reasons for the shortage of prospective fMRI results are unclear, but might be related to publication bias. And although speculative, the structural studies reported by Jacobus and Cheetham likely came from a larger project with fMRI as neuroimaging batteries usually contain both structural and functional assessments. The lack of publications on fMRI data from these groups might suggest no functional differences were identified. And again, as this is speculative, the functional predictors of cannabis use are likely to be small effects, which necessitates a large sample. As a final thought, it might be the case that a non-linear or machine learning approach might be better suited to identify neurobiology predictors as Jacobus and Cheetham and colleagues used traditional mass-univariate approaches. To date, machine learning approaches are in their infancy, as few longitudinal machine learning studies have been reported. This sparse literature will be reviewed at the end of the following section.

1.4 Predictive Modeling of Drug Use

Predictive Modeling Overview

As outlined by Whelan & Garavan, predictive modeling is a technically challenging pursuit with many opportunities for methodological errors leading to inflated interpretations (Whelan and Garavan, 2014). These issues are pertinent to the field of psychiatric neuroimaging (or any field with feature-rich datasets) as the number of independent variables (hundreds of regions of interest or thousands of voxels) predicting
a phenotype exceeds the sample size. In this scenario, estimating a multiple regression model yields perfect fit to the data. In general, model fit statistics increase as the number of parameters increases, and/or the number of subjects decreases (Babyak, 2004). In these scenarios, an uninformed researcher might become overoptimistic and interpret the results of an overfit model (Whelan and Garavan, 2014).

The ultimate goal for prediction analyses is to yield a model that accurately predicts novel observations. The gold standard for predicting novel observations is to test a model on a completely independent external dataset. However, the researcher might not have this option with a limited sample size. To overcome the lack of an external dataset, and to address the challenges in estimating a model with an excessive number of variables, we will consider two remedies that can be used in parallel—cross-validation and regularization (or feature selection more generally).

*Cross-Validation*

Cross-validation is a procedure commonly used to partition the original dataset into subsamples of observations (Wong, 2015). 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. A common practice is to initially set aside a percentage of the data as the validation set, say 10%, and estimate a predictive model on the remaining 90%. Thus, the researcher is able to evaluate how well their predictive model generalizes to the set aside 10%. 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 (ROC AUC) from logistic models, returned from evaluating the model on the test data. For these two statistics, values closer to 1 reflect superior prediction.

ROC curves plot the trade off between sensitivity and 1-specificity at all probability thresholds provided by the classifier (i.e., the logistic model) (Hanley and McNeil, 1982). For ROC AUCs, values from 0.99 – 0.51 reflect diminishing returns, with 0.50 reflecting chance performance. These values reflect the probability of a randomly chosen “case” being ranked higher than a control. To help with interpretation, work by Rice and Harris has shown that the ROC AUC can be equated Cohen’s $d$ and Pearson’s point-biserial correlation, such that a medium effect size Cohen’s $d$=0.5, and $r=0.243$, is equivalent to a ROC AUC=.639 (Rice and Harris, 2005).

One specific form of cross-validation is $k$-fold cross-validation, where $k$ = number of partitions (or, “folds) of the original dataset (Wong, 2015). Each fold contains a similar amount of unique samples from the original dataset (i.e., when $k = 10$ and N=100, each $k$th fold will have 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*
Regularization is a statistical technique used during regression model estimation that attempts to minimize the amount of overfit to the data. (Zou and Hastie, 2005) 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. It is important that all independent variables are standardized prior to regularization so that coefficient penalization is consistent across independent variables. Here, two specific forms of regularization will be considered, LASSO (Least Absolute Shrinkage and Selector Operator; Tibshirani, 1996), and Ridge regression (Hoerl and Kennard, 1970).

LASSO regression rejects complex models in favor of parsimonious models by minimizing the sum of the absolute values of the coefficients (Tibshirani, 1996). In doing so, the LASSO estimator solves for the $\ell_1$-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. The predictors remaining in the model are therefore more important to the outcome measure than the predictors set to zero. LASSO regression is one technique available to researchers when the number of predictors is immense, as the estimation procedure performs feature selection while fitting a model.

Ridge regression seeks to solve problems arising from multicollinearity among predictor variables (Hoerl and Kennard, 1970). 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 $\ell_2$-norm of the design matrix. All predictors are included
during ridge regression (unlike LASSO) but are 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 scenarios with excessive independent variables, and modeling correlated variables might be of theoretical interest, a hybrid approach balancing LASSO and Ridge regression, termed “elastic-net” regularization (Zou and Hastie, 2005), can be used. In elastic-net regularization, LASSO and Ridge are combined using a mixing parameter, $\alpha$ that balances the contribution of the LASSO to Ridge estimation methods. In addition to the $\alpha$ parameter, a second parameter, $\lambda$ controls the magnitude of the shrinkage applied to the coefficients.

During elastic-net model estimation, the $\alpha$ and the $\lambda$ 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 $\alpha$ approaches 1, the LASSO estimator is favored, and when $\alpha$ approaches 0, the Ridge estimator is favored (Zou and Hastie, 2005). Intermediate values of $\alpha$ provide an interpolation between the two estimation procedures.

*Tree-based Estimators*
Most regression models are an extension of the general linear model and assume independent variables (IVs) combine in some linear fashion to predict the dependent variable (DV). However, this might not always be a safe assumption when modeling an excessive number of IVs from disparate sources, as many of these features might exhibit non-linear effects when predicting the DV. In parallel, attempting to include interaction terms (or other non-linear terms) for an already excessive number of IVs would be prohibitive. Regression models also attempt to model the average (singular) relationship between the IVs and DV (See Lemon et al. 2003, for comparison of regression to tree-estimation) (Lemon et al., 2003). However, it is hypothesized that the observations in a sample reach the same outcome through different pathways. Different pathways are especially viable when the outcome is a behavioral measure. Therefore, estimating regression models may not be the optimal way to leverage a large dataset or to probe individual differences.

To address these concerns, non-linear modeling procedures like decision trees and random forests (Breiman, 2001) can serve as candidate models to predict cannabis use initiation. These tree-based models are more easily understood and interpretable non-linear models relative to support vector machines or neural networks. A decision tree consists of series of logical if-then rules that effectively partition the feature space to group observations together (Quinlan, 1986). The optimal set of rules divides the feature space until the observations are adequately separated into their respective outcomes. Trees can be estimated for both categorical or continuous outcomes by minimizing class
impurity or squared error within each partition. In the case of predicting drug use, trees can be constructed to partition all future cannabis users separately from their naïve peers.

An extension of the decision tree is a random forest (Breiman, 2001). During model estimation, a single decision tree is estimated on a randomly selected set of features. This procedure is then repeated many times until many trees built off randomly selected features comprise a “forest”. Random forests are ideal for feature rich datasets as it is equally likely for any feature to be randomly selected during the construction of a tree in the forest. And due to the random selection of features, random forests effectively account for multi-collinearity. And while all randomly selected features (usually restricted to the square root of the total number of features) are considered during tree estimation, only those that separate the classes when split are assigned an if-then rule.

The forest can then be used to predict new observations by taking the majority vote across all trees. Looking across the trees in the forest, one can also determine the features that best predicted the outcome measure when split. A summary decision tree may then be estimated using only those features. Therefore, a predictive profile informed by a tree-based estimator conveys a unique pathway towards the outcome measure, whereas a profile informed from a regression provides a singular pathway for the average individual.

One caveat to this method is the high risk of overfitting, as a decision tree can quickly become complex enough to exactly classify each observation (Schaffer, 1993). Therefore, techniques to resist overfitting must be applied when predicting out of sample observations. These techniques include limiting the depth of the tree and requiring a
minimum number of observations present in a terminal leaf node. In doing so, these rules stop the decision tree from completely isolating each single observation.

**Predictive Modeling of Binge-Drinking by Age 16**

Whelan and colleagues implemented cross-validated regularized regressions 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 highly significant classification (AUC=0.90) and prediction (AUC=0.75). Hence, these methods yielded models that performed well in classifying and predicting independent samples.

When probing the models further, the most reliable features that both classified and predicted adolescent binge drinking were more frequent sexual life experiences and higher novelty seeking, disorderly, and extravagant personalities, and lower conscientiousness personality traits. The brain features that classified age 14 binge drinking included lower GMV of the vmPFC, and lower activations in the left putamen and hippocampus during reward anticipation, and right hippocampus during reward outcome. The brain features that predicted binge drinking were lower activations in pre- and post-central gyri during failed response inhibitions. In sum, the classification was driven by lower activity in regions serving appetitive processing, while the prediction was driven by lower activity in sensory motor areas during failed response inhibitions.

Finally, Squeglia and colleagues recently published a machine learning study predicting the initiation of alcohol use in adolescence (Squeglia et al., 2017). In that
report, researchers used random forests on data collected at age 12-14 to predict moderate
to heavy alcohol use by age 18, relative to a sample of consistently alcohol naïve peers.
Three separate models were generated using an increasing number of feature domains
(demographics only; demographics + neurocognitive; demographics + neurocognitive +
neuroimaging). The prediction accuracies increased with the inclusion of each domain, as
indexed by sensitivity (.60, .67, .74) and specificity (.64, .70, .73) to alcohol initiation.
The most important features driving prediction were largely from the neuroimaging
domain, including lower thickness of the left supramarginal gyrus, lower activation in the
right posterior cingulate and superior temporal gyrus during a working memory task.

Aside from the neuroimaging data, sex, socioeconomic status (SES), and
cognitive measures were also selected as predictors of adolescent alcohol use. The lack of
consistency with Whelan and colleagues are likely due to differences in measurement
(Whelan used voxel-wise data, Squeglia used ROIs). Nonetheless, these results indicated
the highest prediction was achieved with the combination of behavioral and
neuroimaging data. And in keeping with the prediction study by Whalen and colleagues,
significant and meaningful predictions can be made using a machine learning approach to
leverage multi-domain data to uncover profiles predicting drug use in adolescence.

1.5 Overview of the IMAGEN study

The IMAGEN study (Schumann et al., 2010) is large longitudinal study of
adolescent development conducted across eight different sites in Europe (Paris, Dublin,
London, Nottingham, Berlin, Hamburg, Mannheim, Dresden) (www.imagen-
Study procedures included a comprehensive neuroimaging (MRI) battery and blood assays for genetic measurements of the adolescent. Both the adolescent and their parent also completed many psychosocial questionnaires. The study was designed to address many different scientific aims (Schumann et al., 2010) but broadly seeks to understand the relationship between adolescent psychosocial and neurobiological development, and how those data characterize and predict behavioral health issues.

The study employed a convenient community sampling method by targeting the local school systems. Efforts were made to stratify enrollment to capture differences in ethnic and socioeconomic backgrounds. Baseline enrollment started at age 14, and youths were reassessed at ages 16 and 19. At the baseline visit, study protocols were explained to the family, written informed consent was obtained from the parent, and the child provided assent. Local ethics committees at all participating sites approved the study protocols. There were N=2,224 participants enrolled in the baseline sample.

At the baseline visit, parents completed a set of self-report questionnaires related to their personality, drug use levels, and family histories. They also completed a mental health screener for which they reported on their child. The mental health questionnaire used in IMAGEN was the Development and Well-Being Assessment (“DAWBA”) and the affiliated brief screener, the Strengths and Difficulties Questionnaire (“SDQ”) (Goodman, 1997; Goodman et al., 2000).

The adolescent also provided self-report on many of the same questionnaires. Personality was measured via the NEO, TCI, and SURPS questionnaires (Cloninger, 1999; Costa Jr. and McCrae, 1995; Woicik et al., 2009). Drug use frequencies were
measured on an ordinal scale using the European School Survey Project on Alcohol and Other Drugs instrument (ESPAD) (Hibell et al., 1997). Frequencies and valence ratings for various stressful life events was measured via the life events questionnaire (Newcomb et al., 1981). Various cognitive measures were also recorded (Kirby et al., 1999; MacLeod et al., 1986; Robbins et al., 1994) including their performance and verbal IQ levels (Wechsler, 2003).

Blood assays were performed at baseline from which DNA was extracted. Genotype information was collected at 582,892 markers using the Illumina HumanHap610 and HumanHap660 Genotyping BeadChips (San Diego, CA). The 1000 Genomes project reference set of markers (www.internationalgenome.org) was used for imputation after reducing the markers to ~13 million SNPs for European populations.

Adolescents without any MRI contra-indications received a neuroimaging scan. For brain structure, a high-resolution whole-brain anatomical scan (MPRAGE), and a diffusion-tensor imaging (DTI) scan for white-matter fiber tractography were collected. For brain function, three fMRI tasks were administered. The stop signal task measured motor response inhibition (Rubia et al., 2005). The monetary incentive delay task measured reward anticipation and reward outcomes (Knutson et al., 2000). Lastly, a face processing scan that involved passive viewing of angry and neutral faces was collected (Grosbras, 2005). Standardization efforts were in place to ensure all sites used similar acquisition techniques, and all data were submitted to identical preprocessing pipelines.

Adolescents were then followed up at age 16 and 19. The age 16 assessment contained only a brief follow up using psychosocial questionnaires and was completed at
home using an online portal (Psytools, Delosis, London, UK). The age 19 visit also included a follow up using the psychosocial questionnaires, and another neuroimaging scan that was identical to the baseline visit. See www.imagen-europe.com for all standard operating procedures as well as a selection of papers that previously reported on IMAGEN data.

1.6 Current Report

This dissertation contains a collection of studies that uncover the correlates and predictors of cannabis use in adolescence by leveraging data from the IMAGEN study. The first study (in Chapter 2) tested for cross-sectional differences in the neurobiology of adolescents who reported any lifetime cannabis use at baseline (age 14). As informed by the animal and human studies, the face processing task was selected for use as it was hypothesized that adolescents using cannabis would exhibit hyperactivity to social threat processing cues. This study also adds to the apparent gap in the literature relating adolescent cannabis use to the functional neurobiology supporting affective functions.

After characterizing those differences, the longitudinal nature of the IMAGEN dataset was interrogated to determine if the differences observed in Chapter 2 preceded cannabis use in adolescence. Hence, Chapter 3 begins the prediction theme of this dissertation by predicting cannabis use levels at age 19 using a sample of individuals who were cannabis-naïve at age 14. As such, the baseline data was disentangled from the consequences of cannabis exposure.

The remaining studies in Chapters 4 & 5 better characterize a predictive profile related to cannabis use in adolescence by examining data from all three feature domains...
in the IMAGENT study. Cannabis naïve samples at baseline were identified and machine learning was used to identify the predictors of cannabis initiation by age 16 and 19. Prediction models were executed separately by sex in light of the sex-differences discussed above. Post-hoc analyses tested if the predictors were specific to each sex or generalized to the opposite sex. Finally, drug-specificity was assessed by evaluating the identified predictors of cannabis use on an independent sample of binge drinkers. As cannabis use and binge drinking were found to be highly co-occurring, drug-specificity was evaluated again by executing similar machine learning analysis predicting future cannabis use versus future binge drinking.

Results from theses analyses uncovered the predictive profiles of cannabis use in adolescence. These profiles can be used to both stratify risk and inform intervention strategies designed to mitigate use. For instance, the level of risk for cannabis use can be determined by how closely an adolescent aligns with the predictive profiles uncovered here. The psychosocial and neurobiological predictors uncovered here inform treatment strategies. As the literature indicated cannabis use in adolescence is associated with psychosocial, cognitive, and neurobiological differences, the knowledge gained from this dissertation will be translated to help lower cannabis use in adolescence through interventions designed to alter a risk phenotype.
References


CHAPTER 2: CROSS-SECTIONAL FACE PROCESSING DIFFERENCES IN ADOLESCENTS USING CANNABIS

This Chapter has been previously published in the following format:


Abstract

Cannabis use in adolescence may be characterized by differences in the neural basis of affective processing. In this study, we used an fMRI affective face processing task to compare a large group (n = 70) of 14-year olds with a history of cannabis use to a group (n = 70) of never-using controls matched on numerous characteristics including IQ, SES, alcohol and cigarette use. The task contained short movies displaying angry and neutral faces. Results indicated that cannabis users had greater reactivity in the bilateral amygdalae to angry faces than neutral faces, an effect that was not observed in their abstinent peers. In contrast, activity levels in the cannabis users in cortical areas including the right temporal-parietal junction and bilateral dorsolateral prefrontal cortex did not discriminate between the two face conditions, but did differ in controls. Results did not change after excluding subjects with any psychiatric symptomology. Given the high density of cannabinoid receptors in the amygdala, our findings suggest cannabis use in early adolescence is associated with hypersensitivity to signals of threat. Hypersensitivity to negative affect in adolescence may place the subject at-risk for mood disorders in adulthood.
Introduction

Adolescence is a significant period of psychosocial development, with increases in novelty-seeking and risk-taking behaviors (Adriani et al., 1998; Romer et al., 2010; Trimpop et al., 1998). Experimentation with drugs of abuse—especially alcohol, tobacco, and cannabis, is typically initiated during this phase (Chen and Kandel, 1995). As cannabis becomes more available and public opinion trends towards acceptance, adolescents may have increased access to the substance.

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 (SAMHSA, 2014). Chronic use also appears to be growing; in 2008, 5.5% of users aged 12 and up reported near daily use while in 2013 this rate had risen to 8.1% (SAMHSA, 2014). These increasing rates of use are consequential in that about 10% of those who try cannabis will become weekly users in adulthood (Hall and Pacula, 2003). Furthermore, adolescent beliefs about the risks associated with cannabis appear to be declining (Johnston et al., 2011).

Adolescence is also a period of marked neural development including gross volume changes, myelination, synaptic pruning, and receptor proliferation (Spear, 2000). These changes are especially large in the prefrontal cortex (PFC) (Gogtay et al., 2004; Whitford et al., 2007), amygdala, hippocampus, and striatum, and are governed in part by the endogenous cannabinoid system (Bossong and Niesink, 2010). Interestingly, the primary cannabinoid receptor, CB1, is found in high concentrations in these cognitive and affective regions of the brain (Glass et al., 1997; Herkenham et al., 1991; Katona et
al., 2001), and appears to be fully expressed by adolescence (Belue et al., 1995; de Fonseca et al., 1993; Morozov and Freund, 2003; Romero et al., 1997). Studies have shown that exogenous cannabinoids can interfere with the endogenous system (Hoffman et al., 2007; Mato et al., 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 affective neural systems.

Mounting evidence supports the relationship between early cannabis use and mood disorders (Wittchen et al., 2007), even with relatively low levels of use (Cheung et al., 2010). Hence, it is crucial to investigate the consequences of cannabis use on emotional development. Although numerous studies have associated cannabis use in adolescence with an increased likelihood of schizophrenia and/or other affective disorders (Arseneault et al., 2004; Degenhardt and Hall, 2006; Fergusson et al., 2006; Hall, 2006; Linszen and van Amelsvoort, 2007; Manrique-Garcia et al., 2012) there is relatively little research on the impact of cannabis use from a cognitive and affective neuroscience perspective.

The amygdala has a high density of CB1 receptors, notably in the basal and lateral nuclei (Katona et al., 2001). In adulthood, increased amygdala activity is associated with major depressive disorder (Drevets, 2001; Sheline et al., 2001), and generalized social phobia (Evans et al., 2008; Phan et al., 2006). In adolescence, the amygdala was found to yield stronger responses to fearful faces than adults (Thomas et al., 2001), and greater amygdala reactivity may account for adolescent vulnerability to mood disorders (Guyer
et al., 2008a; Monk et al., 2008; Roberson-Nay et al., 2006). In consideration of the amygdala’s role in the endocannabinoid system and affective processing, adolescent vulnerability to mood disorders and propensity for cannabis use, it is important to assess functional differences in this region in cannabis-using teenagers.

Using an animal model, Rubino and colleagues (2008), and Schramm-Sapyta and colleagues (2007) examined the relationship between anxiety and THC exposure in adolescent and adult rats. Findings indicate that adolescent rats exhibit elevated signs of anxiety, depression, and anhedonia when treated with THC compared to placebo. Translating these findings to humans may imply cannabis use in adolescence is related to differences in the generation and regulation of affect.

To examine the impact of cannabis use on brain regions subserving emotional processing, we conducted an fMRI study on 14-year old cannabis users vs. controls using affective face stimuli. Angry and neutral faces provide a robust probe of activity within the amygdala and PFC in adults (Morris et al., 1996; Pessoa et al., 2002; Whalen et al., 1998), as well as children and adolescents (Baird et al., 1999; Thomas et al., 2001). The differential activity of the amygdala to angry versus neutral faces is an excellent index of emotional processing and may relate to psychopathology. However, in order to prevent ceiling effects, we used a set of stimuli that was only mildly negatively valenced on the basis that they may provide a sensitive test of enhanced amygdala reactivity (Grosbras and Paus, 2006).

To date, few study have examined the relationship between cannabis and face processing. Phan and colleagues (2008) recruited healthy adults in a dual-session, double-
blind, placebo-controlled study of THC intoxication and face processing using fMRI. Findings indicate THC attenuates the amygdala response to fearful faces. Similarly, Gruber and colleagues (2009) studied 15 chronic cannabis users vs. matched controls under fMRI during a masked affective face processing task. Results suggest chronic cannabis use is associated with decreased reactivity in the anterior cingulate and amygdala. While both Phan and Gruber’s findings suggest anxiolytic effects in intoxicated adults, these studies do not address whether the effects would replicate in users not intoxicated during scanning, nor does it address whether the effects would generalize to adolescents. Nonetheless, these studies provide evidence that cannabis use is associated with differences in affective processing.

In this relatively large fMRI study (N=140), we investigated the impact of previous cannabis use (n=70) compared to closely matched controls (n=70) in early adolescence using a face processing task during fMRI. To date, there has been no previous research directly studying history of cannabis use with face processing, especially not from a developmental affective neuroscience perspective.

**Methods**

**Participants**

We identified a sample of cannabis-experimenting adolescents (n=70) and matched controls (n=70) from the IMAGEN dataset, a large multi-site longitudinal study of adolescent development (Schumann et al., 2010). The European School Survey Project on Alcohol and Other Drugs (ESPAD) item for lifetime history of cannabis use was used
to identify the cannabis-experimenting group. Subjects provided a self-report based on a scale from 0 to 6, (1=1–2x; 3= 6–9x; 6=40+; see Table 2.1 for complete distribution, and Table 2.2 for substance use age of onset distributions). Subjects who endorsed using other illicit substances were excluded, and any subject exhibiting signs of intoxication were excluded from scanning.

Given the relationship between amygdalar reactivity and psychopathology, subjects completed the Development and Well-Being Assessment (DAWBA; Goodman et al., 2000) to screen for psychopathology symptomology. DAWBA clinical rating scores were obtained from trained DAWBA clinicians who generated clinical rating scores by reviewing parent, teacher, and adolescent DAWBA responses. Final scores consisted of one of three categories: no-diagnosis, unsure, and, sure diagnosis, on any DSM-IV symptom class of psychopathology. From our sample, five of the controls and nine of the cannabis-experimenting group did not complete the DAWBA. Nonetheless, subjects were matched to the best of our ability on the DAWBA as indicated via chi-square analyses.

Controls were identified and matched on sex, handedness, age, verbal comprehension and perceptual reasoning IQ, pubertal development, socioeconomic status, and site. As cannabis use is highly correlated with alcohol and cigarette use (Hall and Pacula, 2003), which often makes it difficult to attribute group differences to the cannabis use per se, controls were also matched on lifetime alcohol and cigarette use. Chi-square tests were performed on the DAWBA, sex, and handedness; t-tests were
performed on the remaining continuous measures (see Table 2.3 for subject information and \( p \)-values).

**Task**

Participants passively viewed a collection of video clips that contained either a person’s face or a control picture (concentric circles). The task was designed and originally implemented by Grosbras and Paus (2006) and required participants to passively view a series of short (2–5s) black-and-white video clips showing a face that started from a neutral expression and progressively turned angry, or, progressively turned to a second neutral expression. The control pictures contained expanding and contracting concentric circles of various contrasts, roughly matching the contrast and motion characteristics of the faces. These control images were designed and originally implemented by Beauchamp and colleagues (2003) and were included to account for neural activity associated with viewing non-biological motion. All stimuli were presented as 18 s blocks, with 4–7 video clips per block during a face block. Each run was comprised of 5 blocks of neutral faces and 5 blocks of angry faces.

**Imaging parameters**

All MRI data were acquired using 3T MRI scanners made by several manufacturers (Siemens, Philips, General Electric, Bruker) in the eight IMAGEN assessment sites (London, Nottingham, Dublin, Mannheim, Dresden, Berlin, Hamburg, and Paris). Important scanning parameters were identical across sites (i.e., field of view,
flip angle and matrix; see Schumann et al., 2010) and followed an extensive program of cross-site standardization. Although our groups were matched on site, each participant’s site was modeled as a nuisance covariate in the statistical analyses. In the present task, 160 volumes per subject were obtained, each comprising 40 slices. The slices were aligned to the connecting line between the anterior and posterior commissure (2.4 mm thickness, 1 mm gap, TR=2.20s, TE=30ms).

*Imaging analysis*

The pre-processing of the EPI data was done within SPM8 (Statistical Parametric Mapping, http://www.fil.ion.ucl.ac.uk/spm/). Time series data were first corrected for slice-timing, then corrected for movement (spatial realignment), non-linearly warped into MNI space (using a custom EPI template), and Gaussian-smoothed at 5mm-FWHM. Activation maps were computed with SPM8, and regressed using a general linear model (GLM) with AR noise model (SPM default) against a design-matrix modeling each event of the stimulus presentation. Contrast images were obtained for the main effect of angry faces and neutral faces, as well as the differential activation for angry vs. neutral faces.

*Preliminary analysis*

A preliminary voxel-wise analysis directly comparing the cannabis-experimenting group to the control group was conducted using the AFNI toolbox (Cox, 1996). We subjected the data to a between-group t-test on the contrast image of angry minus neutral face processing. We detected greater differential activation to angry faces in the
cannabis-experimenting group in small clusters spanning potentially interesting cortical and subcortical areas (dorsolateral prefrontal cortex (dlPFC), temporal parietal junction, fusiform, and right extended amygdala into the striatum). However, at a whole-brain uncorrected p < .005, the clusters were small and consequently prompted a functionally defined region-of-interest analysis.

**Voxel-wise analysis**

The central goal of the voxel-wise analysis was to find unbiased clusters of brain activation that discriminated between angry and neutral faces. All cannabis-experimenting and control subjects were combined and treated as one group in a t-test vs. zero using the angry vs. neutral contrast. Scanning site was used as a nuisance covariate to account for the variance associated with multisite data collection.

**ROI selection**

ROIs were defined based on the results from the above voxel-wise analysis. The alpha-level for cluster detection was determined by running Monte Carlo simulations using AFNI’s 3dClustSim. The smoothness of the data was estimated using 3dFWHMx (details at http://afni.nimh.nih.gov/pub/dist/doc/program help/). Based on a voxel-wise uncorrected alpha of p=.005, a minimum cluster extent was determined to be 112 contiguous voxels, so as to arrive at a corrected ROI-level alpha of p = .01. From these criteria, we identified seven regions that were significantly more active for angry faces relative to neutral faces.
Based on prior knowledge of the importance of the amygdala in affective face processing, left and right anatomically defined amygdala ROIs were also included in the analysis. Amygdala ROIs were obtained using the Eickhoff–Zilles macro label atlas in MNI space distributed within AFNI (Eickhoff et al., 2005). The voxels in the amygdala ROIs were then resampled to match the grid dimensions of the functional data.

**ROI Analysis**

The seven functionally defined clusters, plus the left and right amygdala ROIs, were used to extract the mean BOLD signal from the angry face and neutral face contrasts for all subjects. The mean signal for each ROI were then subjected to a 2-by-2 (group × face type) analyses of variance using SPSS v. 22 (IBM Corp. Armonk, NY). All p-values reported were corrected for multiple comparisons using a modified Bonferroni procedure (Keppel and Wickens, 2004). For display purposes, the mean signal for face type by group was plotted using MATLAB v. R2014a (The MathWorks, Inc., Natick, MA). Lastly, we tested for any correlation between the mean signal per face type within all the ROIs with the level of cannabis use, and age of onset of cannabis, alcohol, and cigarette use.

**Results**

**Subjects**

As shown in Table 2.3, the two groups did not differ in sex, handedness, age, verbal or perceptual IQ, pubertal development, socioeconomic status, total (any) DSM-IV
diagnoses, lifetime alcohol or cigarette use. Further, the cannabis-experimenting group’s mean verbal and perceptual IQ did not significantly differ from the means of the entire IMAGEN sample (N = 1849) at p < .05.

Voxel-wise analysis results

Seven clusters were identified centered on the right and left middle temporal gyrus, right and left inferior frontal gyrus, bilateral anterior cingulate, left cerebellum, and right lingual gyrus (see Table 2.4).

ROI ANOVA Results

As expected given how they were identified, all seven functionally defined ROIs, plus the amygdalae, exhibited a significant main effect of face type \( (F_{9,130}=30.03, p<.001) \). None showed a main effect of group but, instead, five of the nine had significant interactions between face type and group. These five were the left amygdala \( (F_{1,138}=8.54, p<.001) \); right amygdala \( (F_{1,138}=8.54, p=.004) \); right middle temporal gyrus with extent into temporal parietal junction \( (F_{1,138}=5.28, p=.006) \); left inferior frontal gyrus with extent into dlPFC \( (F_{1,138}=4.87, p=.008) \); and right inferior frontal frontal gyrus with extent into dlPFC \( (F_{1,138}=5.71, p=.006) \) (see Figs. 2.1–3 and Table 2.4).

Post hoc tests revealed that within the cannabis-experimenting group, there were significant differences in the bilateral amygdalae with greater activation for the angry faces (right amygdala \( t_{69}=4.02, p< .001 \); left amygdala \( t_{69}=3.15, p=.002 \)) but no effect of face type on activity in the cortical ROIs. Controls showed a different pattern; there were
significant face type differences in all the cortical regions with greater activation for neutral faces, but no effect of face type on the BOLD signal in the amygdalae (right middle temporal gyrus $t_{69}=-7.20, \ p<.001$; left inferior frontal gyrus $t_{69}=-5.13, \ p<.001$; right inferior frontal gyrus $t_{69}=-5.68, \ p<.001$; see Table 2.5 for all post-hoc $t$-test results).

ROI Correlations with Other Drugs

To examine dosage–response effects, we investigated Pearson’s correlation on frequency of cannabis use with the mean signal per face type within each region. Dosage effects within bilateral amygdalae and dlPFC were non-significant at $p<.05$. Interestingly, we detected a significant correlation within the right TPJ cluster with frequency of cannabis use. Both the mean signal related to angry faces ($r=-.25, \ p<.05$), and neutral faces ($r=-.26, \ p<.05$), was correlated with frequency of cannabis use, such that, more frequent cannabis use is associated with less processing by the right TPJ during presentation of both face types.

We also investigated Pearson’s correlation on age of onset of cannabis, alcohol, and cigarette use with the mean signal per face type within each ROI. However, we failed to detect any significant correlations at $p<.05$ between age of onset for any drugs of abuse with any of the ROIs.

Psychopathology Symptomology

The DAWBA clinical rating scores revealed 14 cannabis- experimenters and 10 control subjects ($X^2_{1,122}=1.19, \ p>.05$) were identified as having a “sure” DSM-IV
symptom class diagnoses. Chi-square analyses revealed the only symptom class that significantly differed between the two groups was conduct disorder: $\chi^2_{1,122}=5.55, p<.05$. This finding is consistent with previous studies reporting an association between conduct disorder and cannabis use initiation during adolescence (Castellanos-Ryan and Conrod, 2011; Hopfer et al., 2013).

Influence of Psychopathology

To examine if the conduct disorder finding was related to our results, we first excluded the five subjects with a conduct disorder diagnosis and re-ran the ANOVA and post hoc t-tests. Both the ANOVA and post hoc t-tests results remained the same as the initial analysis with all subjects included. We then tested to see if conduct disorder in the cannabis-experimenting group was correlated with the BOLD signal in any of the ROIs, but failed to detect any significant correlation at $p < .05$.

Lastly, to test if any psychopathology influenced the dataset, we excluded all 14 cannabis-experimenting and 10 control subjects with a strong probability of a DSM-IV category diagnosis from the ANOVA and post hoc t-tests, and reran the analyses. When correcting for multiple comparisons, the left and right amygdala and right TPJ maintained significance on the ANOVA face type $\times$ group interaction. Nonetheless, the same five regions that initially survived correction for multiple comparisons for the full sample analysis still passed significance at an uncorrected $p$-value of $<.05$. Additionally, the post hoc t-test results remained the same. Consequently, with minor exceptions regarding correction for multiple comparisons, results remained largely the same even when
analyzed on sub-groupings devoid of any mental health symptomology. Hence, these findings suggest that mental health symptomology was not contributing to the full sample group differences.

**Discussion**

In this study, we examined the functional neurobiology of angry and neutral face processing in a group of cannabis-experimenting adolescents vs. matched controls using fMRI. We found group-by-face type interaction effects in bilateral amygdala and three clusters of activation that span the right TPJ and bilateral dIPFC. Decomposing these results by face type, we found the cannabis-experimenting group exhibited increased activity to angry faces in the amygdala. Conversely, the control group exhibited increased activity to neutral faces in the cortical regions. Therefore, cannabis use during early adolescence is associated with hypersensitivity to negative affect in the amygdala. While we stress that this study does not permit us to conclude cannabis-experimentation caused the observed functional neurobiological differences, we are confident these differences are associated with the cannabis use status of the participants due to our relatively large sample size (N = 140), carefully matched control group (who did not differ on sex, pubertal development, IQ, site, psychopathology, or alcohol and cigarette use), and a conservative criteria to meet statistical significance.

With regard to the cortical findings, the right TPJ and bilateral dIPFC showed greater activation to neutral faces than angry faces in the control group. The right TPJ has been implicated in theory of mind, social processing, and face processing (Allison et al.,
Furthermore, the right superior temporal gyrus encodes biologically relevant motion (Grossman et al., 2000; Puce and Perrett, 2003; Saygin, 2007). Therefore this cluster may represent a signal of social salience related to the moving face stimuli. In contrast to controls, post hoc t-test results show the cannabis-experimenting group fails to process angry faces differently from neutral faces within the right TPJ (see Fig. 2.2). As this region was also the only region to exhibit significant dosage effects, a higher degree of cannabis experimentation may contribute to a departure from healthy social processing. Interestingly, as none of the regions exhibited a significant correlation with age of onset for any drugs of abuse, we are unable to make claims regarding face processing and cannabis use in relation to age of onset with other drugs.

Considering that the cortical clusters spanned the temporal, parietal, and bilateral frontal lobes, we suggest that the neutral faces demanded more cognitive resources. The neutral faces had greater ambiguity and variability in their content, such as nose twitching, mouth movements, and eye-blinks. Furthermore, all stimuli video clips started from neutral and transitioned to angry or neutral faces. The stimuli that transitioned to angry faces were more explicit during the shift to threat, whereas the transition to another neutral face may have required more cognitive strategies to decode. Hence, the neutral faces may have demanded a greater degree of attention and interpretation by these cognitive systems.

With regard to the amygdala findings, it is unclear whether amygdala hypersensitivity preceded cannabis use or was a consequence of use since this was a
cross-sectional study. If amygdala hypersensitivity preceded use, which might seem most plausible given the low levels of reported use, then it’s possible that these individuals may have been inclined to self-medicate for the drug’s acute anxiolytic effects (Phan et al., 2008). Consistent with this interpretation, recent evidence has identified altered angry face processing in the ventromedial PFC (vmPFC) to predict future binge drinking (Whelan et al., 2014) and the vmPFC is part of a brain circuit that attenuates amygdala activity (Banks et al., 2007; Urry et al., 2006). If, however, the amygdala hypersensitivity is a consequence of cannabis use, then it is likely that this is due to exogenous stimulation of the endocannabinoid system. If confirmed, these findings would raise concerns regarding the risks associated with cannabis consumption and emotional health in adolescent users. Animal studies suggest exogenous cannabinoids inhibit GABAergic neurotransmission in the amygdala (Katona et al., 2001). Interestingly, this effect is magnified when the animal is given THC and placed in a threatening environment (Patel et al., 2004). Together these findings suggest that cannabinoids may compromise the major neuronal inhibitory mechanism within the amygdala and lower the threshold for activation, especially during signals of threat. Consistent with this interpretation, the angry faces used in the task were not exceptionally potent signals of threat yet the cannabis-experimenting group showed a heightened reactivity to them, an effect that is not observed in healthy controls viewing the same stimuli (Grosbras and Paus, 2006).

The amygdala’s role in affective processing serves an important role in evolutionary biology as it directs attention towards aversive stimuli. However, mounting evidence suggests that overrecruitment of the amygdala is associated with various mood
disorders. Greater signal change in the amygdala, specifically during affective face processing, is exhibited by children with anxiety (Thomas et al., 2001), and adults with major depressive disorder (Drevets, 2001; Fu et al., 2008; Sheline et al., 2001) and generalized social phobia (Evans et al., 2008; Phan et al., 2006). Thus, cannabis use in adolescence may contribute to the etiology of mood disorders in adulthood. Moreover, relatively light use by an early age may contribute to an early marker of maladaptive affective processing. Nonetheless, major longitudinal studies are needed to illuminate these hypotheses as the current study is unable to infer causality.

The results reported here are inconsistent with those of Phan and colleagues (2008) and Gruber and colleagues (2009) who both found attenuated amygdala reactivity to threat signals in adults following acute THC administration, and chronic non-intoxicated users, respectively. In contrast, we report trait-related increased amygdala reactivity to threat signals in adolescence. Hence, we report divergent effects in adolescents compared to adults. As previous research demonstrates divergent findings between adolescents and adults during affective face processing (Guyer et al., 2008a,b), we do not hypothesize adolescent data to mirror the adult data. Indeed, our results support the notion that adolescence is period of sensitive affective development that can be perturbed even with very low levels of cannabis experimentation.

The current results are consistent with the animal models of cannabinoid exposure during adolescence (Rubino et al., 2008; Schramm-Sapyta et al., 2007) and suggest that more human research is needed on the long-term effects of cannabis use in adolescence. In consideration of the animal studies and the link between early cannabis use and mood
disorders later in life, acute THC consumption effects in the adolescent brain may be different or, indeed, the long-lasting effects of repeated exposure may be different beyond the acute intoxication phase. As adolescents tend to be more reactive to emotional stimuli, especially face processing in the amygdala, the observed differences in adolescent cannabis users may suggest evidence of maladaptive cognitive and affective systems related to psychosocial development.

Lastly, a notable feature of the present results is that our sample of cannabis users reported relatively low levels of use, but nonetheless exhibited significant differences in processing threat signals. Furthermore, due to the closely matched control group, we excluded a range of possible confounding factors, including mental health comorbidities, which may have accounted for the observed differences. As excluding subjects with mental health comorbidities failed to change the pattern of our results, the findings suggests that very low use of cannabis during early adolescence may compromise healthy emotional reactivity.

An alternative explanation regarding the observed differences in affective face processing may be attributed to unmeasured pre-existing differences in emotional functioning, which might have contributed to the adolescents’ experimentation with cannabis. Indeed, we have previously shown that activation in response to these angry faces in the left PFC predicted binge drinking two years later, which would suggest altered emotional reactivity may precede use (Whelan et al., 2014). However, in the present analyses the measured psychiatric symptomology results failed to show elevated
levels of any of the affective disorders, therefore, it is unclear which preexisting differences, if any, might have been present in the cannabis-experimenting group.

Future studies will be performed on the follow-up (age 16 and 18) data of this project to identify predictive factors contributing to the cannabis use phenotype profile. As this was a cross-sectional study from the baseline IMAGEN dataset, we stress that we are unable to claim cannabis use caused amygdala hypersensitivity to negative affect. To investigate this question, longitudinal data analysis will inform whether hypersensitivity to threat signals precedes use or is a consequence of use, and assessments of psychopathology will clarify if early cannabis use and differences in face processing contribute to the generation of clinically relevant disorders.
References


subjects resolves with antidepressant treatment: an fMRI study. *Biological psychiatry, 50*(9), 651-658.


Tables

Table 2.1: Frequency of cannabis use

<table>
<thead>
<tr>
<th>Frequency</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2 times</td>
<td>49</td>
</tr>
<tr>
<td>3-5 times</td>
<td>7</td>
</tr>
<tr>
<td>6-9 times</td>
<td>7</td>
</tr>
<tr>
<td>10-19 times</td>
<td>2</td>
</tr>
<tr>
<td>20-39 times</td>
<td>3</td>
</tr>
<tr>
<td>40+ times</td>
<td>2</td>
</tr>
<tr>
<td>Any use in month prior to scan</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 2.2: Age of first use by substance

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cannabis (n=70)</td>
<td>Controls (n=70)</td>
</tr>
<tr>
<td>Age of first cigarette (M, SD)</td>
<td>12.73, 1.07</td>
<td>12.64, 1.00</td>
</tr>
<tr>
<td>Age of first wine (M, SD)</td>
<td>12.20, 1.25</td>
<td>12.11, 1.17</td>
</tr>
<tr>
<td>Age of first beer (M, SD)</td>
<td>12.46, 0.98</td>
<td>12.44, 1.04</td>
</tr>
<tr>
<td>Age of first wine cooler (M, SD)</td>
<td>12.90, 1.25</td>
<td>12.97, 0.98</td>
</tr>
<tr>
<td>Age of first spirit (M, SD)</td>
<td>13.25, 0.87</td>
<td>13.21, 0.84</td>
</tr>
<tr>
<td>Age of first cannabis use (M, SD)</td>
<td>13.57, 0.94</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2.3: Demographic information and statistics by group

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cannabis (n=70)</td>
<td>Controls (n=70)</td>
</tr>
<tr>
<td>Males/Females (n)</td>
<td>50/20</td>
<td>41/29</td>
</tr>
<tr>
<td>Left / Right Handedness (n)</td>
<td>6/64</td>
<td>5/65</td>
</tr>
<tr>
<td>Age (M,SD)</td>
<td>14.765, 0.40</td>
<td>14.61, 0.655</td>
</tr>
<tr>
<td>Perceptual Reasoning IQ (M,SD)</td>
<td>104.219, 16.876</td>
<td>105.72, 13.879</td>
</tr>
<tr>
<td>Verbal Comprehension IQ (M,SD)</td>
<td>110.74, 16.84</td>
<td>110.43, 13.329</td>
</tr>
<tr>
<td>Puberty Development Scale (M,SD)</td>
<td>3.60, 0.60</td>
<td>3.8, 0.63</td>
</tr>
<tr>
<td>Socioeconomic Status (M,SD)</td>
<td>18.45, 4.42</td>
<td>18.24, 4.70</td>
</tr>
<tr>
<td>Lifetime Alcohol Use (M,SD)</td>
<td>3.71, 1.63</td>
<td>3.56, 1.32</td>
</tr>
<tr>
<td>Lifetime Cigarette Use (M,SD)</td>
<td>3.106, 2.215</td>
<td>2.54, 2.215</td>
</tr>
<tr>
<td>Lifetime Cannabis Use (M,SD)*</td>
<td>1.70, 1.30</td>
<td>0, 0</td>
</tr>
</tbody>
</table>

* Based on a self-report scale from 0-6. (1=1-2 times; 2=3-5 times; 3=6-9 times; 4=10-19 times; 5=20-39 times; 6=40+ times)
Table 2.4: Anatomically and functionally defined ROIs with group by condition interaction statistics.

<table>
<thead>
<tr>
<th>Peak Voxel Location</th>
<th>Center of Mass Coordinate (MNI)</th>
<th>Cluster size</th>
<th>Group x Condition Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x, y, z</td>
<td>k</td>
<td>F</td>
</tr>
<tr>
<td>Left amygdala</td>
<td>120</td>
<td>8.54</td>
<td>.000</td>
</tr>
<tr>
<td>Right amygdala</td>
<td>139</td>
<td>5.56</td>
<td>.004</td>
</tr>
<tr>
<td>Right middle temporal gyrus, cluster extends into temporal parietal junction (TPJ)</td>
<td>-54, 47, 6</td>
<td>1333</td>
<td>5.28</td>
</tr>
<tr>
<td>Left inferior frontal gyrus, cluster extends into dorsolateral prefrontal cortex (dIPFC)</td>
<td>54, -14, 28</td>
<td>417</td>
<td>4.87</td>
</tr>
<tr>
<td>Right inferior frontal gyrus, cluster extends into dorsolateral prefrontal cortex (dIPFC)</td>
<td>-49, -14, 33</td>
<td>356</td>
<td>5.71</td>
</tr>
<tr>
<td>Left middle temporal gyrus, cluster extends into temporal-parietal junction (TPJ)</td>
<td>53, 51, 9</td>
<td>1181</td>
<td>2.19</td>
</tr>
<tr>
<td>Left cerebellum</td>
<td>12, 78, -39</td>
<td>477</td>
<td>2.36</td>
</tr>
<tr>
<td>Right lingual gyrus</td>
<td>-13, 79, -8</td>
<td>317</td>
<td>1.53</td>
</tr>
<tr>
<td>Bilateral anterior cingulate, cluster extends into ventromedial prefrontal cortex</td>
<td>0, -45, 7</td>
<td>830</td>
<td>3.72</td>
</tr>
</tbody>
</table>

Rows in bold survived a modified Bonferroni correction for multiple comparisons.
Table 2.5: Post-hoc t-test comparison for within-group differences.

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Angry faces vs. Neutral faces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cannabis</td>
</tr>
<tr>
<td>Left amygdala</td>
<td>$t(69) = 4.02, p &lt; .001$</td>
</tr>
<tr>
<td>Right amygdala</td>
<td>$t(69) = 3.15, p = .002$</td>
</tr>
<tr>
<td>Right middle temporal gyrus, cluster extends into temporal parietal junction (TPJ)</td>
<td>$t(69) = -1.21, p = .231$</td>
</tr>
<tr>
<td>Left inferior frontal gyrus, cluster extends into dorsolateral prefrontal cortex (dlPFC)</td>
<td>$t(69) = -0.60, p = .551$</td>
</tr>
<tr>
<td>Right inferior frontal gyrus, cluster extends into dorsolateral prefrontal cortex (dlPFC)</td>
<td>$t(69) = -0.56, p = .576$</td>
</tr>
</tbody>
</table>

Cells in bold are significant at $p < .05$, corrected.

Figures

Figure 2.1: Mean activation for face type by group plotted for right and left amygdala.

Asterisks indicate post-hoc $t$-test differences significant at $p < .05$, corrected. Error bars represent the standard error of the mean.
Figure 2.2: Mean activation for face type by group plotted for the cluster spanning the right temporal parietal junction.

Blue bars represent angry faces, red bars represent neutral faces. Asterisks indicate post-hoc t-test differences significant at $p<.05$, corrected. Error bars represent the standard error of the mean.

Figure 2.3: Mean activation for face type by group plotted for the cluster spanning the right and left dorsolateral prefrontal cortex.

Blue bars represent angry faces, red bars represent neutral faces. Asterisks indicate post-hoc t-test differences significant at $p<.05$, corrected. Error bars represent the standard error of the mean. Cutout: $y = 4, z = 48$. 
CHAPTER 3: AMYGDALA REACTIVITY BEFORE AND AFTER CANNABIS USE IN ADOLESCENCE

3.1. Before: Amygdala Reactivity Predicts Cannabis Use By Age 19

Introduction

Cross-sectional analyses from Chapter 2 (Spechler et al., 2015) identified significant group by face-type interactions across several cortical and subcortical regions when comparing a group of adolescents reporting cannabis use by age 14 relative to a group of matched controls. Of particular interest, heightened bilateral amygdala reactivity to angry faces were observed in individuals who reported any lifetime cannabis use at baseline. This initial investigation characterizing the functional neurobiology of adolescent cannabis use was unable to determine if the amygdala effect was predictive, or a consequence, of cannabis use. Fortunately, the longitudinal nature of the IMAGEN study can be interrogated to approach this question by analyzing the face processing data in a sample of teens who were cannabis naïve at baseline and then report use later in adolescence.

As reported in Chapter 2, there was no correlation between either the left or right amygdala activations with cannabis use age of onset. Additionally, no correlation was found using the baseline level of cannabis use. It is therefore possible that heightened amygdala reactivity was a pre-existing difference in cannabis users, rather than a consequence arising from use. Given this lack of association with age of use onset and use levels, the hypothesis guiding the subsequent analyses is that heightened amygdala reactivity to angry faces at age 14 will predict the level of cannabis use by age 19.
Previous studies indicated that heightened amygdala activations, specifically to emotionally evocative faces, may be characteristic of emotional dysregulation in adolescence (van den Bulk et al., 2014; Monk et al., 2008; Thomas et al., 2013). And while chronic cannabis use (Gruber et al., 2009) and acute administration of THC (Phan et al., 2008) have been implicated in the attenuation of amygdala activations to evocative faces, there is mounting evidence that cannabis use may negatively impact adolescent development (as discussed in Chapter 1). Adolescents with pre-existing heightened amygdala reactivity may be vulnerable to initiate and maintain cannabis use as a means to down-regulate their amygdala activity and achieve anxiolytic effects. In doing so, these adolescents also risk experiencing some consequences of their use. Further research is therefore required to confirm dysregulated amygdala processing as a risk factor for cannabis use. If substantiated, these findings may implicate amygdala reactivity as a prognostic biomarker in the identification of vulnerable adolescents. These findings would also inform safe treatment methods by directly targeting this biomarker via biofeedback mechanisms (Zotev et al., 2011), pharmaceuticals (Arce et al., 2008; Paulus et al., 2005), or indirectly via cognitive behavioral therapy (McClure et al., 2007).

For the analyses in the current section, bilateral amygdala reactivity to angry faces measured at baseline (age 14) served as the independent variables in a linear regression model predicting the level of cannabis use by the age 19 assessment of the IMAGEN study. Critically, all participants were selected for being cannabis-naïve at baseline. Other variables potentially influencing amygdala reactivity and/or related to future cannabis use were modeled as nuisance covariates, including baseline cigarette, alcohol, and anxiety
levels. Therefore, any significant effects related to the amygdala were identified over and above the influence of these measures.

**Methods**

*Participants*

Participants from the IMAGEN study were selected based on their reported drug use levels at all time points from the ESPAD survey (Hibell et al., 1997). Starting with the full baseline sample (N=2,224), there were n=2,045 individuals who reported no lifetime cannabis use by age 14. From these n=2,045, there were 571 who reported cannabis use by age 19. Only those with reliable reporting patterns (age 16 level <= age 19 level) were selected. No exclusions were made for other drug use levels at any time point, although tests for drug specificity were completed (detailed later).

From those 571, there were 40 who did not supply a face processing scan, and an additional 6 excluded for excessive head motion. Therefore, 525 participants were included in the subsequent analyses (see Table 3.1.1 for sample characteristics). Across these 525, their lifetime cannabis use by age 19 data was plotted. These levels followed a U-shape distribution with relatively high levels at the opposite tails, with lower intermediate use levels (See Figure 3.1.1 and Table 3.1.2).

There were no controls in the primary linear regression analyses (described below) in order to test if amygdala reactivity would exhibit a dose-response relationship in predicting the level of future use in an analysis restricted to users. As a secondary analysis, a control sample was identified to determine if the future cannabis users differed
as a whole from a sample of participants who were cannabis-naïve at all time points. Hence, from the full sample of participants with ESPAD data at baseline (N=2,224), there were n=648 identified as being cannabis-naïve at all time points. From those 648, there were 46 who did not supply a face processing scan, and an extra 8 who were excluded due to excessive head motion. Therefore, 594 participants were included in the cannabis-naïve sample.

**fMRI Data**

The face processing fMRI data were analyzed using standard preprocessing methods as described in Chapter 2. During image realignment, head motion estimates were obtained for each of the three translation and rotation directions. From these motion estimates, framewise displacement (Power et al., 2012) was calculated. The mean framewise displacement across the entire run (mean FD) constituted a single summary statistic for head motion and was used as a nuisance covariate in the regression analyses (described below). Participants exceeding a mean FD >0.5mm were excluded for quality assurance purposes.

Whole-brain task activation maps were estimated using the GLM, and contrast images were obtained for angry faces vs. control images, and neutral faces vs. control images. In keeping with the methods of Chapter 2, bilateral amygdala regions of interests (ROIs) using the “Eickhoff–Zilles” macro label atlas (Eickhoff et al., 2005) were used to extract the mean values from each whole-brain contrast image.
Nuisance Covariates

A set of nuisance covariates was included to adjust for potentially confounding factors influencing both amygdala reactivity and cannabis use levels. These covariates included age at baseline scan, pubertal development scale (Carskadon and Acebo, 1993), sex, handedness, performance and verbal IQ, mean FD, and site of scanning acquisition. Lifetime cigarette and alcohol use from the baseline ESPAD survey were also modeled as covariates. Finally, given the relationship between amygdala activation and anxiety, the baseline DAWBA band score (Goodman et al., 2000) for generalized anxiety disorder (DSM-IV) was included as the final covariate. The band score is an ordinal measure estimated by a computer algorithm using the pattern of responses from the DAWBA instrument and reflects the probability of receiving a DSM-IV diagnosis. See Table 3.1.3 for the probability bands for each level of this measure for each group. Bivariate correlations between anxiety and the amygdala activation, and, anxiety and the outcome measure were also reported to address the self-medication hypothesis.

Regression Models

Linear regression analyses were constructed to predict cannabis use by age 19 from a set of measures collected at age 14 including left and right amygdala reactivity to angry faces. The regression models were also estimated in a stepwise fashion in three blocks, starting with all the nuisance covariates, followed by the left amygdala, and finishing with the right amygdala. This order was implemented to obtain an adjusted $R^2$ signifying the percent variance explained following the inclusion of each amygdala as a
predictor after first modeling the nuisance covariates. Similar regression models were estimated using bilateral amygdala activations to neutral faces. Although angry faces was the primary contrast of interest given the finding from Chapter 2 indicating angry faces elicits the most robust activations. All statistical analyses were conducted using SPSS v.24 (Armonk, NY: IBM Corp).

Results

Primary Regression Analyses (Future Cannabis Users Only)

525 participants were included in a multiple linear regression model predicting their level of cannabis use by age 19 with a set of independent variables measured at baseline. Results indicated that the full model containing all variables at age 14 significantly predicted cannabis use by age 19 ($F_{19,504} = 3.48$, $p < .001$), explaining roughly 8% of the variance in future cannabis use. Within this full model adjusted for all nuisance covariates, the right amygdala reactivity to angry faces significantly predicted the level of cannabis use by age 19 (Right amygdala: $\hat{\beta} = 1.11$, $p < .05$). The positive beta value indicates that an increase in right amygdalar activation to angry faces at age 14 predicts an increase in the level of future cannabis use. Moreover, sex ($\hat{\beta} = -.50$, $p < .05$) and baseline cigarette use ($\hat{\beta} = .23$, $p < .05$) also emerged as significant predictors within the full model. When estimating a stepwise regression model, it was found that after including all other predictors, the inclusion of the right amygdala activation to angry faces explained an extra 1% of the variance ($\Delta R^2 = .01$, $p < .05$) in future cannabis use.
The left amygdala activation to angry faces was not a significant predictor when adding it into the stepwise regression either before or after the inclusion of the right amygdala, nor was it a significant predictor when considering a simple regression model excluding all other predictors. Moreover, when executing identical analytic procedures using the bilateral amygdala activations to neutral faces, no significant effects were observed. Therefore, when considering angry and neutral face processing in the left and right amygdala, only the activation to angry faces in the right amygdala emerged as a significant predictor of cannabis use later in adolescence.

*Associations With Anxiety Levels*

Interestingly, baseline anxiety did not emerge as a significant predictor within the multiple regression model. Baseline anxiety was included as a covariate because anxiety has been previously reported to predict both cannabis use (Agosti et al., 2002; Buckner et al., 2008), and heightened amygdala activations (Monk et al., 2008). To address these potential relationships in the current dataset, Pearson’s bivariate correlations between baseline anxiety, follow-up cannabis use, and the baseline amygdala activations were estimated. Results indicated that, again, no relationship was found between baseline anxiety and future cannabis use, or, baseline anxiety and any of the amygdala activations (left or right; angry or neutral) (all \( p > .05 \)). Spearman’s rho correlation was also estimated given the ordinal nature of the measure, but null results were reproduced. Hence, baseline anxiety had no influence on future cannabis use when considering the
two measures in isolation, or in the presence of the other predictors. See the following Section 3.2 for analyses testing for changes in anxiety levels from baseline to follow up.

**Correlated Measures**

Concerns with multicolinearity among the predictor variables can be addressed by estimating the variance inflation factor (VIF), which signifies the extent to which a regression coefficient’s standard error is inflated given the correlation with the other independent variables. Generally, VIFs greater than or equal to 5 indicate serious multicolinearity and compromises the interpretation of the results. Considering the significant predictors, the VIF for sex (VIF=1.5), baseline cigarettes (VIF=1.3), and the right (VIF=1.9) amygdala activation to angry faces, were well below values of concern. And while the left amygdala was not a significant predictor, its VIF was also low (VIF=1.9).

Sex was identified as a significant predictor of future cannabis use within the multiple regression model. As between-group t-tests indicated that males exhibit higher activations to angry faces than females ($t=3.5, p<.05$), and also use more cannabis ($t=2.4, p<.05$), it could be argued that the amygdala effect is a proxy for sex. This argument is countered by the low VIF on sex and the right amygdala coefficients. Those results indicated stable coefficient estimates with low colinearity with each other despite their modest correlation. Lastly, the low VIF for the left amygdala indicated that it is unlikely that its failure to predict cannabis use was due to colinearity with the right amygdala.
Secondary Regression Analyses (Inclusion of Cannabis-Naïve Participants)

As a final analysis to determine that the reported effects discriminates all future users from controls, the linear regressions were repeated with the inclusion of the cannabis-naïve sample. Here, the results were consistent with the primary analysis, and also indicated a substantial improvement in the overall $R^2$. Results indicated the full model containing all variables at age 14 significantly predicted cannabis use by age 19 ($F_{19,1097} = 4.34$, $p<.001$), explaining roughly 15% of the variance in future cannabis use. Consistent with the cannabis-only regression model, the right (but not left) amygdala activation to angry faces significantly predicted the level of cannabis use by age 19 (Right amygdala: $\beta=.74$, $p<.05$). Lastly, when estimating a stepwise regression model, the inclusion of the right amygdala activation to angry faces explained an extra .3% of the variance ($\Delta R^2=.003$, $p<.05$) in future cannabis use. The lower explained variance in this secondary model is likely due to the addition of noise. See Figure 3.1.2 for the positive linear relationship between the baseline right amygdala reactivity adjusted for all other covariates in the model plotted against future cannabis use level. A dose-response relationship was observed such that the lowest reactivity is exhibited by the controls, and higher reactivity exhibited with increasing cannabis use levels at follow up (see Figure 3.1.2). And in line with the primary regression results, no significant effects were observed using the bilateral amygdala activations to neutral faces.

Drug Specificity
As the sample of future cannabis users were not excluded on the basis of their other drug use levels at age 19 (follow up), a test of drug specificity is warranted. When testing the follow up data, it was found that cannabis use level significantly correlated with alcohol ($r=.20$, $p<.001$) and cigarette use ($r=.40$, $p<.001$) levels. Therefore, similar linear regression analyses predicting the level of alcohol and cigarette use at follow up were necessary to determine if baseline amygdala reactivity is a risk factor that generalizes to predict other drug use.

To start, a linear regression model predicting follow up alcohol use was estimated using the same baseline covariates and right and left amygdala reactivity to angry faces. The full model significantly predicted follow up alcohol use ($F_{19,504}=2.83$, $p<.001$), with verbal IQ ($\beta=.01$, $p<.05$) and baseline alcohol ($\beta=.08$, $p<.05$) identified as significant predictors of follow up use. Rerunning the model without baseline alcohol was still significant ($F_{19,504}=2.44$, $p<.001$). Neither the left nor right amygdala emerged as significant predictors in these models.

Results were consistent using a similar model to predict follow up cigarette use ($F_{19,504}=5.2$, $p<.001$), with baseline age ($\beta=-.65$, $p<.05$) and cigarettes ($\beta=.48$, $p<.05$) identified as significantly predicting follow up use. Rerunning without baseline cigarettes was still significant ($F_{19,504}=2.83$, $p<.001$). Likewise, neither the left nor right amygdala reactivity to angry faces emerged as significant predictors in these models. These analyses suggest the right amygdala reactivity to angry faces specifically predicted cannabis use at follow up.
Discussion

This Section 3.1 builds on the cross-sectional findings of Chapter 2 by indicating the right amygdala reactivity to angry faces likely precedes cannabis use in adolescence. This effect is supported by the dose-response relationship predicting the level of use five years later, and also by the finding that the lowest baseline amygdala activations were exhibited by the cannabis-naïve sample. This study is supported by the longitudinal design, where all participants were selected on the basis of being naïve to cannabis at the baseline scan. And while it would be incautious to assert the amygdala activations caused later cannabis use, these findings suggested that amygdala activations to angry faces might be considered a specific risk factor for cannabis use in adolescence.

The finding that the right, but not left, amygdala activation was significantly predictive is consistent with the literature. In a meta-analysis of neuroimaging studies on the amygdala, data suggests that the right amygdala is specific to rapid detection of threatening stimuli as right-sided activations are commonly reported in studies using temporally masked presentation (e.g., when a threatening stimulus presentation is brief and immediately followed, or “masked”, by an alternate stimulus) (Costafreda et al., 2007). While the task paradigm used in the IMAGEN study is by no means a masked presentation, each stimulus involves a short presentation (2-5 seconds) of faces starting from a neutral expression shifting to either a neutral or angry expression (Grosbras, 2005). It is plausible that a brain system sensitive to detecting threatening stimuli may also be engaged by this shift. This interpretation is supported by two studies that
demonstrated rapid right-sided amygdala activations to angry faces using temporally precise magnetoencephalography (Dumas et al., 2013; Hung et al., 2010).

Interestingly, sex and baseline cigarette use also emerged as significant predictors of cannabis use five years later. With regard to sex, this finding is in line with epidemiological studies of adolescent drug use indicating that males use both cigarettes and cannabis at higher rates than females (Johnston et al., 2018). For baseline cigarette use, this finding is also consistent with the literature implicating cigarette use as a robust predictor of cannabis use (Agrawal et al., 2012).

In considering the significant reactivity of the right amygdala, the positive linear relationship uncovered from the regression analysis indicated more amygdala activation predicts more cannabis use. Hence, individuals with the most exaggerated amygdala activations to signals of threat might be vulnerable to use more frequently. The results of these analyses point to a self-medication interpretation. Phan and colleagues (2008) previously demonstrated that acute administration of THC attenuates amygdala reactivity to angry faces. Therefore, the motivation to consume and maintain cannabis use in these individuals is hypothesized to be driven, in part, through negative reinforcement properties achieved by the interaction between THC and amygdala function. However, the lack of a correlation between baseline anxiety level and amygdala reactivity fails to support this framework. Other measures of life stress or psychiatric symptomatology might be useful to better explain the reasons for baseline hyperactivity in this sample.

It will also be important to determine how amygdala reactivity might change following cannabis use before discounting the self-medication hypothesis. In keeping
with this framework, amygdala reactivity is expected to decrease over time with protracted use. A similar pattern in anxiety levels is also hypothesized to follow cannabis use. See the subsequent Section 3.2 for analyses testing these hypotheses.

The implication of the current Section 3.1 is that heightened amygdala reactivity to angry faces may be considered a biomarker predictive of cannabis use in adolescence. Mitigating risk associated with adolescent use might be achieved by targeting this biomarker. Research conducted by Paulus and colleagues, and Arce and colleagues, provided converging evidence on the attenuation of amygdala reactivity via common psychiatric medications. In these studies, researchers demonstrated that acute administration of the anxiolytic lorazepam (Paulus et al., 2005) and three weeks use of the antidepressant escitalopram (Arce et al., 2008), significantly reduced amygdala reactivity to angry faces. In tandem with the Phan report on THC, cannabinoids, lorazepam, and escitalopram, may all be effective in normalizing dysregulated affect by targeting the same biomarker (despite their differing pharmacological properties). While rigorous studies are needed, it is hypothesized that lorazepam or escitalopram might have secondary effects of minimizing cannabis use in vulnerable adolescents by attenuating their amygdala reactivity, thus disrupting their risk phenotype.
Tables

Table 3.1.1: Demographic Information for Each Group

<table>
<thead>
<tr>
<th>Measure</th>
<th>Future Cannabis Use Sample (n=525)</th>
<th>Cannabis- naïve Sample (n=594)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years ((M, SD))</td>
<td>14.39, .39</td>
<td>14.44, .41</td>
<td>.06</td>
</tr>
<tr>
<td>PDS ((M,SD))</td>
<td>2.88, .57</td>
<td>2.95, .56</td>
<td>.04</td>
</tr>
<tr>
<td>Sex (M, F)</td>
<td>282, 243</td>
<td>226, 368</td>
<td>.00</td>
</tr>
<tr>
<td>Handedness (L, R)</td>
<td>46, 479</td>
<td>65, 529</td>
<td>.23</td>
</tr>
<tr>
<td>Performance IQ ((M,SD))</td>
<td>109.27, 13.33</td>
<td>110.48, 14.02</td>
<td>.14</td>
</tr>
<tr>
<td>Verbal IQ ((M,SD))</td>
<td>113.49, 13.05</td>
<td>112.23, 13.55</td>
<td>.11</td>
</tr>
</tbody>
</table>

All tabulated measures collected at the baseline assessment (age 14). Future cannabis use participants \((n=525)\) were selected for being cannabis-naïve at age 14 and then report cannabis use by age 19. Cannabis-naïve participants \((n=594)\) were selected for being cannabis–naïve at all time points. PDS: Pubertal development scale (Carskadon and Acebo, 1993). P-value from chi-square test for between-group differences.
Table 3.1.2: Cigarette and Alcohol Use Levels for Each Group

<table>
<thead>
<tr>
<th></th>
<th>Future Cannabis Use Sample (n=525)</th>
<th>Cannabis-Naïve Sample (n=594)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 1-2x 3-5x 6-9x 10-19x 20-39x 40+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline Cigarettes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n)</td>
<td>357 83 24 18 12 9 22</td>
<td>528 40 7 7 8 2 2 .01</td>
<td></td>
</tr>
<tr>
<td>Baseline Alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n)</td>
<td>91 132 113 76 68 28 17</td>
<td>199 164 104 65 39 16 7 .01</td>
<td></td>
</tr>
</tbody>
</table>

Baseline cigarette and alcohol use from the ESPAD instrument (Hibell et al., 1997) and reflects lifetime usage by age 14. P-value from chi-square test for between-group differences.

Table 3.1.3: Anxiety Band Score for Each Group

<table>
<thead>
<tr>
<th></th>
<th>Future Cannabis Use Sample (n=525)</th>
<th>Cannabis-Naïve Sample (n=594)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;.1% .5% 3% 15% 50%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety Band Score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n)</td>
<td>380 123 0 18 3</td>
<td>424 126 0 35 8</td>
<td>.12</td>
</tr>
</tbody>
</table>

Anxiety Band Score from the DAWBA instrument (Goodman et al., 2000) and represents the probability of receiving a generalized anxiety disorder diagnosis for individuals contained in that band level. P-value from chi-square test for between-group differences.
Data from the ESPAD survey (Hibell et al., 1997) represents the level of lifetime cannabis use by age 19. All N=525 participants were selected from the IMAGEN study for reporting no cannabis use at the baseline assessment (age 14).
Figure 3.1.2: Adjusted Right Amygdala Reactivity By Cannabis Use Level (N=1019)

Right amygdala reactivity at baseline adjusted for all other covariates in linear regression model containing the controls. Plotted against cannabis use level at follow up (age 19). The sample of controls was consistently cannabis-naïve at all time points (n=594). Error bars reflect +/- 2 standard deviations at each use level.
3.2. After: Amygdala Reactivity Following Cannabis Use

Introduction

Chapter 2 and the preceding Section 3.1 provided evidence that amygdala reactivity to angry faces both correlated with concurrent use and predicted later use. The remaining temporal characteristic is to determine if amygdala reactivity changed following cannabis use. The longitudinal nature of the IMAGEN study can be leveraged to study the extent to which cannabis use may influence amygdalar functional development. In this Section 3.2, analyses were conducted on amygdala reactivity at age 14 and age 19. The individuals from Chapter 2 were studied since they were hypothesized to yield the most robust changes in amygdala reactivity as they had the most chronic cannabis use in the IMAGEN sample (initiating by age 14). This sample was compared to a closely matched sample of individuals who were cannabis-naïve at all time points.

The comparison sample was also used to illuminate typical amygdalar functional development. Previous studies on amygdalar functional development provided evidence that amygdala reactivity to angry faces increases across development. For instance, in the first developmental study on face processing using fMRI, Thomas and colleagues reported that angry face processing in the left amygdala increased from childhood to adulthood (Thomas et al., 2001). These findings have since been reproduced in a larger sample by Todd and colleagues (Todd et al., 2011), although evidence for a developmental decrease has also been reported (Guyer et al., 2008). This Section 3.2 was
therefore useful in examining both typically-developing, and cannabis-using, functional development using large samples from the IMAGEN study.

In considering the relationship between cannabis and neurodevelopmental processes, research on the endogenous cannabinoid system indicated the primary cannabinoid receptor (CB1) proliferates across prefrontal, striatal, and medial temporal lobe regions from early development through adolescence (Mato et al., 2003). Researchers also consistently identified high CB1 densities in the amygdala, with specificity for basal and lateral nuclei (Glass et al., 1997; Katona et al., 2001; Mailleux and Vanderhaeghen, 1992). Given the localization of these receptors, the endogenous cannabinoid system is putatively involved in regulating emotional and stress responses (Marco and Viveros, 2009). Any perturbations of this system by exogenous cannabinoids used during adolescence may precipitate changes to the functional neurobiology and emotional well being of the individual. Therefore, patterns of both amygdala reactivity and anxiety levels from baseline to follow up were examined within the context of cannabis use in adolescence.

**Methods**

*Participants*

The individuals from Chapter 2 who reported cannabis use at the baseline assessment (n=70) were reevaluated at the age 19 (follow up) assessment. From the starting 70, there were 25 who were lost due to attrition. From the remaining 45, there were 7 subjects excluded for inconsistent reporting in their drug use levels (e.g., age19 <
Therefore, n=38 subjects were included in the final sample. A comparison of these 38 to the 25 who dropped out indicated the two samples did not differ on various demographic measures (See Table 3.2.1 for comparison). Cannabis use patterns at baseline and follow up were tabulated for the retained 38 participants. Generally, the 38 participants exhibited an escalation in their cannabis use levels over time (Table 3.2.2).

Next, from the n=594 consistently cannabis-naïve participants identified in Section 3.1 on the basis of having acceptable face processing data at baseline, there were 546 who supplied a face processing scan at follow up. From the 546, there were three participants excluded due to excessive head motion. A comparison sample of n=38 individuals was then selected from this pool of 543 participants who were cannabis-naïve at all time points. The comparison sample was selected to be perfectly matched on sex and site, and best matched on handedness, IQ, baseline PDS, baseline and follow up anxiety levels, baseline and follow up age, baseline and follow up head motion (FD), and baseline alcohol use. Unfortunately, it was not possible to best match on baseline cigarette or follow up cigarette and alcohol use. The full sample of n=543 participants was also used in select analyses to characterize neurotypical patterns of amygdala reactivity and anxiety levels over time. See Table 3.2.3 for sample characteristics for the cannabis group, matched comparison group, and full sample of controls.

*Baseline and Follow Up Amygdala Reactivity*

Standardization efforts were implemented to ensure the follow up neuroimaging session was identical to the baseline scan. Moreover, the fMRI data for each time point
was processed using an identical analytic pipeline. Whole-brain contrast images were estimated for the angry vs. control images, and neutral vs. control images. In similar fashion to Chapter 2 and Section 3.1, the same left and right amygdala ROI was used to extract the mean values for angry faces and neutral faces for all subjects.

The data were then submitted to a 2 x 2 (group [cannabis, controls] by time [baseline, follow up]) repeated measures analysis of covariance (ANCOVA) with a test for the interaction between the two factors, for the left and right amygdala. To adjust for variation in baseline and follow up cigarette and alcohol use, a mean centering approach was used. For both measures, the mean of the two time points was computed, and used to center the baseline and follow up measure (Winer et al., 1991). These three measures for alcohol, and three measures for cigarettes, were included as covariates in the model. Follow up analyses on these models were conducted using paired-samples $t$-tests within the cannabis group and control groups, whereby baseline amygdala reactivity was compared to follow up. Finally, to examine changes in anxiety levels, similar repeated measures ANCOVA models were estimated using the DAWBA band scores for generalized anxiety disorder.

**Results**

*Comparison of Retained vs. Lost Cannabis Users*

Chi-square and $t$-tests were used to determine if the 25 cannabis using participants who dropped out of the study prior to the age 19 assessment were different from the 38 retained individuals. These two groups were largely similar on their demographic
characteristics, except for verbal and performance IQs which were significantly lower in the lost sample ($p<.05$). However, IQ was not correlated with baseline amygdala reactivity, and the two groups did not differ on their baseline drug use levels, anxiety levels, or amygdala reactivity (Table 3.2.1). Therefore, the retained sample is largely representative of the full sample of $n=70$ studied in Chapter 2.

**ANCOVA Findings**

A repeated measures ANCOVA model indicated there was no significant interaction effect for the left ($F_{1,70}=0.33$, $p=.57$), or right amygdala ($F_{1,70}=1.9$, $p=.17$), and no significant main effect of group, time ($ps >.05$). Given these null results, a similar ANCOVA model was estimated using the full sample of cannabis-naïve participants ($n=543$). This model was also adjusted for sex and age as significant between-group differences were observed (Table 3.2.3). Again, null results for the interaction for the left ($F_{1,576}=1.17$, $p=.68$), and right amygdala ($F_{1,576}=3.02$, $p=.07$), and main effects were reproduced (all $p>.05$). As the interaction trended to significance for the right amygdala, the adjusted means for both amygdalae were plotted for the cannabis users and matched controls for exploratory purposes (Figures 3.2.1-2). Finally, given the lack of an association uncovered by modeling the two groups together, exploratory analyses were conducted to analyze amygdala reactivity within each group separately.

**Paired-samples Tests**
Paired-samples $t$-tests were conducted comparing baseline to follow up reactivity to angry faces for the left and right amygdala for each group. To start, the full sample of cannabis-naïve participants ($n=543$) were analyzed to determine a neurotypical pattern of functional development. While there was no significant change for the left amygdala ($t=1.2$, $p>.05$), there was a significant increase in follow up activations observed in the right amygdala ($t=2.9$, $p<.005$). When analyzing the matched sample of controls ($n=38$), this effect was reproduced as the right amygdala exhibited a robust increase at follow up ($t=3.7$, $p<.005$), while the left amygdala approached a significant increase ($t=1.8$, $p<.05$). In considering the cannabis users ($n=38$), there was no significant change from baseline to follow up for either the left ($t=-.30$, $p>.05$) or right amygdala ($t=-.53$, $p>.05$).

For reference, there was no significant change when analyzing neutral face processing at either the left or right amygdala, within any group. Therefore, it is specifically the right amygdala activations to angry faces that showed an increase in reactivity in typically developing adolescents only.

*Adjusted Means for Baseline and Follow Up by Group*

The adjusted means for the right and left amygdala along with their standard errors were plotted at baseline and follow up for each group for (See Figures 3.2.1 and 3.2.2). These graphs visually depicted an interaction with group and time despite the null results from the ANCOVA models. As reflected by the paired-samples $t$-tests, the matched control group exhibited a clear increase in reactivity from baseline to follow up in the right amygdala (Figure 3.2.1), and to a lesser extent in the left amygdala (Figure
3.2.2). For the cannabis users, an opposite pattern emerged, such that a marginal decrease in reactivity from baseline to follow up was reflected in the right and left amygdala although statistically non-significant.

**Anxiety Levels at Baseline and Follow Up**

As previous research has supported relationships between amygdala reactivity and anxiety levels, the DAWBA band scores for generalized anxiety at baseline and follow up were analyzed in a similar fashion. A 2 (Group: cannabis, controls) by 2 (Time: baseline, follow up) repeated measures ANOVA model estimated group changes in anxiety levels over time. Results indicated there was no significant interaction ($F_{1,72}=.08$, $p=.79$), however, a highly significant main effect of time ($F_{1,72}=24.5$, $p<.001$) was identified, with anxiety levels increasing from baseline to follow up (See Table 3.2.2). There was no main effect of group. These results were consistent using the full sample of controls (n=543). Consistent with the patterns reported in Section 3.1, no significant bivariate correlation between anxiety level and left or right amygdala was detected at follow up.

**Discussion**

The statistical analyses from this section suggests that amygdala reactivity does not significantly change following heavy cannabis use in adolescence. However, the control groups exhibited a significant increase in activation from baseline to follow up. This effect informs a typically developing pattern of functional development related to
angry face processing in the amygdala, and is consistent with the smaller developmental studies reported by Thomas and colleagues (2001) and Todd and colleagues (2011).

The null results for the cannabis group indicated these individuals failed to exhibit change over time. One possible interpretation is that cannabis use is associated with precocious development. The significantly higher levels of amygdala reactivity at age 14 reported in Chapter 2 and Section 3.1 might suggest these individuals developed faster than their non-using peers. This interpretation is supported by the neurotypical effect that indicated higher amygdala activations at age 19. The lack of a significant change at age 19 in the cannabis users would indicate a ceiling effect of the amygdala if these individuals were already at an advanced stage of amygdala reactivity at age 14.

A group by time interaction effect was visually depicted in Figures 3.2.1-2 but should be interpreted cautiously given the null ANCOVA results. Statistical significance may have emerged if 25 adolescents from the baseline sample were not lost to attrition. And while it is impossible to assume the lost participants would exhibit a similar pattern of amygdala reactivity at follow up as the retained sample, the finding that these 25 were no different from the retained sample might support this hypothesis (Table 3.2.1).

In terms of the anxiety level, the main effect of time demonstrated an increase in anxiety levels from age 14 to 19, while the lack of a main effect of group indicated both groups increased at the same rate. Throughout this Chapter, it was hypothesized that repeated exposure to an anxiolytic like THC would decrease their anxiety levels over time. And while the amygdala reactivity did not significantly change in cannabis users, the negative linear trend depicted in Figures 3.2.1-2, which contrasts with the increase in
cannabis naïve control, might have been interpreted as a decrease in anxiety. Yet, the null results from the anxiety analyses failed to support this interpretation, as anxiety level was not related to cannabis exposure nor was there a significant interaction with group.

It is important to note that the anxiety measure used here reflected the probability of receiving a generalized anxiety disorder (DSM-IV) diagnosis. Therefore, this measure is sensitive to more clinically relevant anxiety. While the cannabis users might not have clinical levels of anxiety per se, they might experience more reinforcing properties under acute exposure relative to individuals without hyperactive amygdalae. Unfortunately no data exists regarding their motivations or intentions for cannabis use. Therefore, these analyses were only able to determine if cannabis use predicted a change in anxiety disorder diagnoses, and unable to probe nuances related to their motivation or subjective experiences during cannabis use.

Finally, the IMAGEN study is a rich dataset containing other measurements that might reflect the nuances influencing cannabis use and therefore better characterize their predictive profile. The following Chapters will consider measures from the brain, behavioral, and genetic domains to uncover a comprehensive predictive profile of cannabis use in adolescence.
Tables

Table 3.2.1: Retained Sample (n=38) vs. Lost Sample (n=25) of Cannabis Users

<table>
<thead>
<tr>
<th>Measure</th>
<th>Retained Sample (n=38)</th>
<th>Lost Sample (n=89)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (M, SD)</td>
<td>14.6, .42</td>
<td>14.7, .38</td>
<td>.29</td>
</tr>
<tr>
<td>Sex (M, F)</td>
<td>25, 13</td>
<td>19, 6</td>
<td>.39</td>
</tr>
<tr>
<td>Handedness (L, R)</td>
<td>2, 36</td>
<td>4, 21</td>
<td>.16</td>
</tr>
<tr>
<td>PDS (M,SD)</td>
<td>2.9, .47</td>
<td>3.0, .49</td>
<td>.59</td>
</tr>
<tr>
<td>Anxiety (M,SD)</td>
<td>0.58, 1.0</td>
<td>0.32, .84</td>
<td>.08</td>
</tr>
<tr>
<td>Verbal IQ (M, SD)</td>
<td>115.4, 17.2</td>
<td>106.5, 16.0</td>
<td>.04</td>
</tr>
<tr>
<td>Performance IQ (M,SD)</td>
<td>111.3, 15.3</td>
<td>97.7, 15.1</td>
<td>.01</td>
</tr>
<tr>
<td>Cannabis (M, SD)</td>
<td>1.4, 1.1</td>
<td>2.0, 1.5</td>
<td>.32</td>
</tr>
<tr>
<td>Cigarettes (M, SD)</td>
<td>2.5, 1.9</td>
<td>3.5, 2.2</td>
<td>.22</td>
</tr>
<tr>
<td>Alcohol (M, SD)</td>
<td>3.9, 1.6</td>
<td>3.6, 1.7</td>
<td>.29</td>
</tr>
<tr>
<td>R. Amygdala Reactivity to</td>
<td>.24, .27</td>
<td>.26, .38</td>
<td>.80</td>
</tr>
<tr>
<td>Angry Faces (M, SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. Amygdala Reactivity to</td>
<td>.25, .29</td>
<td>.32, .24</td>
<td>.26</td>
</tr>
<tr>
<td>Angry Faces (M, SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison of the retained cannabis users who provided follow up (age 19) data versus the participants who dropped out of the IMAGEN study. All tabulated measures collected at baseline (age 14). P-values from chi-square and t-tests for between-group differences. Significant differences detected for IQ only (p<.05).

Table 3.2.2: Baseline and Follow Up Cannabis Use Level

<table>
<thead>
<tr>
<th>Time Point</th>
<th>1-2x</th>
<th>3-5x</th>
<th>6-9x</th>
<th>10-19x</th>
<th>20-39x</th>
<th>40+</th>
<th>SUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 14</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>3-5x</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>6-9x</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>10-19x</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20-39x</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>40+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Cross-tabulation of cannabis use levels at baseline (age 14) and follow up (age 19) in the cannabis using sample (n=38). Overall, the majority of the sample increased their cannabis use level over time.
Table 3.2.3: Characteristic Information for Groups in Follow Up Analyses

<table>
<thead>
<tr>
<th>Measure</th>
<th>A. Cannabis Users (n=38)</th>
<th>B. Matched Controls (n=89)</th>
<th>C. Full Sample of Controls (n=543)</th>
<th>A vs. B p</th>
<th>A vs. C p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSL Age (M, SD)</td>
<td>14.6, .42</td>
<td>14.5, .37</td>
<td>14.4</td>
<td>.39</td>
<td>.02</td>
</tr>
<tr>
<td>FU Age (M, SD)</td>
<td>19.4, .96</td>
<td>19.3, .65</td>
<td>18.9, .67</td>
<td>.54</td>
<td>.01</td>
</tr>
<tr>
<td>Sex (M, F)</td>
<td>25, 13</td>
<td>25, 13</td>
<td>210, 336</td>
<td>1.0</td>
<td>.01</td>
</tr>
<tr>
<td>Handedness (L, R)</td>
<td>2, 36</td>
<td>4, 34</td>
<td>61, 485</td>
<td>.40</td>
<td>.26</td>
</tr>
<tr>
<td>PDS (M,SD)</td>
<td>2.9, .47</td>
<td>2.8, .50</td>
<td>2.9, .56</td>
<td>.41</td>
<td>.95</td>
</tr>
<tr>
<td>BSL Anxiety (M,SD)</td>
<td>0.58, 1.0</td>
<td>0.32, .84</td>
<td>0.45, .89</td>
<td>.30</td>
<td>.40</td>
</tr>
<tr>
<td>FU Anxiety (M,SD)</td>
<td>1.34, .97</td>
<td>1.16, .60</td>
<td>1.32, .74</td>
<td>.49</td>
<td>.20</td>
</tr>
<tr>
<td>Verbal IQ (M, SD)</td>
<td>115.4, 17.2</td>
<td>110.1, 10.6</td>
<td>112.3, 13.2</td>
<td>.11</td>
<td>.18</td>
</tr>
<tr>
<td>Performance IQ (M,SD)</td>
<td>111.3, 15.3</td>
<td>108.8, 11.8</td>
<td>110.5, 13.8</td>
<td>.42</td>
<td>.74</td>
</tr>
<tr>
<td>BSL Cigarettes (M, SD)</td>
<td>2.5, 1.9</td>
<td>1.0, 1.5</td>
<td>0.22, .78</td>
<td>.01</td>
<td>.01</td>
</tr>
<tr>
<td>FU Cigarettes (M, SD)</td>
<td>5.8, .81</td>
<td>1.5, 2.0</td>
<td>1.2, 1.9</td>
<td>.01</td>
<td>.01</td>
</tr>
<tr>
<td>BSL Alcohol (M, SD)</td>
<td>3.9, 1.6</td>
<td>2.9, 1.5</td>
<td>1.4, 1.4</td>
<td>.25</td>
<td>.01</td>
</tr>
<tr>
<td>FU Alcohol (M, SD)</td>
<td>5.8, .55</td>
<td>4.8, 1.5</td>
<td>4.4, 1.7</td>
<td>.01</td>
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</tr>
</tbody>
</table>

All measures were collected at baseline except where noted as follow up (Age 19, “FU”). Drug use measured on an ordinal scale from 0-6 (See table 3.2.1 for levels) using the ESPAD instrument. A.) Sample of cannabis users from Chapter 2 who provided suitable imaging data at the follow up assessment. B.) Sample of matched controls who were cannabis-naïve at all time points. C.) Full sample of controls who were cannabis-naïve at all time points. P-values from chi-square and t-tests for between-group differences.
Figures

Figure 3.2.1: Right Amygdala Reactivity to Angry Faces by Time (N=76)

Plotted adjusted means for angry face processing in the right amygdala for the cannabis users (n=38; Green lines) and matched control (n=38; Blue lines) samples at baseline and follow up. Error bars represent +/- 1 standard error of the mean. Note, no significant differences were identified using repeated measures ANCOVA, nor using a paired-samples t-test within the cannabis users ($p$s>.05). Only a paired-samples t-test within the controls exhibited a significant increase from baseline to follow up ($p$<.05).
Figure 3.2.2: Left Amygdala Reactivity to Angry Faces by Time (N=76)

Plotted adjusted means for angry face processing in the left amygdala for the cannabis users (n=38; Green lines) and matched control (n=38; Blue lines) samples at baseline and follow up. Error bars represent +/- 1 standard error of the mean. Note, no significant differences were identified using repeated measures ANCOVA, nor using a paired-samples t-test within either group (p>.05).
References


4.1. Predicting the Initiation of Cannabis Use By Age 16

This Chapter has been previously published in the following format:


Abstract

Cannabis use initiated during adolescence might precipitate negative consequences in adulthood. Thus, predicting adolescent cannabis use prior to any exposure will inform the aetiology of substance abuse by disentangling predictors from consequences of use. In this prediction study, data were drawn from the IMAGEN sample, a longitudinal study of adolescence. All selected participants (n = 1,581) were cannabis-naïve at age 14. Those reporting any cannabis use (out of six ordinal use levels) by age 16 were included in the outcome group (N = 365, males n = 207). Cannabis-naïve participants at age 14 and 16 were included in the comparison group (N = 1,216, males n = 538). Psychosocial, brain and genetic features were measured at age 14 prior to any exposure. Cross-validated regularized logistic regressions for each use level by sex were used to perform feature selection and obtain prediction error statistics on independent observations. Predictors were probed for sex- and drug-specificity using post-hoc logistic regressions. Models reliably predicted use as indicated by satisfactory prediction error statistics, and contained psychosocial features common to both sexes.
However, males and females exhibited distinct brain predictors that failed to predict use in the opposite sex or predict binge drinking in independent samples of same-sex participants. Collapsed across sex, genetic variation on catecholamine and opioid receptors marginally predicted use. Using machine learning techniques applied to a large multimodal dataset, we identified a risk profile containing psychosocial and sex-specific brain prognostic markers, which were likely to precede and influence cannabis initiation.

**Introduction**

Cannabis use in adolescence is associated with a range of adversity in adulthood including cannabis dependence (DSM-IV) (Hall and Degenhardt, 2009; Moss et al., 2014b), 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 and Laeber, 2014; Malone et al., 2010), and diminished life attainment goals (e.g., socioeconomic factors; (Fergusson and Boden, 2008). These findings are supported by animal models linking adolescent cannabis exposure with detrimental outcomes in adulthood (O’Shea, 2004; Quinn et al., 2008a). However, in humans, it is difficult to assert a causal role for cannabis in subsequent outcomes as any negative outcomes arising from use could be related to a number of factors confounded with the choice to initiate use (Jackson et al., 2016).

Results from the 2013 National Survey on Drug Use and Health indicated that nearly 25% of 10th graders reported ever trying cannabis (NSDUH, 2014). From 2005 to 2010 rates of cannabis-related emergency room visits increased 54% in males and 42% in
females aged 15-17 years (NSDUH, 2014). Moreover, beliefs concerning the risk of use are declining (Johnston et al., 2011) despite the increase in drug potency relative to previous decades (ElSohly et al., 2016). These trends are a source of concern as *in vitro* models indicate that delta-9-tetrahydrocannabinol (THC), a psychoactive compound in cannabis, could be more toxic in adolescent than in adult tissue (Pope et al., 2003; Quinn et al., 2008a; Renard et al., 2016; Rubino et al., 2015; Schneider, 2008), and human studies suggest early, compared to adult, initiation of cannabis is associated with worse outcomes (Brook et al., 2011; Coffey and Patton, 2016).

Global studies suggest cannabis use is typically initiated prior to age 18 (Degenhardt et al., 2008). Thus, adolescence might be a developmental period during which initiation can be best predicted. Investigations of the risk factors associated with cannabis initiation commonly report features like temperament (Creemers et al., 2010), delinquent behaviors (Bree and Pickworth, 2005), alcohol and tobacco use (von Sydow et al., 2002), and parental (Day et al., 2006) and peer influences (Ellickson et al., 2004), while rarely considering any neurobiological or genetic contributions. Incorporating these domains may uncover biobehavioral processes that are specific to the initiation of cannabis use. Therefore, we sought to uncover a comprehensive risk profile of adolescent cannabis use by predicting the initiation of use via a large multimodal biobehavioral dataset.

Prior studies have stressed the importance of attending to sex differences in substance abuse research. Indeed, males and females differ in their biological response to cannabis, such that females produce more psychoactive THC metabolites (Narimatsu et
al., 1991) and exhibit elevated gene expression levels of both CB1 & CB2 cannabinoid receptors (Onaivi et al., 1999) relative to males. Behaviorally, female cannabis users endorse more positive subjective ratings associated with abuse liability to smoked cannabis (vs. placebo; (Cooper and Haney, 2014). Moreover, converging evidence using animal (Fattore et al., 2007) and human studies (Hernandez-Avila et al., 2004; Schepis et al., 2011) indicates the transition from cannabis use initiation to regular use is accelerated in females. Hence, the identification of a predictive profile may identify sex-specific etiological mechanisms while also informing sex-specific interventions to attenuate the risk of ever becoming a user.

While prediction analyses can illuminate the nature of drug initiation, these studies are rare as they necessitate large, longitudinal samples, especially when feature-rich domains are considered (Whelan and Garavan, 2014). Large samples are also needed for cross-validation schemes to ensure predictive models are tested on independent samples. Hence, we modeled our analytic approach on a related study using the IMAGEN dataset in which Whelan and colleagues developed predictive models which identified multi-domain features at age 14 that predicted binge drinking at age 16 (Whelan et al., 2014a). Given this work, we hypothesized cannabis use could be predicted in a similar fashion using multi-domain data from the IMAGEN sample. We extend the methods of Whelan and colleagues by identifying multi-domain risk profiles for each sex while considering a range of subsequent cannabis use levels. In doing so, we identify predictive features that are both common and unique between the sexes, and between future cannabis use and binge drinking. While we anticipate replicating many
psychosocial predictors and uncovering a sparse set of brain and genetic predictors, these exploratory analyses are data driven. In an era where large multisite neuroimaging projects and big datasets are becoming more prevalent, we leverage machine learning techniques to uncover a sparse set of predictors of cannabis use from a large multidomain set of variables that generalize to predict use in independent samples.

**Methods and Materials**

Full details of the multisite IMAGEN study (Schumann et al., 2010) are available in the online Standard Operating Procedures (https://imagen-europe.com/). Imaging acquisition parameters and quality assurance procedures were standardized across site to ensure comparable data (see Schumann et al., 2010 for standardization of procedures across sites). The IMAGEN study conformed to the ethical standards outlined by Declaration of Helsinki and was approved by ethics committees at each site including King’s College, London; Central Institute of Mental Health, Mannheim; Charite, Universitätsmedizin Berlin; University Medical Center Hamburg- Eppendorf; University of Nottingham; Trinity College Dublin; Institut National de la Sante et de la Recherche Medicale, Orsay. After description of the IMAGEN study to the participants and their parents, written informed consent was obtained. Individuals who provided assent were studied at age 14 & 16.

*Participants*
Inclusion was determined by a self-report drug use questionnaire (using the “ESPAD”, described below). Participants from the baseline sample (age 14) who provided ESPAD data and were cannabis-naïve were eligible for inclusion (n=2,018). At age 16, n=1,581 participants (78% of the cannabis-naïve sample) provided usable data (see Table 4.1.1 for evaluation of participants unavailable for follow-up) and were thus included in the analysis. Participants reporting any level of cannabis use by age 16 were assigned to the outcome groups (n=365). Participants who remained cannabis-naïve at age 14 and 16 were assigned to the comparison group (n=1,216).

The European School Survey Project on Alcohol and Drugs (ESPAD; Hibell et al., 1997) was administered at age 14 and 16 using Psytools (London, UK). Lifetime usage was measured on an ordinal scale: 0, 1=1-2x, 2=3-5x, 3=6-9x, 4=10-19x, 5=20-39x, 6=40x+. See Table 4.1.2 for sample demographics and drug use levels.

Data

Participants were extensively characterized at age 14 using psychosocial (of parent and child), neuroimaging, and genetic assessments (see supplemental materials). Psychosocial data were largely self-reported, and included demographics, summary scores for personality dimensions (Cloninger, 1999; Costa Jr. and McCrae, 1995; Woicik et al., 2009), frequency of candidate life events (Newcomb et al., 1981), cognitive (Robbins et al., 1994) and intelligence (Wechsler, 2003) assessments, and drug use levels of the parent and child (additional features described in supplemental materials). Genetic data included 108 candidate single nucleotide polymorphisms (SNPs) on genes 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 based on the summation of those eight risk-alleles (Cornelis, 2009). Brain data included three fMRI tasks designed to engage cognitive processes associated with substance abuse (reward processing, motor response inhibition, and social affective (face) processing; see supplemental materials for task specifics) and one structural MRI scan. Whole-brain fMRI contrast maps (generated using a standard GLM) and gray matter volume maps (GMV; generated using voxel-based morphometry) were each parcellated into 278 regions of interest (ROIs) (Shen et al., 2013). All data (except the cannabis use outcome) were collected at age 14 and used to predict cannabis use by age 16, and all predictors (n variables=2,413; see Table 4.1.3 for summary of predictor variables) from each domain were considered during predictive model estimation.

Statistical Analyses

The overall analytic procedure was designed to accomplish three goals: (1) perform feature selection to identify the predictors of light to heavy use in males and females separately; the selected features then informed post-hoc analyses to (2) probe the identified predictors for sex- and drug-specificity, and (3) assess the relative contribution of each data domain to the prediction of cannabis use initiation.

Feature Selection
Six prediction analyses were conducted for each sex in order to predict each level of use via the ESPAD scale (use levels of 1 and above (Males n=207; Females n=158), levels 2 and above (Males n=172; Females n=120), and so on up to level 6). Predictive models were estimated using elastic-net regularization (Zou and Hastie, 2005) with logistic regression to perform feature selection (from \( n \) variables=2,413) and reduce model overfit. The elastic-net minimizes both the sum of the squared and absolute values of the regression coefficients, effectively setting some coefficients to zero, thereby performing feature selection during model estimation. Elastic-net parameters (see supplemental information) were tuned on independent samples (via nested \( k \)-fold cross-validation), and then final models were tested on an independent internal validation set. These analyses were implemented using the “glmnet” function in MATLAB (v. R2014a, Natick, MA).

\( k \)(10)-fold cross-validation was used during model estimation to evaluate predictive models on independent observations. Partitioning a completely external validation set would have reduced an already small group of interest. Therefore, internal validation using \( k \)-fold cross-validation was used as a proxy for external validation. During \( k \)-fold cross-validation, the full sample of data is partitioned into subsamples of data, where \( k \) equals the number of partitions (or “folds”) of the original starting sample. \( k \)-fold cross-validation then becomes an iterative process whereby a single fold is set aside as the test sample (“test fold”), and a “training model” is estimated on the observations in the remaining \( k-1 \) folds (“training folds”). The training model is then used
to predict the observations in the set aside test fold, thereby ensuring the independence of the test fold sample. This procedure returns $k$ final models.

Each of the six sex-specific prediction analyses were run 100 times to account for the subtle differences in results incurred due to the random assignment of participants to folds. Results were thresholded to identify only the predictors that were present in at least six final models (from $k=10$) across all 100 runs within a use level analysis. Predictors passing this threshold were selected for use in post-hoc analyses. See Figure 4.1.1 for a schematic of the analytic method.

The area under the curve (AUC) of the receiver-operating characteristic (ROC) was calculated based on the model’s ability to predict cannabis use in the independent samples segregated during cross-validation. Broadly, here the ROC AUC represents the probability that a randomly selected individual from the outcome group will be predicted as a future user (Fawcett, 2006). Null-hypothesis significance testing on the AUC was conducted using a Mann-Whitney U-test (Mason and Graham, 2002) (significance set using a Bonferroni corrected $p<.008 (p<.05/6$ models)) to test the hypothesis that models predicted independent samples better than chance.

Features selected from each use level analysis were then used in post-hoc analyses described below. Correlations between each identified feature and cannabis use were also analyzed using Pearson’s point-biserial correlation to predict any level of future use in a binary fashion.

Specificity Analyses
Sex-specificity was assessed by including the selected features of male cannabis use as the independent variables of a logistic regression model estimated on the female sample (and *vice versa*). Drug-specificity was assessed by including the selected features of male cannabis use as the independent variables of a logistic regression model estimated on an independent sample of binge drinking males (and likewise for females). The binge drinking sample contained new individuals (n=400) who were naïve to binge drinking at age 14 (with a maximum of 2 lifetime drinks), but endorsed binge drinking episodes (i.e., being drunk from alcoholic beverages) by age 16 (see Table 4.1.4 for binge drinking sample demographics).

*Domain Contribution Analyses*

The selected features for each sex were also modeled in a hierarchical fashion to measure the relative change in model fit after the inclusion of each domain-specific set of predictors. Model fit for all *post-hoc* regressions were determined using a chi-square goodness of fit statistic and the delta Akaike information criterion of model selection ($\Delta$AIC; Akaike, 1974).

*Results*

Feature selection analyses predicting each use level returned a range of ROC AUC values (Males: AUC=0.65–0.74, $p=1.4\times10^{-8}$–$5.3\times10^{-10}$; Females: AUC=0.74–0.82, $p =1.8\times10^{-16}$–$5.5\times10^{-13}$), indicating high accuracy in predicting independent samples for each use level (Figure 4.1.2). Best performance was achieved predicting $\geq$20 uses for
males (AUC=.74, \( p=5.3 \times 10^{-10} \)) and \( \geq 10 \) uses for females (AUC=. 82, \( p=5.5 \times 10^{-13} \)). For context, in a study using only psychosocial features to predict the initiation of cannabis use, authors reported a final predictive logistic regression model returning a ROC AUC=.78 (von Sydow et al., 2002). Additionally, Whelan and colleagues reported a cross-validated ROC AUC=.75 in their study of brain, psychosocial, and genetic predictors of binge drinking (Whelan et al., 2014a). Hence, the AUCs reported here are in line with previous research, while the AUCs from the female models reflect an even higher degree of cross-validated prediction than what has been previously reported.

**Selected Psychosocial Predictors**

Six psychosocial predictors were found to be common to both sexes, including greater lifetime alcohol and cigarette use, parental lifetime cannabis use, novelty-seeking personality and the disorderliness personality subscale (Cloninger, 1999), and less-negative feelings towards deviant behaviors (Newcomb et al., 1981). Post-hoc regressions indicated these predictors returned strong model fit for the full sample (males and females) for all levels of cannabis use \( (\chi^2_{6, N=1539}=184.02, \ p=4.7 \times 10^{-37}; \ \Delta AIC=175.02) \), and also predicted binge drinking \( (\chi^2_{5, N=379}=29.58, \ p=1.8 \times 10^{-5}; \ \Delta AIC=19.58) \) in an independent sample. See Figure 4.1.4 for a summary of all identified predictors and their point-biserial correlation with use initiation.

Male-specific predictors included greater parental novelty-seeking (Cloninger, 1999) and sensation seeking personality. While these parental personality traits measure similar constructs, partial correlations indicated parent sensation seeking predicted use
(r_{739}=.10, p=.005) after accounting for parent novelty seeking personality (r_{740}=.10, p=.007). Furthermore, although personality traits are heritable, partial correlations also indicated child novelty-seeking personality predicted use (r_{739}=.14, p=2.1 \times 10^{-4}) after accounting for parent novelty-seeking personality, r_{740}=.10, p=.007).

Female-specific predictors included greater extravagant personality subscale (Cloninger, 1999) in both the parent and daughter. The extravagant subscale assesses overspending behaviors and diminished planning, and conveys a tendency to approach reward cues. Similar to males, greater extravagance of both the parent and daughter made separate contributions to the prediction (post-hoc partial correlation between the outcome measure and child extravagance r_{823}=.12, p=3.6 \times 10^{-4}, after accounting for parent extravagance r_{824}=.16, p=6.0 \times 10^{-6}). Additionally, greater impulsive personality subscale (Cloninger, 1999), frequent sexual experiences, and higher verbal IQ, predicted female use.

Selected Brain Predictors

For males, six functional and two structural brain features predicted cannabis use. For females, fifteen functional and two structural brain features predicted use with no overlap with the predictors for males. Post-hoc point-biserial correlations indicated that five regions for males, and sixteen regions for females, significantly predicted any level of use across each sample. See Figures 4.1.3-4 for visualization of all brain features and direction of effects.
Sex- and Drug-specificity

Post-hoc regressions confirmed that male-specific brain predictors of use returned strong model fits when estimated on the male sample ($\chi^2_{8,N=745}=24.3$, $p=.002$; $\Delta$AIC=8.3), as did the female-specific brain predictors estimated on the female sample ($\chi^2_{17,N=836}=101.7$, $p=4.3\times10^{-14}$ $\Delta$AIC=67.7). The male-specific brain predictors failed to predict use in females ($\chi^2_{8,N=836}=9.9$, $p=.272$; model with predictors $\Delta$AIC=6.1 relative to the base rate model) and failed to predict binge drinking in males ($\chi^2_{8,N=180}=8.3$, $p=.405$; model with predictors $\Delta$AIC=7.6 relative to the base rate model). Likewise, the female-specific brain predictors failed to predict use in males ($\chi^2_{17,N=745}=18.8$, $p=.341$; model with predictors $\Delta$AIC=15.2 relative to the base rate model), and failed to predict binge drinking in females ($\chi^2_{17,N=220}=16.6$, $p=.482$; model with predictors $\Delta$AIC=17.4 relative to the base rate model). See Table 4.1.5 for all sex- and drug-specific post-hoc regression summaries.

Genetic Predictors Sex-specific feature selection analyses did not identify any SNPs, therefore, as a post-hoc exploratory analysis, we collapsed across sex and reran the analyses with only the genetic predictors (plus nuisance covariates). This analysis returned an ROC AUC range = 0.54–0.61; $p=.01–1.4\times10^{-6}$ (See Figure 4.1.5). We note that given the relatively small $p$-values, these models do not pass a Bonferroni correction, and as the highest use level analysis (use level 6) yielded a non-significant prediction (AUC=.53, $p=.23$), only results from the uncorrected significant models (use level 1-5) were probed further. Moreover, the genetic multidimensional scaling factors plus demographic covariates inflated model performance. With that in consideration, two
SNPs on genes coding for the $\beta_2$-adrenergic receptor, one SNP on a gene coding for the $\alpha_{1b}$-adrenergic receptor, two SNPs on genes coding for the DRD1 receptor, and five SNPs on genes coding for the $\mu_1$-opioid receptor, predicted cannabis use. Post-hoc analyses suggested three SNPs were significantly related to cannabis use for the male sample ($\beta_2$-adrenergic: rs1042711, rs1801704; and DRD1: rs1174661), whereas none of the SNPs were significant for the female sample (see Table 4.1.6 and Figure 4.1.6 for SNP statistics, including their correlation with the outcome measure across the entire sample).

When including these ten SNPs in a post-hoc hierarchical logistic regression predicting cannabis use, the model exhibited strong fit to the full sample after first modeling the nuisance covariates ($\Delta\chi^2_{9,N=1581}=25.7$, $p=.002$; $\Delta\text{AIC}=7.7$). However, these SNPs returned poor model fits to the full sample of binge drinkers after first modeling the nuisance covariates ($\Delta\chi^2_{9,N=312}=9.03$, $p=.435$; $\Delta\text{AIC}=9$ relative to the model with nuisance covariates only).

**Domain Contribution Effects**

The psychosocial predictors were entered first and significantly improved model fit relative to the base rate model for the male sample ($\chi^2_{8,N=742}=94.5$, $p=5.5\times10^{-17}$; $\Delta\text{AIC}=78.53$) and the female sample ($\chi^2_{11,N=826}=134.1$, $p=2.5\times10^{-23}$; $\Delta\text{AIC}=112.13$). Next, the brain predictors were added and significantly improved model fit for the male sample ($\Delta\chi^2_{8,N=742}=17.3$, $p=.027$; $\Delta\text{AIC}=1.3$) and the female sample ($\Delta\chi^2_{17,N=826}=101.1$, $p=5.8\times10^{-14}$; $\Delta\text{AIC}=67.1$). Finally, the ten SNPs were added and significantly improved model fit for the male sample ($\Delta\chi^2_{10,N=742}=24.2$, $p=.007$; $\Delta\text{AIC}=6.2$) but not the female
sample ($\Delta \chi^2_{9, N=826} = 6.5$, $p = .689$; psychosocial and brain model $\Delta \text{AIC} = 11.5$). These findings held irrespective of the order in which each domain was entered. Thus, while psychosocial data alone can be used to significantly predict use, models containing both psychosocial and sex-specific brain features return superior fits, highlighting the utility of capturing individual neurobiological differences in predicting adolescent cannabis use.

**Discussion**

**Psychosocial Findings**

The six shared psychosocial predictors replicate previous findings establishing alcohol and tobacco as predictors of cannabis use (Hall and Pacula, 2003; Siegel et al., 2014), as are novelty-seeking and disorderliness personality traits (Hale et al., 2003; Sher and Trull, 1994), and parental transmission of drug use (Brook et al., 2001; Kandel et al., 1978; Kosty et al., 2015). As these features also predicted binge drinking, they may be considered general risk factors for adolescent drug use. In considering the parental influence, parents with behaviorally disinhibited personality traits, coupled with a history of cannabis use, were found to increase risk for use in their children, mirroring previously published studies (Day et al., 2006; Kerr et al., 2015). Moreover, less-negative feelings towards deviant behaviors may signal a predisposition towards conduct disorder, which previous literature has linked to cannabis use (Crowley et al., 1998). Risk of use was also identified for females exhibiting higher verbal IQ, which has been implicated in cannabis experimentation (Fried et al., 2002). Additionally, higher impulsivity, extravagance, and
sexual experiences are consistent with the novelty-seeking phenotype of individuals most likely to initiate substance use.

**Brain Findings**

For males, the brain predictors were largely related to cerebellar activation differences during response inhibition. Animal models suggest the lateral cerebellum is involved in motor preparation and inhibition via projections to cortical motor and inhibitory regions through the thalamus (Middleton and Strick, 2001). Additionally, the cerebellar regions identified have also been implicated in a network underlying motor inhibitory control (Stevens et al., 2007). Thus, hypoactivity in all three cerebellar regions may suggest a compromised motor inhibitory control system constitutes a neurobiological vulnerability that influences the initiation of cannabis consuming behaviors. Moreover, larger GMV in the right medial prefrontal cortex (PFC) might indicate a neurodevelopmental delayed maturation in regions supporting executive functioning. This finding is supported by studies reporting an adolescent male-specific increase in PFC volume with alcohol use disorder (Medina et al., 2008) and conduct use disorder (Brito et al., 2009).

In females, a structural-functional finding in the right pre-supplemental motor area (pre-SMA) predicted cannabis use. As myelination proliferates during adolescence, especially in motor areas requiring expedited signal propagation (Paus, 1999), higher GMV and activity during failed inhibitions observed in the right pre-SMA suggests a functional consequence of delayed cortical maturation. This structural finding is notable
for the female sample as cortical maturation (thinning) occurs earlier in females compared to their male peers (Giedd, 2004).

Additionally, lower activity compared to non-users in the right inferior frontal gyrus (IFG) during failed inhibitions was predictive of cannabis use in females. As the right IFG is a key region implicated in the stop task (Garavan et al., 1999), lower activity is notable as hypoactivity here is also associated with cigarette use (Spechler et al., 2016). As our test for drug-specificity was restricted to binge drinking, some brain predictors might generalize to other drugs of abuse not tested here. In the orbitofrontal cortex (OFC), females also displayed lower bilateral activations during successful inhibitions, and lower right-sided GMV. The volumetric finding is concordant with Cheetham and colleagues who reported lower OFC GMV at age 12 predicts use at age 16, with only the right OFC remaining significant after accounting for poly-drug use (Cheetham et al., 2012b), thus underscoring the right OFC specificity to cannabis initiation. Furthermore, as other studies have correlated OFC hypoactivity with adolescent substance use (Whelan et al., 2012), the anterior prefrontal cortex might be especially valuable for inquiry relating female-specific neurobiological pathways with substance abuse.

For females, more predictors related to face processing were identified. Specifically, lower processing of neutral faces in the right superior frontal and lingual gyri. Previous studies suggest neutral faces can be misperceived as threatening, especially in individuals with social anxiety disorder (Cooney et al., 2006; Yoon and 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) these results suggest a female-specific pathway towards cannabis use. Additionally, higher female-specific activation to angry faces in the ventromedial prefrontal cortex is notable given this region’s involvement in emotion regulation (Urry et al., 2006).

**Genetic Findings**

The number of predictive \( \mu_1 \)-opioid receptor SNPs highlights the importance of the opioid system in substance abuse. Opioid and cannabinoid systems co-localize in the striatum (Rodriguez et al., 2001) and exhibit reciprocal signaling (Robledo et al., 2008). However, the biobehavioral effects orchestrated by these systems remain unclear in humans. Animal models suggest the \( \mu_1 \)-opioid receptor is specifically involved in reinforcement as \( \mu_1 \)-opioid receptor knockout mice failed to exhibit THC-induced conditioned place preference compared to \( \delta_1 \)-knockout and wild-type mice (Ghozland et al., 2002). Hence, our findings that cannabis users had a greater number of risk alleles for both DRD1 SNPs and three \( \mu_1 \)-receptor SNPs suggest alterations in their neurobiological processing of rewards. As these findings were uncovered from exploratory models that were not as robust to predict use as the multi-domain models, larger GWAS studies or candidate SNP analyses are needed to reinforce these results.

**Conclusions**

In this large longitudinal study, we offer evidence that psychosocial and sex-specific neurobiological predictors of cannabis use preceded, and likely influenced,
teenage cannabis consuming behaviors. Hence, these analyses identified individual differences at age 14 that predict later cannabis use, and thus have potential for guiding proactive interventions. Despite having thousands of multi-domain variables per individual, prediction with high generalizability was achieved with a sparse set of sex-specific brain and psychosocial features, and six shared psychosocial features. And while the psychosocial data alone was found to predict both cannabis and binge drinking, the addition of the brain features improved cannabis prediction and augmented the sex-specificity of the findings.

The superior prediction of the female sample suggests they exhibit a more distinct predictive profile at age 14, despite having lower levels of subsequent use. These findings are clinically meaningful given the female-specific vulnerability towards accelerated dependency. Moreover, the fMRI findings highlight the sex-specific psychological processes potentially driving the initiation of cannabis use in adolescence. Thus, our findings underscore the importance of attending to sex-differences in addiction research, and fulfills the recent NIH policy for investigators to examine sex-differences in biobehavioral research (Clayton and Collins, 2014).

Limitations of this study include the absence of measures of peer influences. The addition of these variables, as well as interactions between features, might yield a higher AUC, as the reported AUCs indicate a departure from perfect prediction. Future analyses to identify how psychosocial, brain, and genetic feature interact to influence the likelihood of cannabis use are needed. Additionally, the convenient community sampling of predominantly white Europeans may impact generalizability to other populations.
Finally, despite predicting high levels of use (e.g., ≥40 uses by age 16), it is unknown if these individuals will meet DSM-V diagnostic criteria for cannabis use disorder later in life. However, by design of the analysis, all participants were early initiators of cannabis, with the heavy users always present in the prediction models. Therefore, these predictors may signify risk for higher use. Still, the heavy users only encompassed a small proportion of the sample, therefore even larger studies are needed. And while our predictive models generalized to independent observations via internal cross-validation, a completely set aside external validation set was not possible due to the limited sample sizes. As such, the gold standard remains a completely independent external validation set. Studies assessing the degree by which cross-validated prediction metrics may differ by cross-validation scheme are also needed (although Whelan et al., 2014 reports similar AUCs for internal and external validation). Taken together, our findings supply new hypotheses to be tested using additional time points from the ongoing IMAGEN and larger ABCD (www.ABCDstudy.org) studies.
References


Tables

Table 4.1.1: Comparison of Age 16 Dropouts vs. Retained Sample

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</tr>
<tr>
<td>SES (M,SD)</td>
<td>17.01, 4.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Retained Sample (n=1581)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (M,SD)</td>
<td>14.5, 0.42</td>
<td></td>
<td></td>
<td>.002</td>
</tr>
<tr>
<td>Sex (Male, Female)</td>
<td>745, 836</td>
<td></td>
<td></td>
<td>.051</td>
</tr>
<tr>
<td>Handedness (L,R)</td>
<td>169, 1412</td>
<td></td>
<td></td>
<td>.174</td>
</tr>
<tr>
<td>PDS (M,SD)</td>
<td>3.5, 0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perceptual IQ (M,SD)</td>
<td>108.11, 13.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal IQ (M,SD)</td>
<td>111.2, 13.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SES (M,SD)</td>
<td>18.00, 3.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Participants who completed the baseline ESPAD assessment and reported no lifetime cannabis use but then were unavailable for follow up assessment two years later were assigned to the dropout sample. Compared to the retained sample, the dropout sample had significantly higher age, and lower IQs and SES.
Table 4.1.2: Participant Demographics

<table>
<thead>
<tr>
<th>Measure</th>
<th>Groups</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Cannabis Use by Age 16 (n=207)</td>
<td>Comparison Group (n=538)</td>
<td>$p$</td>
<td>Females</td>
<td>Cannabis Use by Age 16 (n=158)</td>
<td>Comparison Group (n=678)</td>
<td>$p$</td>
</tr>
<tr>
<td>Age (M,SD)</td>
<td>14.50, 0.47</td>
<td>14.52, 0.39</td>
<td>.54</td>
<td>14.51, 0.53</td>
<td>14.54, 0.42</td>
<td>.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handedness (L,R)</td>
<td>25, 182</td>
<td>66, 472</td>
<td>.94</td>
<td>18, 140</td>
<td>60, 618</td>
<td>.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDS (M,SD)</td>
<td>2.65, 0.49</td>
<td>2.54, 0.55</td>
<td>.01</td>
<td>3.22, 0.39</td>
<td>3.17, 0.44</td>
<td>.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perceptual IQ (M,SD)</td>
<td>108.11, 13.55</td>
<td>108.18, 14.56</td>
<td>.95</td>
<td>109.40, 13.49</td>
<td>107.77, 13.23</td>
<td>.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal IQ (M,SD)</td>
<td>114.19, 13.267</td>
<td>112.07, 13.14</td>
<td>.05</td>
<td>112.93, 12.29</td>
<td>109.22, 13.80</td>
<td>.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SES (M,SD)</td>
<td>18.52, 3.97</td>
<td>17.88, 3.82</td>
<td>.05</td>
<td>18.26, 3.94</td>
<td>17.88, 3.68</td>
<td>.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cannabis Use Levels</td>
<td>1 2 3 4 5 6</td>
<td>(N) 62 35 26 24 23 37</td>
<td></td>
<td>1 2 3 4 5 6</td>
<td>56 39 19 20 9 15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PDS: Puberty Development Scale (Carskadon & Acebo, 1993); SES: Socioeconomic status. Cannabis use levels from the ESPAD and measured on an ordinal scale (1=1–2x, 2=3–5x, 3=6–9x, 4=10–19x, 5=20–39x, 6=40+) All data (with the exception of cannabis use) were obtained at age 14. All demographics measures were also included as predictors in feature selection analyses.
Table 4.1.3: Summary of data used as independent variables in predictive modeling.

<table>
<thead>
<tr>
<th>Domain</th>
<th>Measures</th>
<th>Data points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychosocial</td>
<td>Demographics</td>
<td>• 80 measures</td>
</tr>
<tr>
<td></td>
<td>Cognitive assessments</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Personality assessment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Life-events questionnaires</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baseline cigarette &amp; alcohol use</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parent personality and drug use</td>
<td></td>
</tr>
<tr>
<td>Genetic</td>
<td>A-priori SNPs</td>
<td>• 108 SNPs</td>
</tr>
<tr>
<td></td>
<td>• Cannabinoid Receptor</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Catecholamine Receptors</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Opioid Receptors</td>
<td></td>
</tr>
<tr>
<td>Structural</td>
<td>Total GMV</td>
<td>• 1 total GMV</td>
</tr>
<tr>
<td>Neuroimaging</td>
<td>Gray-Matter Volume ROIs</td>
<td>• 278 GMV ROIs</td>
</tr>
<tr>
<td>Functional</td>
<td>Reward Processing Task</td>
<td>• 1946 ROIs</td>
</tr>
<tr>
<td>Neuroimaging</td>
<td>• (2 Contrasts)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stop Signal Task</td>
<td>• 278 per contrast</td>
</tr>
<tr>
<td></td>
<td>• (2 Contrasts)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Face Processing Task</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• (3 Contrasts)</td>
<td></td>
</tr>
</tbody>
</table>

**Total predictors per subject**: 2413

A related analysis including psychopathology measures was conducted but did not improve predictive performance. Site was also modeled in the analysis and yielded Paris (data not shown) as a significant predictor due to the higher prevalence of cannabis use at age 16 for both sexes.

Table 4.1.4: Binge Drinking Sample Demographics.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Groups</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Binge Drinkers by age 16 (n=208)</td>
<td>Comparison Group</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=192)</td>
<td></td>
</tr>
<tr>
<td>Age (M,SD)</td>
<td>14.5, 0.41</td>
<td>14.5, 0.39</td>
<td>.71</td>
</tr>
<tr>
<td>Sex (Male, Female)</td>
<td>103, 105</td>
<td>77, 115</td>
<td>.06</td>
</tr>
<tr>
<td>Handedness (L,R)</td>
<td>21, 171</td>
<td>20, 188</td>
<td>.67</td>
</tr>
<tr>
<td>PDS (M,SD)</td>
<td>2.8, 0.6</td>
<td>2.9, 0.6</td>
<td>.61</td>
</tr>
<tr>
<td>Perceptual IQ (M,SD)</td>
<td>106.2, 13.5</td>
<td>105.8, 14.3</td>
<td>.78</td>
</tr>
<tr>
<td>Verbal IQ (M,SD)</td>
<td>109.5, 13.1</td>
<td>108.5, 14.5</td>
<td>.51</td>
</tr>
<tr>
<td>SES (M,SD)</td>
<td>18.1, 3.7</td>
<td>17.8, 3.8</td>
<td>.79</td>
</tr>
</tbody>
</table>

All participants at baseline reported no lifetime binge drinking episodes and a maximum of 2 lifetime alcoholic drinks. Participants who then went on to report any level of binge drinking by age 16 were included in the binge drinking at age 16 sample, compared to participants who endorsed a maximum of 2 lifetime drinks.
Table 4.1.5: Post-hoc Regression Model Summaries.

<table>
<thead>
<tr>
<th>Cannabis Predictive Features</th>
<th>Test Sample</th>
<th>Model Fit</th>
<th>( \chi^2, p )</th>
<th>( \Delta AIC^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shared Psychosocial Features</strong></td>
<td>Binge Drinking</td>
<td>29.6, ( p &lt; .01 )</td>
<td>19.6 (base rate model – model with predictors)</td>
<td></td>
</tr>
<tr>
<td><strong>Male Brain Features</strong></td>
<td>Females: Cannabis Use</td>
<td>9.9, ( p &gt; .05 )</td>
<td>6.1 (model with predictors – base rate model)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Males: Binge Drinking</td>
<td>8.3, ( p &gt; .05 )</td>
<td>7.6 (model with predictors – base rate model)</td>
<td></td>
</tr>
<tr>
<td><strong>Female Brain Features</strong></td>
<td>Males: Cannabis Use</td>
<td>18.8, ( p &gt; .05 )</td>
<td>15.2 (model with predictors – base rate model)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Females: Binge Drinking</td>
<td>16.6, ( p &gt; .05 )</td>
<td>17.4 (model with predictors – base rate model)</td>
<td></td>
</tr>
<tr>
<td><strong>Shared Genetic Features</strong></td>
<td>Binge Drinking</td>
<td>9.03, ( p &gt; .05 )</td>
<td>9 (model with predictors – nuisance model)</td>
<td></td>
</tr>
</tbody>
</table>

Features identified from each cannabis predictive modeling scenario were used to probe sex- and drug-specific effects. Male & Female shared psychosocial predictors of cannabis use also predicted binge drinking by age 16. Male brain predictors and female brain predictors failed to model cannabis use in the opposite sex, or, binge drinking in the same sex. \( \Delta AIC \) always in reference to the better fitting model. \( \Delta AIC = AIC_{model_j} - AIC_{min} \) and reflects the relative increase in information gained from the \( AIC_{min} \) (better) model. Values >=2 favor the \( AIC_{min} \) model.
Table 4.1.6: Statistics and Frequencies for Cannabis Predictive SNPs.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Gene</th>
<th>HW P value</th>
<th>MAF</th>
<th>Major: Minor Alleles</th>
<th>Imputation Quality (R²)</th>
<th>Association with age 16 Cannabis Use</th>
<th>Genotype (% Hₘᵢₙᵢᵩ : HT : Hₘₐᵢᵦᵩ)</th>
<th>Minor Allele Effect On Cannabis Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1042711</td>
<td>ADRB2</td>
<td>.86</td>
<td>.122</td>
<td>T:C</td>
<td>.97</td>
<td>.06 .02</td>
<td>12:54:34 16:55:30</td>
<td>Protection</td>
</tr>
<tr>
<td>rs1801704</td>
<td>ADRB2</td>
<td>.86</td>
<td>.122</td>
<td>T:C</td>
<td>.97</td>
<td>.06 .02</td>
<td>12:54:34 16:55:30</td>
<td>Protection</td>
</tr>
<tr>
<td>rs6888306</td>
<td>ADRA1b</td>
<td>.92</td>
<td>.099</td>
<td>C:T</td>
<td>.89</td>
<td>.03 .25</td>
<td>3:33:64 4:32:64</td>
<td>Protection</td>
</tr>
<tr>
<td>rs686</td>
<td>DRD1</td>
<td>.85</td>
<td>.135</td>
<td>A:G</td>
<td>.85</td>
<td>-.03 .23</td>
<td>12:51:37 12:44:44</td>
<td>Risk</td>
</tr>
<tr>
<td>rs11746641</td>
<td>DRD1</td>
<td>.84</td>
<td>.060</td>
<td>T:G</td>
<td>.64</td>
<td>-.05 .05</td>
<td>4:25:71 2:22:76</td>
<td>Risk</td>
</tr>
<tr>
<td>rs2281617</td>
<td>OPRM1</td>
<td>.88</td>
<td>.098</td>
<td>G:T</td>
<td>.86</td>
<td>.01 .72</td>
<td>2:23:76 1:23:76</td>
<td>Risk</td>
</tr>
<tr>
<td>rs563649</td>
<td>OPRM1</td>
<td>.91</td>
<td>.158</td>
<td>G:A</td>
<td>.89</td>
<td>.03 .27</td>
<td>0:14:86 1:13:86</td>
<td>Protection</td>
</tr>
<tr>
<td>rs10485057</td>
<td>OPRM1</td>
<td>.89</td>
<td>.094</td>
<td>A:G</td>
<td>.87</td>
<td>.02 .41</td>
<td>1:13:86 1:14:85</td>
<td>Protection</td>
</tr>
<tr>
<td>rs1074287</td>
<td>OPRM1</td>
<td>.90</td>
<td>.256</td>
<td>A:G</td>
<td>.99</td>
<td>-.04 .15</td>
<td>9:34:57 8:29:63</td>
<td>Risk</td>
</tr>
<tr>
<td>rs511420</td>
<td>OPRM1</td>
<td>.87</td>
<td>.097</td>
<td>T:C</td>
<td>.99</td>
<td>-.04 .09</td>
<td>2:18:80 1:17:83</td>
<td>Risk</td>
</tr>
</tbody>
</table>

Table 4.1.7: Frequency of Selected Male Features

<table>
<thead>
<tr>
<th>Domain</th>
<th>Feature</th>
<th>Analysis Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\geq 1x$</td>
</tr>
<tr>
<td>Psychosocial</td>
<td>Lifetime Cigarette Use</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Parental Cannabis Use</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Feelings of Deviance</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Lifetime Alcohol Use</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Sensation Seeking Personality (Parent)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Disorderly Personality</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Novelty Seeking Personality</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Novelty Seeking Personality (Parent)</td>
<td>28</td>
</tr>
<tr>
<td>Structural MRI</td>
<td>L. Mid-Cingulate Cortex</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>R. Medial Prefrontal Cortex</td>
<td>0</td>
</tr>
<tr>
<td>Functional MRI</td>
<td>Stop Success: R. Midbrain-Thalamus</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Stop Success: L. Inferior Temporal Gyrus</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>Stop Success: L. Post-Lateral Hemisphere</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Stop Success: L. Anterior Cerebellum</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Stop Success: L. Paravermis</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Neutral Faces: R. Midbrain-Thalamus</td>
<td>0</td>
</tr>
</tbody>
</table>

Count of the number of runs (out of 100) that a predictor was selected in at least 6 of 10 final models.
### Table 4.1.8: Frequency of Selected Female Features

<table>
<thead>
<tr>
<th>Domain</th>
<th>Feature</th>
<th>Analysis Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≥1x</td>
</tr>
<tr>
<td>Psychosocial</td>
<td>Lifetime Cigarette Use</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Lifetime Alcohol Use</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Novelty Seeking Personality</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Parental Cannabis Use</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Extravagant Personality (Parent)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Feelings of Deviance</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Disorderly Personality</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Verbal IQ</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Impulsive Personality</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Frequency of Sexual Life Events</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Extravagant Personality</td>
<td>33</td>
</tr>
<tr>
<td>Structural MRI</td>
<td>R.Pre-Supplementary Motor Area</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>R.Middle Frontal Gyrus</td>
<td>0</td>
</tr>
<tr>
<td>Functional MRI</td>
<td>Stop Success: L. Orbital Frontal Cortex</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Stop Success: R. Orbital Frontal Cortex</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Stop Success: R.Middle Temporal Gyrus</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Stop Success: R.Middle Temporal Gyrus</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Stop Failure: L.Middle Temporal Gyrus</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Stop Failure: R.Post-Central Gyrus</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Stop Failure: R.Inferior Frontal Gyrus</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Stop Failure: R.Pre-Supplementary Motor Area</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Stop Failure: L.Lateral Paravermis</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Stop Failure: L.Pre-Post Central Gyrus</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Angry Faces: R.Anterior Cerebellum</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Angry Faces: L.Ventromedial Prefrontal Cortex</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Neutral Faces: R. Superior Frontal Gyrus</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Reward Anticipation: L.Middle Frontal Gyrus</td>
<td>100</td>
</tr>
</tbody>
</table>

Count of the number of runs (out of 100) that a predictor was selected in at least 6 of 10 final models.
Table 4.1.9: Analysis of Head Motion

<table>
<thead>
<tr>
<th>Sex</th>
<th>Task</th>
<th>Mean Framewise Displacement: Age 16 Users vs. Comparison Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Age 16 Users vs. Comparison Group</td>
</tr>
<tr>
<td>Males</td>
<td>Faces</td>
<td>$t_{720} = -0.73, p &gt; .05$</td>
</tr>
<tr>
<td></td>
<td>MID</td>
<td>$t_{684} = -0.85, p &gt; .05$</td>
</tr>
<tr>
<td></td>
<td>Stop Signal</td>
<td>$t_{669} = -1.69, p &gt; .05$</td>
</tr>
<tr>
<td>Females</td>
<td>Faces</td>
<td>$t_{806} = -2.09, p = .04$</td>
</tr>
<tr>
<td></td>
<td>MID</td>
<td>$t_{772} = -0.22, p &gt; .05$</td>
</tr>
<tr>
<td></td>
<td>Stop Signal</td>
<td>$t_{765} = -1.00, p &gt; .05$</td>
</tr>
</tbody>
</table>

Framewise displacement was calculated from the six-directional head motion parameters estimated during image realignment. 2-sample $t$-tests on the participants endorsing any 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, with the exception of the faces task for females. The modest motion effect detected for the faces task in females is driven by outliers in the comparison sample. Exclusion of these participants does not affect predictive model performance. Furthermore, the faces task predictors were lower activity (with one exception) in the cannabis use sample therefore, any motion effects are likely non-influential.
First, data are divided into $k(10)$ outer-folds. $k$-1 outer-folds are then divided into $k(10)$ nested subfolds. Elastic-net regularized logistic regression applied to $k$-1 subfolds, during which the $\alpha$, $\lambda$ parameters are tuned by finding the optimal pair returning the highest AUC when it’s model is tested on the $k$th subfold. The iterative process is completed for the $k(10)$ subfolds, generating 10 final nested models. The 10 nested models are ranked by their AUC returned when tested on each respective test-fold. The highest-ranking model is then tested on the outer fold, and used to generate the reported test AUC. This process is repeated $k$-times, and the entire procedure executed 100 times.
Figure 4.1.2: Mean Receiver-operating characteristic (ROC) AUC For Each Use Level by Sex

Mean ROC AUC indicates the performance of the predictive models on independent samples across 100 runs for each use level by sex.
Panels A&B: Brain regions where age 16 cannabis users displayed higher average group-level activation or gray matter volume relative to their non-using peers. **Panel A:** Male Specific Predictive ROIs. Stop Success refers to successful inhibition trials minus implicit baseline during the stop signal task; ROI (red) in left inferior temporal gyrus. GMV ROI (yellow) in right medial prefrontal cortex. **Panel B:** Female Specific Predictive ROIs. Stop Failure refers to failed inhibition trials minus implicit baseline during the stop signal task; ROIs (pink) in left lateral paravermis, left midbrain, left pre- & post-central gyrus, right post-central gyrus. Angry Faces refers to passive viewing of angry faces minus control images; ROI (orange) in left ventromedial prefrontal cortex. Reward Anticipation refers to the processing of monetary reward cues; ROI (dark green) in left middle frontal gyrus. Stop Failure & GMV overlapping ROI (purple) in right pre-supplementary motor area.
Panels C&D: Brain regions where age 16 cannabis users displayed lower average group-level activation or gray matter volume relative to their non-using peers.

Panel C: Male Specific ROIs. Stop Success ROIs (dark blue) in left cerebellum include the anterior cerebellum, paravermis, and posterior-lateral portion of the left hemisphere. GMV ROI (bright green) in left middle cingulate. Neutral Faces (passive viewing of neutral faces minus control images) & GMV overlapping ROI (teal) in right midbrain with extent into thalamus.

Panel D: Female Specific ROIs. Angry Faces ROI (light blue) in right cerebellar tonsil. Stop Success ROIs (dark blue) in bilateral orbitofrontal cortex and two contiguous regions in the right middle temporal gyrus. GMV ROI (bright green) in right middle frontal gyrus. Neutral Faces ROIs (maroon) in right superior frontal gyrus and lingual gyrus. Stop Failure ROI (dark yellow) in right inferior frontal gyrus.
Figure 4.1.4: Correlations Between Identified Predictors and Outcome Measure by Sex.

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). Triangles=personality (from TCI & SURPS). Squares=Life Event (from LEQ). Pentagon=Verbal IQ. Diamonds = Neuroimaging data.
Figure 4.1.5: Receiver-operating characteristic (ROC) mean AUC for Gene-specific analysis

ROC AUC indicates the performance of the predictive models on independent samples. This plot visualizes the mean AUC across 100 runs for each use level collapsed across sex.
Table 4.1.6: Correlations Between Identified SNPs and Outcome Measure by Sex.

<table>
<thead>
<tr>
<th>GENE</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rs1042711</td>
<td>rs1042711</td>
</tr>
<tr>
<td></td>
<td>rs1801704</td>
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</tr>
<tr>
<td>ADRB2</td>
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<td>rs6888306</td>
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<tr>
<td>ADRA1b</td>
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<tr>
<td>DRD1</td>
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<td>rs11746641</td>
</tr>
<tr>
<td>OPRM1</td>
<td>rs563649</td>
<td>rs563649</td>
</tr>
<tr>
<td></td>
<td>rs10485057</td>
<td>rs10485057</td>
</tr>
<tr>
<td></td>
<td>rs1074287</td>
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<tr>
<td></td>
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</tbody>
</table>

Pearson’s point-biserial correlation ($r$) between SNP and outcome. Error bars represent 95% confidence intervals generated from 5000 bootstrap samples.
4.2: Predicting The Initiation Of Cannabis Use By Age 19: Exploratory Analyses

Introduction

As discussed in Chapter 1, early age of onset for cannabis use predicts worse functional outcomes later in life (Lisdahl et al., 2013). Therefore, predicting initiation by age 16 was likely the most impactful cannabis prediction analysis that can be conducted using the IMAGEN dataset. Those analyses provided evidence that cannabis use by age 16 is reliably predicted using multi-domain data collected at age 14 prior to exposure. Results from those analyses identified sex- and drug-specific psychosocial and neurobiological predictors that might inform etiological mechanisms and provide targets for interventions related to cannabis use by age 16.

As the IMAGEN study also contained assessments at age 19, a natural extension of this line of inquiry is to also predict cannabis initiation by age 19. If the age 16 predictors identified from Chapter 4.1 (future cannabis users vs. all non-users) were reproduced, then that would underscore their predictive validity. A reproduction might also be used to characterize those features as trait-like predictors, meaning, those features are predictive of cannabis use regardless of the age of initiation. Alternatively, if the predictors were not reproduced, that might highlight their specificity to early initiation.

These analyses were conducted for exploratory purposes to test if any level of cannabis use by age 19 can be predicted using multi-domain data collected at age 14, regardless of the future use level. Hence, the technique in Chapter 4.1 that involved predicting each increasing use level was not used here. That technique dramatically added complexity to an already complicated analysis, but was justified in light of the
importance to identify the predictors of early initiation. Moreover, the majority of the
predictors from Chapter 4.1 were identified from the most lenient cannabis use threshold
(ESPAD 1>=1; Tables 4.1.7-8). Therefore, these exploratory analyses reported here were
most likely to identify the greatest number of predictors to compare to the predictors from
Chapter 4.1. Lastly, setting a low threshold to include all future cannabis users also
provided the most statistical power.

Methods

Participants

Participants from the IMAGEN study were selected based on their reported drug
use levels at all time points from the ESPAD survey (Hibell et al., 1997). From the full
baseline sample (N=2,224), there were n=648 (Females n=398) identified as being
cannabis-naïve at all time points and were therefore used in the comparison groups. Next,
there were n=313 (Females n=145) who were cannabis-naïve at age 14 and 16 and then
reported any cannabis use by age 19. In keeping with the theme of sex-specificity, the
samples were separated by sex. See Table 4.2.1 for demographic information for each
group including their cannabis use levels by age 19.

Data Analysis

Similar data analytic procedures from the preceding section were used here. In
brief, k(10)-fold cross-validated logistic regression with elastic-net regularization was
used to predict cannabis initiation by age 19 separately for each sex. All predictors were drawn from the age 14 assessment only.

As these analyses were exploratory in nature, the increasing use level technique was not used here. Therefore, the analysis was designed to predict any amount of cannabis use (ESPAD >1) by age 19 vs. controls. The predictors selected from these analyses were identified using the same threshold as Chapter 4.1 (i.e., present in at least six final models (from k=10) across all 100 runs). Finally, sex-specificity was assessed in a similar fashion by including the selected predictors in a post-hoc logistic regression model estimated on the opposite sex.

Results

Prediction Accuracies

Prediction model accuracies indicated a significant prediction of age 19 cannabis use in females (mean ROC AUC=.63, $p=1.8\times10^{-6}$), but not males (mean ROC AUC=.52, $p>.05$).

Feature Selection

When probing the results from the female-specific analysis, only two predictors passed feature selection threshold and they were higher baseline alcohol use and novelty seeking personality (Cloninger, 1999). The results from the male-specific analysis were not probed given the null prediction accuracies.
**Sex-specificity**

In keeping with the methods of Chapter 4.1, the two psychosocial predictors (alcohol and novelty seeking personality) from the female specific analysis were used to estimate a post-hoc logistic regression model on the male sample. Findings indicated that these two psychosocial predictors returned strong model fits when tested on the full male sample ($\chi^2_{2,N=418}=13.5, p=.001; \Delta AIC=9.4$). As there were only two predictors, the regression coefficients were probed in an exploratory fashion. Findings indicated only novelty seeking personality emerged as a significant predictor of cannabis use by age 19 for males (Adj.OR 1.03 [95% CI: 1.01-1.05], $p<.05$).

**Discussion**

These results indicated that age 19 cannabis use can be predicted for females but not males using machine learning applied to a large set of multi-domain data collected at age 14. The predictive profile identified from the female-specific analysis contained two psychosocial predictors: higher baseline alcohol use and novelty seeking personality. Tests for sex-specificity indicated that novelty seeking personality generalized to predict cannabis use by age 19 in males.

Here, males were not predicted using machine learning, but were predicted using the single novelty seeking personality predictor. This may have been due to the loss of statistical power incurred when the sample was split during cross-validation prior to model estimation. As the post-hoc model was estimated on the full sample of males, the
modest odds ratio and confidence interval might reflect this subtle effect was observed due to the increase in statistical power.

Baseline alcohol predicted cannabis use by age 19 for females, but not males. This finding is consistent for females relative to the results in Chapter 4.1 and suggests higher baseline alcohol use is a trait-like characteristic that predicts cannabis use throughout adolescence and into adulthood.

In these analyses, novelty seeking personality predicted females using machine learning, and predicted males in a post-hoc fashion. Novelty seeking personality was also identified as a predictor common across the sexes in Chapter 4.1. This converging evidence indicated that novelty seeking personality is also trait-like, and confers risk for cannabis use regardless of sex or age of initiation.

Translating these findings into treatment is challenging, however, personality-targeted intervention programs may be useful (Conrod, 2016). These interventions are grounded in cognitive-behavioral therapies, and are tailored to the individual following measurement on the substance use risk profile scale (SURPS) personality instrument (Woicik et al., 2009). Although SURPS data was collected in IMAGEN and used in all the machine learning analyses here, only the Temperament and Character-Inventory (TCI) (Cloninger, 1999) measurement of novelty seeking personality was identified as predictive. The SURPS measurement that is broadly in line with novelty seeking personality is the sensation seeking personality trait, which measures sensitivity to rewards (Woicik et al., 2009).
Personality-targeted intervention programs have been effective in adolescent samples. Mahu and colleagues tested this intervention technique at high schools with students who were designated high risk for cannabis use (Mahu et al., 2015). Following a brief personality-targeted intervention in 9th grade (ages 13-14), adolescents were followed up every six months for two years. Authors reported that children from schools receiving the intervention exhibited a reduction in cannabis use frequency 12 and 18 months later compared to control schools. Of note, authors also reported that individuals receiving a targeted intervention for an elevated sensation seeking personality demonstrated a delay in the onset of cannabis use (Mahu et al., 2015). Therefore, targeting novelty seeking personality traits in a similar fashion as the Mahu report might be effective in altering the risk phenotype for both males and females. A delay in the onset of use might generalize to the samples characterized using the TCI novelty seeking personality trait following a similar personality-targeted intervention.

These analyses used only the predictors measured at age 14. The IMAGEN study also assessed participants at age 16. Future analyses could incorporate the age 16 data, and could, for example, also incorporate changes in relevant measures from age 14 to 16. For example, the life events questionnaire at age 16 (Newcomb et al., 1981) is expected to reflect the stressful life experiences that are more proximal in time to cannabis use at age 19. Moreover, the difference in those measures would reflect severity of change and is hypothesized to better predict cannabis use. Likewise, baseline cigarette use was a strong predictor in Chapter 4.1, but not reproduced here. It would be interesting to test if an increase in cigarette use at age 16 predicts cannabis use by age 19 as the literature
commonly reports a correlation between those two drugs (Agrawal et al., 2012). Finally, the brain data were absent from these results. Future analyses could also incorporate the age 14 brain data with the age 16 psychosocial data to perform mediation analyses linking the brain to behavior two and five years later. For example, a path from one the baseline predictors of cannabis use uncovered in Chapter 4.1, might predict performance on a cognitive measure at age 16, which in turn, might predict cannabis use at age 19.
References


Table 4.2.1: Participant Demographic Information by Sex

<table>
<thead>
<tr>
<th>Measure</th>
<th>Males (N=418)</th>
<th></th>
<th></th>
<th>Females (N=543)</th>
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<tbody>
<tr>
<td></td>
<td>Cannabis Use by Age 19 (n=168)</td>
<td>Comparison Group (n=250)</td>
<td>P</td>
<td>Cannabis Use by Age 19 (n=145)</td>
<td>Comparison Group (n=398)</td>
<td>P</td>
</tr>
<tr>
<td>Age (M,SD)</td>
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<td>14.5, 0.42</td>
<td>.30</td>
<td>14.5, 0.4</td>
<td>14.6, 0.4</td>
<td>.11</td>
</tr>
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<td>Handedness (L,R)</td>
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<td>29, 221</td>
<td>.38</td>
<td>10, 135</td>
<td>39, 359</td>
<td>.30</td>
</tr>
<tr>
<td>PDS (M,SD)</td>
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<td>2.5, 0.56</td>
<td>.33</td>
<td>3.1, 0.5</td>
<td>3.2, 0.4</td>
<td>.16</td>
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<td>Perceptual IQ (M,SD)</td>
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<td>111.0, 15.1</td>
<td>.01</td>
<td>108.5, 13.2</td>
<td>109.3, 13.0</td>
<td>.52</td>
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<tr>
<td>Verbal IQ (M,SD)</td>
<td>112.3, 12.9</td>
<td>113.4, 13.1</td>
<td>.40</td>
<td>110.5, 12.7</td>
<td>110.4, 13.9</td>
<td>.97</td>
</tr>
<tr>
<td>SES (M,SD)</td>
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<td>18.2, 3.7</td>
<td>.54</td>
<td>18.7, 3.4</td>
<td>18.0, 3.6</td>
<td>.03</td>
</tr>
</tbody>
</table>

PDS=Puberty Development Scale (Carskadon and Acebo, 1993). SES=Socioeconomic Status. Cannabis Use Levels from the ESPAD and measured on an ordinal scale (1=1-2x, 2=3-5x, 3=6-9x, 4=10-19x, 5=20-39x, 6=40x+). All measures (with the exception of cannabis use) were obtained at age 14. All demographics measures were also included as predictors in feature selection analyses. The comparison group was cannabis-naïve at all time points.
CHAPTER 5: DIFFERENTIAL PREDICTION OF FUTURE CANNABIS USE VS. FUTURE BINGE DRINKING

Introduction

It is important to consider drug specificity in analyses identifying risk factors and predictors of a single substance as it is uncommon for individuals to only use one drug of abuse. For instance, data from a nationally representative sample of adolescents indicated that cannabis is commonly used in tandem with alcohol and tobacco and only 1% of individuals who use cannabis do so exclusively (Patrick et al., 2018). When considering cannabis and alcohol use together, Terry-McElrath and colleagues report that 13% of teens use the two drugs concurrently (defined as separate use occasions within the past 30 days), whereas 21% of teens use the two drugs simultaneously for overlapping intoxication (Terry-McElrath et al., 2014).

Alcohol is the most commonly used drug during adolescence (Johnston et al., 2018), however many teens with binge drinking episodes might not necessarily have experiences using cannabis. Instead, for the adolescents who use cannabis, it may be considered common for these teens to engage in excessive alcohol consumption. Therefore, when predicting future cannabis use in adolescence it is important to consider the extent to which teens also go on to binge drink. Evidence of concurrent drug use could undermine the strength of the study conclusions, as it is unclear if the prediction accuracy or predictors themselves are indicative of cannabis use per se or a likelihood to consume drugs, specifically alcohol, more generally.
Guided by this framework, the study in Chapter 4 contains an important caveat that the future cannabis users also reported some binge drinking by age 16. The examination of drug specificity in that Chapter involved testing the sex-specific predictors on an independent sample of future binge drinkers only. Therefore, a stricter test of drug specificity targeting a typical pattern of cannabis use is required.

To this end, a differential prediction analysis between the future cannabis use sample (which also contained some binge drinking) and future binge drinking without future cannabis use will be performed. These analyses are therefore much more rigorous than Chapter 4. In considering these very similar behavioral phenotypes, it is hypothesized that the prediction accuracy in these analyses will not be as large as those reported in Chapter 4.

Few investigations have used neuroimaging to explicitly predict or compare teens with concurrent cannabis use and binge drinking (“cannabis+binge drinking”) to teens with binge drinking only. While the study by Whelan and colleagues used very similar methods to classify and predict binge drinking, the authors compared binge drinking teens to a sample with very light alcohol use (maximum two lifetime alcohol uses, no binge drinking), and did not comment on the amount of co-occurring cannabis use in their samples (Whelan et al., 2014b). Three cross-sectional structural neuroimaging studies compared teens with cannabis+binge drinking experiences to teens with only binge drinking experiences. Medina and colleagues found that binge drinkers exhibited smaller left hippocampal volumes, and larger right-to-left hippocampal asymmetry relative to the cannabis+binge drinking groups (Medina et al., 2007b). Jacobus and
colleagues used diffusion tensor imaging and found the binge drinking only group had lower fractional anisotropy (a measure of white matter integrity) relative to teens with cannabis+binge drinking, in four major white matter tracts across the brain (Jacobus et al., 2009). Finally, Schweinsburg and colleagues used a working memory fMRI task and reported the cannabis+binge drinking group exhibited lower activations than the binge drinking only group in bilateral prefrontal and right temporal and occipital regions (Schweinsburg et al., 2005).

Together, these findings underscore the use of neuroimaging in delineating group differences between cannabis+binge drinking and binge drinking only adolescents. In both structural studies, authors posited that cannabis might have provided some neuroprotective properties relative to alcohol. In the fMRI study, authors suggested that the cannabis+binge drinking group might have compromised their attentional processing abilities. And while all authors were unable to assert causality, they were less inclined to interpret their findings as being predictive of use. Therefore, the analyses here are tailored to address a gap in the literature by identifying a generalizable predictive profile characterizing a typical pattern of cannabis use in adolescence.

Critically, the analysis in this Chapter will, by design, compare two groups who are matched on their levels of future binge drinking. The distinctive characteristic of one group will therefore be their future cannabis use. Any predictors that separate these two groups are hypothesized to be specific to the combination of future cannabis use and binge drinking. Moreover, as the results from Chapter 4 demonstrated that the shared psychosocial predictors of future cannabis use generalized to predict future binge
drinking, it is hypothesized that the psychosocial predictors will no longer discriminate the two groups, and instead, the discriminating predictors will be from the neuroimaging and/or genetic domains.

Methods

Participants & Data

All data were drawn from the IMAGEN study. Binge drinking levels reported by age 16 were tabulated for both the male and female samples of future cannabis use studied in Chapter 4 (now referred to as “cannabis+binge drinking”), as well as the binge drinking levels for the future binge drinking only group (See Figure 5.1). Participants from the starting samples of cannabis+binge drinking and future binge drinking groups were then randomly sampled without replacement to identify a reduced sample of two groups perfectly matched on future binge drinking levels. For example, in the male starting sample, there were 32 participants in the cannabis+binge drinking group, and 50 in the binge drinking only group, who reported a binge drinking level of 1-2x by age 16 (see Table 5.1 or Figure 5.1). Therefore, 32 participants from the binge drinking only group use level 1-2x were randomly selected for inclusion to match the size of the future cannabis+binge drinking group. Similar logic was executed for each use level for each sex. This procedure resulted in n=148 females (74 per group), and n=178 males (89 per group) who were matched on the level of future binge drinking. See Tables 5.2 and 5.3 for baseline demographic information and drug levels by age 16 for the samples used in the differential prediction analyses.
The same set of multi-domain predictors (2,412 total predictors) collected at baseline (age 14) was used in the differential prediction analyses, except for baseline alcohol use. The exclusion of baseline alcohol use was necessary so that data from the participants in the binge drinking only group were minimally confounded with consequences of early alcohol use. Thus, the future binge drinkers were restricted to have used alcohol a maximum of 1-2x in the life by age 14.

Analysis

Similar logistic regressions with elastic-net regularization (Zou and Hastie, 2005) as explained in Chapter 4 were applied separately for each sex. The main difference is that given the reduced sample sizes, a smaller $k$-fold cross-validation scheme was implemented ($k=5$). All other procedures applied, including the nested $k(5)$-fold cross-validation scheme to tune the alpha and lambda parameters for the elastic-net. Likewise from Chapter 4, these procedures were looped 100 times to account for the randomness of participants being assigned to the $k$-folds.

As preliminary analyses using regularized logistic regressions returned very sparse results for the male sample, the prediction analyses were conducted again using a random forest model (Breiman, 2001). This model was chosen based on the hypothesis that non-linear relationships between the predictors might better differentiate between two very similar behavioral phenotypes. When estimating the random forest models, a similar 5-fold cross validation scheme was implemented, including a nested 5-fold cross validation scheme for random forest parameter tuning. These tuning parameters included
the number of decision trees (100 to 500 trees), and the number of samples required for a "leaf" node (defined as the minimum number of samples to be contained within a partition of the decision tree). Generally, more decision trees the better, however, an excessive number of decision trees risks fitting many trees using poor predictors. The minimum samples per leaf may be considered a method to resist overfitting. By requiring a larger number of samples in a leaf, the decision tree is restricted from becoming overly complex and fitting to the noise and nuances of small samples. As above, the entire analytic scheme was run 100 times to account for the random assignment of participants to folds.

After running the logistic regression and random forest analyses separately for each sex, the predictors were evaluated for sex-specificity using post-hoc analyses. Predictors identified from the logistic regression analyses for one sex were used in a post-hoc regression model to predict the opposite sex. For the results of the random forests, in keeping with tree-based estimators, the predictors for one sex were used to estimate a single decision tree to predict the opposite sex. Prediction model accuracy was evaluated for all scenarios using the area under the receiver-operating characteristic curve (ROC AUC). Null-hypothesis significance testing on the ROC AUC was conducted using a Mann–Whitney U-test (Mason and Graham, 2002).

Results

Drug Use Levels
The exact levels of future binge drinking in the cannabis+binge drinking group and the future binge drinking only group was plotted for each sex (see Figure 5.1). The distribution of binge drinking within the cannabis+binge groups was nearly uniform across the use levels for males and females, whereas the future binge drinking only groups were more skewed to lighter binge drinking. These data suggest that while it is likely for future cannabis users to also binge drink, the patterns of use are not consistent across the two drugs. Therefore, identifying subsamples of individuals matched on binge drinking (described below) removes the confound of predicting co-occurring use, and substantiates future cannabis use as the defining outcome measure.

Prior to the pseudo-random selection process to match groups on future binge drinking levels, it was found that 89% of females, and 91% of males in the future cannabis using groups from Chapter 4 also indicated some level of future binge drinking. Therefore, 11% and 9% of participants for each sex who had not initiated any binge drinking were initially excluded from the sample. Of the remaining samples, participants were randomly sampled without replacement from the larger group within each binge drinking level (described above). This procedure yielded n=178 males (89 per group; see Table 5.2) and n=148 females (74 per group; see Table 5.3) who were then passed forward to the differential prediction analysis (See Figure 5.2 for graphs of matched samples use levels). For each sex, chi-square and t-test analyses on various baseline demographic measures indicated the two groups did not differ on age, handedness, pubertal development, SES or IQs (with the exception of lower performance IQ in the
male cannabis+binge drinking group, see Table 5.3). Nonetheless, all demographics were also included as candidate predictors in the analysis.

Predictive Models Performance

When evaluating the logistic regression differential prediction analyses on independent samples, results indicated significant predictions in the female sample: mean ROC AUC=.6567 \((p<5.92\times10^{-6})\), and the male sample: mean ROC AUC=.6140 \((p<4.6\times10^{-4})\). When evaluating the random forest prediction analyses on independent samples, results were nearly identical for females: mean ROC AUC=.6593 \((p<4.23\times10^{-6})\) and consistent for males: mean ROC AUC=.6286 \((p<9.25\times10^{-5})\), relative to the AUCs from the logistic regression analyses. Hence, the prediction accuracy was consistent across the two machine learning algorithms.

Permutation Tests

The AUCs reported above may be considered modest relative to those reported in Chapter 4. To further investigate the prediction accuracies for these analyses, a non-parametric test on the significance of the ROC AUCs was performed. Here, the prediction analyses were run an additional 100 times while randomly shuffling the group labels. These analyses empirically derive a distribution of ROC AUCs over the null hypothesis. The ROC AUCs from the true label analysis are then compared to this null distribution. Given the very similar AUCs across the two machine learning algorithms, the permutation analyses were conducted using the random forest algorithm.
These permutation analyses returned a mean ROC AUC=.50 for both males and females, indicating the models failed to predict the two randomly labeled groups better than chance. For both males and females, the true label AUC results for both the logistic and random forest models were greater than 95 of the 100 ROC AUC derived from the random label permutation analysis ($p<.05$). Taken together, these results provide strong supporting evidence that for each sex, the two groups were distinct and the performance of the prediction analyses were significantly above chance levels.

*Features Selected*

As the differential prediction models were fitted within a 5-fold cross validation framework (thus supplying 5 final models), and looped 100 times, a threshold was necessary to perform feature selection. Therefore, in keeping with the threshold from Chapter 4, a feature must have been present in at least half of the final models (here, a minimum 3 of 5) across all 100 runs. With this threshold in place, the logistic regression analyses identified four features for females, and one feature for males. After interrogating the random forest analyses, five features for females, and three features for males were identified.

Starting with the four female features from the logistic regression analyses, higher baseline cigarette use and less negative feelings towards deviant behaviors in the future cannabis+binge drinkers were the two psychosocial predictors identified. From the brain domain, higher activations in the left superior cerebellum during reward anticipations was identified in the cannabis+binge drinking group (Figure 5.3). The final predictor was
rs521674, a SNP on a gene coding for the alpha-2A adrenergic receptor, with a higher number of risk alleles present in the future binge drinking only group. See Table 5.4 for summary of SNP rs521674 allele frequencies by group.

Moving to the five female features identified from the random forest analyses, only higher baseline cigarette use was reproduced from the logistic results. The other four predictors were brain predictors from the stop signal task. During stop success, lower activations in the right inferior temporal and paracentral lobule, and left middle frontal gyrus, were identified in the cannabis+binge drinking group. Finally, during stop failures, lower activation in the left inferior frontal gyrus was identified in the cannabis+binge drinking group. See Figure 5.3 for all female brain predictor results.

Considering the male results, only higher baseline cigarette use in the future cannabis+binge drinking group was identified from the logistic regression analyses. For the random forest analyses, higher baseline cigarette use was also observed, and less negative feelings towards deviant behaviors in the cannabis+binge drinking group was identified. Finally, higher gray matter volume in the right inferior temporal lobe was identified in the cannabis+binge drinking group (Figure 5.4). Interestingly, this was the same ROI that exhibited lower activations during stop success trials for future female cannabis+binge users.

**Sex-Specificity**

Like Chapter 4, the differential prediction analyses returned a unique set of brain predictors for each sex. Sex-specificity was tested in a similar manner as Chapter 4, such
that a *post-hoc* logistic regression model using the female-specific brain predictors was estimated on the entire female sample, and then tested on the entire male sample. This was done only for females because the male analysis identified only one shared predictor (baseline cigarette use) using a logistic regression model.

To begin, estimating a *post-hoc* logistic regression model using only the cerebellar ROI during reward processing returned a ROC AUC=.65 (\(p<8.2\times10^{-4}\)) when tested on the entire female sample, and a ROC AUC=.48 (\(p>.05\)) when tested on the entire male sample. Thus, cerebellar activations to rewards may be considered a female-specific differential predictor of future cannabis+binge drinking. For reference, estimating a *post-hoc* logistic regression model using all the female-specific predictors (feelings of deviance, SNP rs521674, and the cerebellar ROI) returned a ROC AUC=.80 (\(p<1.5\times10^{-10}\)) when tested on the entire female sample. When tested on males, that model returned a ROC AUC=.61 (\(p<.05\)). This modest prediction is likely driven by the feelings toward deviant behaviors predictor, which was identified for males using the random forest model, and approached selection threshold for the male logistic model (64 of 100 runs). See Table 5.5 for the number of runs each predictor was selected by the alternate analytic model.

Turning to the predictors uncovered from the random forest analyses, sex-specificity was assessed in a similar way using a decision tree. Here, a *post-hoc* decision tree using the four female-specific brain predictors from the stop signal task was estimated on the females. This decision tree returned a ROC AUC=.83 (\(p<2.1\times10^{-12}\)) when tested on the full female sample, and failed to predict better than chance when
tested on the full male sample, ROC AUC=.53 \((p>.05)\). Taken together with the result of the cerebellar ROI from the logistic regression analysis, all brain predictors for females, regardless of the model from which they were identified, exhibit clear sex-specificity in differentially predicting future cannabis use vs. binge drinking.

For the male results, only the random forest identified a brain predictor. A single post-hoc decision tree was estimated on the male sample using the GMV ROI. This decision tree returned a ROC AUC=.74 \((p<1.6\times10^{-8})\) when tested on the full male sample, and failed to predict better than chance when tested on the full female sample, ROC AUC=.49 \((p>.05)\). Hence, the random forest identified brain predictor exhibited sex-specificity for males. For reference, estimating a single post-hoc decision tree using the two male-specific predictors (feelings towards deviant behaviors and the GMV ROI) returned a ROC AUC=.76 for males \((p<1.5\times10^{-9})\). This decision tree predicted marginally better than chance when tested on the full female sample, ROC AUC=.57 \((p<.05)\), and was likely driven by the feelings towards deviant behaviors predictor.

Comparison of Predictors Identified from Random Forests vs. Logistic Regression

With the exception of baseline cigarette use, the predictors identified from the random forest were different than the predictors identified from the logistic regression. A likely interpretation of these discrepant findings is that the random forest is capable of modeling predictors exhibiting both linear and non-linear relationships. To assess this possibility, the predictors identified form the random forest analyses were used to estimate a post-hoc logistic regression model and a decision tree for each sex. It was
hypothesized that the AUCs for the logistic regression models would be lower than the
decision trees, thus supporting the argument that the tree-based estimators achieve their
results by capturing non-linearities among the features.

The four female-specific brain predictors identified from the random forest
analysis were first used in a post-hoc logistic regression model on the full female sample.
This model returned a ROC AUC=.71 \((p<5.2\times10^{-6})\). Using these predictors in a post-hoc
decision tree returned a ROC AUC=.83 \((p<2.1\times10^{-12})\). Similarly for males, the one male-
specific brain predictor identified from the random forest model was used to estimate a
post-hoc logistic regression model on the full male sample. This model returned a ROC
AUC=.70 \((p<2.4\times10^{-6})\). Using this predictor to estimate a post-hoc decision tree returned
a ROC AUC=.76 \((p<1.5\times10^{-9})\).

In all cases, using predictors identified from the random forest analyses returned
inferior prediction in a logistic regression model relative to the decision tree model.
Interestingly, the post-hoc logistic regression models were still highly significant when
predicting each sample, although to a lesser extent than the decision trees. These results
indicate that tree-based estimators are more flexible in modeling relationships. And while
the predictors uncovered from the random forests exhibit some degree of non-linearity (as
the post-hoc decision tree returned superior performance), they also exhibit linear
relationships as the logistic regression also return highly significant prediction. Post-hoc
analyses using predictors identified from the logistic regression analyses to estimate
decision trees were not conducted. This is because decision trees are robust in capturing
linear relationships and will inevitably perform as well as a logistic regression.
Influence of Baseline Cigarette Use

Given the finding that baseline cigarette use was the most robust predictor, the logistic regression and random forest analyses were executed again with this variable excluded. Any fluctuation in the ROC AUC would indicate the extent to which the prediction accuracy is dependent on baseline cigarette use. Additionally, the dominant relationship between baseline cigarette use and the outcome measure might interfere with the likelihood of other predictors being selected within the context of the regularization procedure. As regularization favors sparse models, the predictors that were less correlated with the outcome measure were unlikely to coexist in a model containing baseline cigarettes. This problem is less of an issue with random forests, but similar logic applies if baseline cigarettes was part of the randomly selected set of predictors. Therefore, rerunning the analyses without cigarettes might permit discovery of novel predictors.

For females, the logistic regression analyses returned a mean ROC AUC=.6196 ($p<.01$), whereas the random forest analyses returned a mean ROC AUC=.6077 ($p<.05$). Relative to the original analyses with baseline cigarette use included, the logistic regression model was slightly more resilient ($\Delta$AUC=.0371) than the random forest ($\Delta$AUC=.0516). These results indicate that baseline cigarette use is important, but not critical, to differentially predict future cannabis+binge drinking in females. Probing the logistic regression analyses further, all three predictors identified from the original analyses (feelings of deviance, SNP rs521674, and the cerebellar ROI during reward outcome) passed threshold for feature selection, along with 12 new predictors across all
domains. Probing the random forest analyses, only two of the four stop task ROIs were reproduced (right inferior temporal and left middle frontal gyrus during stop success) from the original analyses. One additional ROI during stop success (right cerebellum) and neutral face processing (left visual cortex) were identified from the random forest. In both scenarios, the models attempted to compensate for the loss of the best predictor through other predictors, as indexed by the many new predictors selected by the logistic regression analysis, and the lack of a consistency in the predictors identified by the random forests.

For the male sample, a different finding emerged. The logistic regression analyses returned a mean ROC ACU=.49, and the random forest returned a mean ROC AUC=.54 ($p>.05$). Therefore, the exclusion of baseline cigarettes eliminated the ability of either model to significantly predict between the two groups. For exploratory purposes, the predictors selected from these non-significant models were probed to determine if there was any consistency with the original analyses despite their non-significant prediction. Interestingly, both the right temporal GMV ROI and feelings towards deviant behaviors predictors were identified as the top predictors across both of the non-significant analyses, along with frequency of sexual life events. These results indicated that GMV ROI and feelings towards deviant behaviors predictors explained a very small portion of the variance, and must be modeled with baseline cigarette use to predict between the groups. Frequency of sexual life events was identified presumably as a proxy for baseline cigarette use as these two predictors were correlated in males ($r=.29, p<.001$).
Discussion

The analyses reported here better model the predictive profile of teenagers who use cannabis in adolescence. As it is rare for an adolescent to use cannabis exclusively, these prediction analyses target a typical pattern of adolescent cannabis use by differentially predicting cannabis and binge drinking versus binge drinking only. This chapter was also distinguished by its exploratory use of two competing machine learning algorithms. As elastic-net regularized logistic regression was used successfully in Chapter 4 to develop predictive profiles containing brain and behavioral data, the use of regularized regression in this chapter returned very sparse profiles. While these sparse results here may seem in conflict with Chapter 4, those results were an amalgamation of six analyses predicting each increasing use level. Implementing a similar technique for the differential predictions outlined here would not be feasible due to the very low sample sizes within each use level.

Starting with the cross-validated prediction accuracies, the random forest analyses returned surprisingly similar ROC AUCs relative to the logistic regression analyses, indicating one model is not necessarily superior to the other. This is beneficial in that both results may be considered a form of internal replication, and reaffirms the significant differential prediction between the two groups. And while both analyses returned a very sparse set of shared and unique predictors within each sex, the difference between the predictors identified is likely due to the flexibility of the random forest model.

Higher baseline cigarette use predicted future cannabis+binge drinking across all models and sexes. This predictor was also identified in Chapter 4 as being predictive of
cannabis use vs. controls for both sexes. These consistent results across samples, models, and sexes provided strong evidence that cannabis use in adolescence is likely to follow from early cigarette use. The similarities in route of administration for the two drugs may influence transition from one to the other as experiences smoking a cigarette may facilitate smoking cannabis.

Epidemiological data indicated a downward trend in cigarette use in teens (Johnston et al., 2018), and researchers are just starting to study how these trends might impact cannabis use. Miech and colleagues assert that despite the observed decrease in cannabis risk perception, a hypothesized increase in cannabis use has not been observed due to the decrease in cigarette use levels (Miech et al., 2017). This interpretation suggests lowering cigarette use would be protective against cannabis use. Therefore, policies and programs designed to discourage cigarette use in youths are already showing efficacy in helping to delay cannabis use. However, considering the change in landscape regarding e-cigarette use, it will be important to monitor how e-cigarettes might influence cannabis use in adolescence.

The other identified psychosocial predictor was less negative feelings towards deviant behaviors taken from the life events questionnaire (Newcomb et al., 1981). This measure asks the teen to assign a valence score to the idea of engaging in deviant behaviors (e.g., stealing something valuable). Given the nature of this measure, the finding reported here is likely signaling a propensity for poor conduct. This interpretation is consistent with Pedersen and colleagues who demonstrated that subclinical conduct
disorder predicted cannabis initiation in adolescence for both sexes, with a stronger association reported for females (Pedersen et al., 2001).

Interestingly, the feelings towards deviant behaviors predictor were identified for both sexes but using different models (Females: logistic regression; Males: random forests). For males, this predictor was selected less often during the logistic regression model (64 of 100 runs) but nearly passed threshold for females with the random forest model (95 of 100; Table 5.5). Post-hoc analyses supported these trends, and indicated the inclusion of this predictor in a decision tree modestly improved prediction for males ($\Delta$AUC=.02), whereas the inclusion of this predictor (and rs521674) in a logistic regression model dramatically improved prediction for females ($\Delta$AUC=.15).

Given the superiority of the post-hoc logistic model predicting cannabis use in females relative to the decision tree predicting cannabis use in males, the relationships between the feelings toward deviant behavior predictor and the other variables may be characterized as linear for females, and non-linear for males. This result underscores the use of the random forest model, as one might have assumed feelings toward deviant behaviors to be female-specific if only a linear model was used. As Chapter 4 only used a logistic regression model for both the prediction analyses and post-hoc tests, it is possible that some of the sex-specific predictors for one sex might generalize to the opposite sex if non-linear model was used. This finding highlights the need for prediction analyses to consider both types of relationships when modeling human behavior.

As baseline cigarette use and less negative feelings toward deviant behaviors were also identified in Chapter 4 and generalized to predict binge drinking, it is unusual that
they would also emerge as differential predictors here. Indeed, this effect counters the initial hypothesis that the differential predictors will be from the brain and genetic domain. Given this effect, it is likely the case that there is a linear relationship with these two psychosocial predictors and the three samples (controls, binge drinking only, and cannabis+binge drinking). In other words, the controls have the lowest levels on these two measures, followed by the future binge drinking only, with the cannabis+binge drinking groups exhibiting the highest levels. This pattern would explain why these two measures are consistently identified as a predictor of cannabis use relative to the two comparison samples.

In support of the initial hypothesis, a unique finding for females was SNP rs521674 identified from the logistic model. For this SNP, more risk alleles were present in the future binge drinking only group (Table 5.5). This finding is in line with a study by Clarke and colleagues who reported a correlation between this SNP and a family history of alcoholism in a sample of individuals with alcohol use disorder (Clarke et al., 2012). And while the exact predictive mechanism is difficult to ascertain, it is likely that these future binge drinking only adolescents initiated alcohol abuse for different reasons, one of which being this genetic variation.

From the female brain results, the logistic analyses identified that the future cannabis+binge drinking group exhibited higher activations in the superior cerebellum during reward outcome (Figure 5.3). This predictor was also selected by the random forest model, albeit at a slightly lower threshold (91 out of 100 runs; Table 5.5). This finding is consistent with a previous neuroimaging study on adolescent binge drinking by
Cservenka, Jones, & Nagel (2015). In that study, lower activations during reward outcome in the left superior cerebellum correlated with binge drinking levels later in adolescence. Moreover, the peak voxel location reported in that study is contained in the ROI identified here. Therefore, this study adds converging evidence on blunted reward processing in the cerebellum as predictive of adolescent binge drinking. And while the cerebellum is classically implicated during motor functioning, recent work has identified cortical-cerebellar circuits involved during cognitive and affective processing (Strick et al., 2009), and have incorporated the cerebellum into brain-based models of addiction (Moulton et al., 2014).

In the random forest analyses, all the brain predictors identified for females were from the stop signal task. When referencing the brain predictors from Chapter 4, 10 out of the 17 female brain predictors were from the stop task so this task may be especially useful in developing brain-based predictive profiles. The effects observed here were all lower activations in the future cannabis+binge group. This pattern is broadly consistent with Schweinsburg and colleagues who reported lower activations in prefrontal regions for cannabis+binge drinking adolescents (Schweinsburg et al., 2005).

The lower activations reported here might indicate that these individuals failed to recruit sufficient processing resources to execute inhibitory control behaviors. Lower activations in the left middle frontal gyrus and left inferior frontal gyrus were implicated during stop success and stop failures (respectively). This finding may suggest that in these individuals, the left hemisphere failed to supplement the predominantly right-sided “braking” system (Aron, Robbins, & Poldrack, 2014). Moreover, lower activations in the
right paracentral lobule and inferior temporal region have both been implicated in the motor (Zhang et al., 2015) and visual processing (Boehler et al., 2010) features of the stop signal task. Taken together, these results suggest that a compromised network of regions supporting inhibitory control constitutes a female-specific risk profile.

For males, the sole brain predictor was higher gray matter volume in the right inferior temporal gyrus (Figure 5.4). As healthy neurodevelopment is characterized by gray matter volume reduction over time (Giedd et al., 1999; Spear, 2000b), this effect might suggest a neurodevelopmental delay at this region. The finding of lower stop success activation in this same region for females might indicate that this delayed neurodevelopmental process only manifests as a functional difference later in life. As neurodevelopment begins sooner for females (Lenroot et al., 2007), this interpretation may explain why males exhibit structural differences, whereas females exhibit functional differences at this region during the age 14 scan. Future analyses could be conducted to determine if this functional difference is observed in the male sample using the age 19 assessment of the IMAGEN study.

The post-hoc analyses suggested that the brain predictors from the random forest analyses were the most robust predictors. For instance, for females, the highest ROC AUC was found using the predictors from the random forest in a post-hoc decision tree (ROC AUC=.83). Using the four female brain predictors identified from the random forest to estimate a post-hoc logistic regression model returned superior prediction (ROC AUC=.71) than using the single brain predictor identified from the logistic regression analyses in a post-hoc logistic regression model (ROC AUC=.65). Therefore, using non-
linear analyses (random forest) to inform a *post-hoc* linear model (logistic regression) was superior to a consistently linear model course of inquiry. These results indicate that looking across the *post-hoc* results, the four stop task brain predictors are superior predictors than the reward task predictor. And as the random forest analyses only uncovered brain predictors passing threshold for males, it can be concluded that random forests are better suited to identifying generalizable brain predictors.
References


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### Table 5.1: Future Binge Drinking Levels For Each Starting Sample by Sex

<table>
<thead>
<tr>
<th>Binge Drinking Level</th>
<th>Starting Samples to Later Match on Binge Drinking Levels</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (N=315)</td>
<td>Females (N=270)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Future Cannabis+Binge Drinking Group (n=208)</td>
<td>Future Binge Drinking Only Group (n=107)</td>
<td>Future Cannabis+Binge Drinking Group (n=159)</td>
<td>Future Binge Drinking Only Group (n=111)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>23</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1-2x</td>
<td>32</td>
<td>50</td>
<td>26</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>3-5x</td>
<td>33</td>
<td>30</td>
<td>25</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>6-9x</td>
<td>27</td>
<td>14</td>
<td>25</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>10-19x</td>
<td>29</td>
<td>9</td>
<td>32</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>20-39x</td>
<td>31</td>
<td>3</td>
<td>20</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>40+</td>
<td>33</td>
<td>1</td>
<td>17</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

These data reflect the amount of binge drinking within the starting samples. All Future Binge Drinking Only participants were cannabis naïve. Referencing these levels, two subgroups for each sex were randomly selected for differential prediction analyses. First, the 23 male and 14 female participants from the Future Cannabis+Binge Drinking sample with a binge drinking level of zero were excluded. Then, within each use level, the larger group was randomly subsampled to match the size of the smaller group (see Tables 5.2 and 5.3 for resulting analytic samples). See Figure 5.1 for graphical representation.
Table 5.2: Demographic Information and Drug Use Levels for Male Analytic Samples

<table>
<thead>
<tr>
<th>Measure</th>
<th>Males (N=178)</th>
<th>Future Cannabis+Binge Drinking Group (n=89)</th>
<th>Future Binge Drinking Only Group (n=89)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (M,SD)</td>
<td></td>
<td>14.4, .45</td>
<td>14.5, .38</td>
<td>.41</td>
</tr>
<tr>
<td>Handedness (L, R)</td>
<td></td>
<td>13.76</td>
<td>12, 77</td>
<td>.83</td>
</tr>
<tr>
<td>PDS (M,SD)</td>
<td></td>
<td>2.6, .49</td>
<td>2.5, .55</td>
<td>.12</td>
</tr>
<tr>
<td>Verbal IQ (M,SD)</td>
<td></td>
<td>114.3, 14.7</td>
<td>110.2, 13.8</td>
<td>.06</td>
</tr>
<tr>
<td>Performance IQ (M,SD)</td>
<td></td>
<td>108.5, 15.4</td>
<td>110.2, 13.8</td>
<td>.04</td>
</tr>
<tr>
<td>SES (M,SD)</td>
<td></td>
<td>18.7, 4</td>
<td>17.9, 3.7</td>
<td>.16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug Use Level</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binge Drinking by Age 16</td>
<td>32</td>
<td>30</td>
<td>14</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>32</td>
<td>30</td>
<td>14</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>Cannabis Use by Age 16</td>
<td>39</td>
<td>10</td>
<td>8</td>
<td>6</td>
<td>9</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>.00</td>
</tr>
</tbody>
</table>

The two groups were pseudo-randomly selected from the starting sample (Table 5.1) to be matched on binge drinking levels. P value reflects significant between-group differences determined via chi-square (for handedness) and t-tests. All demographic information measured at baseline, and were also included as candidate predictors in the differential prediction analyses. PDS: Pubertal Development Scale (Carskadon and Acebo, 1993). SES: Socioeconomic Status. Drug levels from the ESPAD and measured on an ordinal scale (1=1–2x, 2=3–5x, 3=6-9x, 4=10-19x, 5=20-39x, 6=40+).
Table 5.3: Demographic Information and Drug Use Levels for Female Analytic Samples

<table>
<thead>
<tr>
<th>Measure</th>
<th>Females (N=148)</th>
<th>Future Cannabis+Binge Drinking Group (n=74)</th>
<th>Future Binge Drinking Only Group (n=74)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (M,SD)</td>
<td></td>
<td>14.5, .48</td>
<td>14.5, .47</td>
<td>.70</td>
</tr>
<tr>
<td>Handedness (L, R)</td>
<td></td>
<td>9, 65</td>
<td>5, 69</td>
<td>.26</td>
</tr>
<tr>
<td>PDS (M,SD)</td>
<td></td>
<td>3.2, .37</td>
<td>3.1, .42</td>
<td>.20</td>
</tr>
<tr>
<td>Verbal IQ (M,SD)</td>
<td></td>
<td>112.5, 11.8</td>
<td>108.5, 15.1</td>
<td>.08</td>
</tr>
<tr>
<td>Performance IQ (M,SD)</td>
<td></td>
<td>110.1, 12.6</td>
<td>105.8, 13.8</td>
<td>.06</td>
</tr>
<tr>
<td>SES (M,SD)</td>
<td></td>
<td>19, 3.9</td>
<td>18, 3.7</td>
<td>.10</td>
</tr>
</tbody>
</table>

The two groups were pseudo-randomly selected from the starting sample (Table 5.1) to be matched on binge drinking levels. P value reflects significant between-group differences determined via chi-square (for handedness) and t-tests. All demographic information measured at baseline, and were also included as candidate predictors in the differential prediction analyses. PDS: Pubertal Development Scale (Carskadon and Acebo, 1993). SES: Socioeconomic Status. Drug levels from the ESPAD and measured on an ordinal scale (1=1–2x, 2=3–5x, 3=6–9x, 4=10-19x, 5=20-39x, 6=40+).
Table 5.4: Summary of SNP rs521674 distribution for Females

<table>
<thead>
<tr>
<th>SNP</th>
<th>Major: Minor Alleles</th>
<th>Future Cannabis+Binge Drinking Group (n=74)</th>
<th>Future Binge Drinking Only Group (n=74)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs521674</td>
<td>T:A</td>
<td>41: 27: 6</td>
<td>57: 0: 17</td>
<td>.001</td>
</tr>
</tbody>
</table>

Count of the number of risk alleles for the two groups. Hminor: Homozygote minor (high risk alleles, A/A). HT:heterozygote major (A/T). Hmajor: Homozygote major (low risk alleles, T/T). A higher number of risk alleles were present in the future binge drinking only group. P-value derived from a chi-square analysis. SNP rs521674 located on CHR10: 111075832, coding for the ADRA-2A receptor.

Table 5.5: Summary of Predictors Selected

<table>
<thead>
<tr>
<th>Sex</th>
<th>Domain</th>
<th>Predictor</th>
<th>Primary Model</th>
<th>Secondary Model (Selection Frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Psychosocial</td>
<td>Lifetime Cigarettes</td>
<td>RF &amp; LR</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feelings of Deviant Behaviors</td>
<td>LR</td>
<td>RF (95)</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>Reward Outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>L. Superior Cerebellum</td>
<td>RF &amp; LR</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stop Success</td>
<td>R. Inferior Temporal Lobe</td>
<td>RF</td>
<td>LR (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. Paracentral Lobule</td>
<td>RF</td>
<td>LR (43)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. Middle Frontal Gyrus</td>
<td>RF</td>
<td>LR (1)</td>
</tr>
<tr>
<td></td>
<td>Stop Failures</td>
<td>L. Inferior Frontal Gyrus</td>
<td>RF</td>
<td>LR (0)</td>
</tr>
<tr>
<td></td>
<td>Genetic</td>
<td>rs521674</td>
<td>LR</td>
<td>RF (11)</td>
</tr>
<tr>
<td></td>
<td>Psychosocial</td>
<td>Lifetime Cigarettes</td>
<td>RF &amp; LR</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feelings of Deviant Behaviors</td>
<td>RF</td>
<td>LR (64)</td>
</tr>
<tr>
<td>Males</td>
<td>Brain</td>
<td>Gray Matter Volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. Inferior temporal lobe</td>
<td>RF</td>
<td></td>
<td>LR (71)</td>
</tr>
</tbody>
</table>

The primary model denotes the model for which the predictor passed the initial threshold for identification: 3 of 5 final models across all 100 runs. The secondary model denotes the model for which the predictor did not pass the initial threshold, and the selection frequency denotes the number of runs for which the predictor was selected by the secondary model. RF: Random Forest. LR: Logistic Regression.
Figure 5.1: Future Binge Drinking Levels in Starting Sample by Sex

A: Binge drinking levels by age 16 for starting sample of males. B: Binge drinking levels by age 16 for starting sample of females.
Figure 5.2: Future Binge Drinking Levels in Analytic Sample by Sex

A: Binge drinking levels by age 16 for analytic sample of males. B: Binge drinking levels by age 16 for analytic sample of females. Binge drinking levels were perfectly matched.
Figure 5.3: Female Brain Predictors Identified for Each Model

For the logistic regression results, higher activations to reward outcomes in the left superior cerebellum was identified as a differential predictor. For the random forest results, lower activations in the left middle frontal gyrus, right inferior temporal gyrus, and the right paracentral lobule during stop success, and lower activations in the left inferior frontal gyrus during stop failure, were identified as differential predictors.

Figure 5.4: Male Brain Predictor Identified From the Random Forest.

Only the random forest analysis identified a single predictor from the brain domain for males. Higher gray matter volume in the right inferior temporal lobe was identified as a differential predictor.
CHAPTER 6: DISCUSSION & CONCLUSIONS

6.1: General Discussion

Overall Objectives

This dissertation identified a set of brain and behavioral features that characterized cannabis use in adolescence. Findings were reported within the context of prediction as age 14 data was used to predict cannabis initiation or level of cannabis use two or five years later. And even though Chapter 2 contained a cross-sectional analysis, Chapter 3 provided evidence that the observed amygdala hyperactivity is likely a pre-existing difference.

Chapters 4 and 5 searched beyond the amygdala and identified a comprehensive risk profile that contained predictors from the psychosocial and brain domains collected prior to cannabis exposure. Therefore, this dissertation improves on the largely cross-sectional body of work by uncovering sex- and drug-specific predictors that were disentangled from any consequences of cannabis use. And although prediction was the dominant theme throughout this dissertation, exploratory analyses also tested for changes in amygdala reactivity and anxiety levels following an escalation of cannabis use from age 14 to 19.

Results from these analyses are discussed here in terms of their utility in stratifying risk and informing interventions. For risk stratification, these results help gauge severity of risk for cannabis use by how closely an adolescent fits the identified risk profiles. For instance, a female who drinks, smokes cigarettes, and exhibits a novelty seeking personality may be considered more at risk than her peer who only drinks. The
neurobiological predictors further aid in risk stratification if neuroimaging batteries ever become commonly administered as a screening tool in behavioral health. Furthermore, the identified predictors are discussed in terms of their ability to inform treatment strategies. Those treatments are hypothesized to attenuate the likelihood of cannabis use by altering the risk phenotypes for males and females.

*Data Analysis Philosophy*

Throughout this dissertation two lines of inquiry were pursued, hypothesis- and data-driven (machine learning) analyses. Starting with the hypothesis-driven analyses, a theoretical framework related to amygdala reactivity was used to characterize and predict adolescent cannabis use. For the data-driven analyses, additional measures from the IMAGEN study were explored without being limited by a specific theoretical framework and uncovered a set sex-specific psychosocial and neurobiological predictors.

Hypothesis-driven studies have the advantage of being more transparent and may lead to more interpretable findings guided by a theoretical framework. Alternatively, data-driven studies provide a unique opportunity to make discoveries that were not specifically theorized *a-priori*. This characteristic of data-driven studies was reflected by the set of multimodal brain measures that predicted cannabis use by age 16. However, only the hypothesis-driven line of inquiry yielded a brain measure that predicted cannabis use by age 19. Therefore, both approaches can be used to make valuable insights into the relationship between the brain to behavior.
Moreover, these two lines of inquiry are not in opposition. This notion is demonstrated by the post-hoc analyses in Chapters 4 & 5 that were informed by the data-driven analyses. Given the high-dimensionality of the brain data (1,946 ROIs; Table 4.1.3), it was impossible to theorize or test hypotheses related to each measure or combination thereof. Instead, machine learning was required to identify the most robust and generalizable brain features. Those results were then passed forward to test hypotheses related to sex and drug-specificity. This approach embraced the high-dimensionality of the brain data while employing a set of best practices (Whelan and Garavan, 2014) that rigorously examined relationships between adolescent cannabis use and the underlying neurobiology. Hence, this line of inquiry facilitated the discovery of many novel neurobiological predictors of adolescent cannabis use.

6.2: Main Findings & Implications

Chapters 2 & 3 Findings

Chapters 2 & 3 together demonstrated that amygdala hyperactivity to angry faces was likely a pre-existing difference that partially predicted the level of cannabis use five years later. This dose-response prediction of cannabis use, and the lack of a significant difference in amygdala reactivity at age 19, indicated that the amygdala is a predictive biomarker, rather than a marker of a cannabis-related effect.

As the amygdala has been implicated in threat monitoring processes (Fox et al., 2015; Mobbs et al., 2010), these results might suggest an exaggerated threat monitoring system confers risk for cannabis use in adolescence. Chapter 3.2 attempted to confirm
this by testing for associations between amygdala reactivity and anxiety levels. However, this association was not confirmed and indicated that the DAWBA band score for generalized anxiety is not a suitable measure to probe this construct for this sample.

For the anxiety level data, a significant main effect of time indicated that anxiety levels increased from baseline into late adolescence. No main effect of group, or a group-by-time interaction was uncovered, which indicated cannabis use did not influence symptoms of generalized anxiety. Instead, the main effect of time suggested it was typical to report higher anxiety levels later at age 19. This pattern of results is consistent with Van Oort and colleagues who previously reported that generalized anxiety disorder symptom levels peaked in late adolescence (Van Oort et al., 2009). This phenomenon might be due to the exploratory nature of early adolescence where lower anxiety levels may be evolutionary beneficial to motivate autonomy (Spear, 2000b). These youths may then experience more stress and uncertainty later in adolescence as they first become independent outside of the household (i.e., for college, military, new careers, etc.).

Chapter 3.2 tested for changes in amygdala reactivity from age 14 to 19 with chronic cannabis use. No significant differences were detected, although plotting the data visually displayed a decrease in amygdala reactivity with chronic cannabis use (Figures 3.2.1-2). Despite the null statistical results, these analyses motivated a comparison of the relatively large sample of typically developing (cannabis-naïve) adolescents. Those analyses indicated healthy amygdala development was characterized by an increase in activation from age 14 to 19. The observed pattern of amygdala reactivity might also be characteristic of adolescence and early adulthood more generally. In tandem with the
interpretation of the anxiety data, lower threat monitoring responses in adolescence might facilitate exploration with novel environments and elevations in novelty seeking personality, whereas a higher threat monitoring system in adulthood is beneficial to maintain survival and independence (Spear, 2000b).

Chapters 2 & 3 Implications

The meaning of the amygdala findings were difficult to dissect. Considering the role of the amygdala in threat monitoring (Fox et al., 2015), it is plausible that these data reflected an attentional-bias to negative affect or an inability to suppress automatic threat detection in favor of goal-directed behavior. However, it is difficult to make these conclusions because the passive viewing face processing task did not have any task performance measures.

Previous fMRI studies on adolescent psychopathology characterized both generalized and social anxiety, as well as depression, in terms of amygdala hyperactivity to evocative faces (Beesdo et al., 2009; Lau et al., 2009; Monk et al., 2008; Yang et al., 2010). To better decompose this apparent lack of disorder specificity, van den Bulk and colleagues correlated amygdala activations to faces with dimensional scores of anxiety and depression in diagnosed children (DSM-IV) compared to controls (van den Bulk et al., 2014). Results indicated that the right (but not left) amygdala activation to happy, neutral, and angry faces significantly correlated with anxiety, but not depression scores. Anxiety dimension scores explained 28% of the variance in right amygdala activations to angry face within the clinical sample, but was not correlated in controls (van den Bulk et
al., 2014). Although the cannabis users reported here were not selected for having an anxiety disorder diagnosis, and their DAWBA band score levels did not differ from the cannabis-naïve sample (Table 3.1.3), their pattern of amygdala reactivity were in line with the papers above. And while that should not lead to the conclusion that the cannabis users were an anxious sample, their similarities are worth considering. Nonetheless, the lack of a correlation between anxiety scores and amygdala reactivity reported here was consistent with the null effects reported by van den Bulk and colleagues for their control sample (van den Bulk et al., 2014). Although, the van den Bulk report used a dimensional measurement tool for anxiety (Revised Child Anxiety and Depression Scale) (Chorpita et al., 2000) which was likely better suited to identify a linear relationship with amygdala reactivity than the ordinal nature of the DAWBA band score.

Despite the lack of a suitable measure for anxiety, the baseline amygdala reactivity may have signaled the child was under some level of distress more generally. As these youths continued their cannabis use throughout the intervening five years, the follow up amygdala reactivity indicated a visual decrease in activation relative to baseline (Figure 3.2.1-2). Those findings were interpreted as broadly supporting a negative reinforcement model of addiction, as proposed by Koob (Wise and Koob, 2014). While this model asserts positive reinforcement is necessary for the initial repetition of drug taking, it is the removal of distress that maintains and escalates drug use. Here, the distress experienced by the individual, as reflected by heightened amygdala reactivity at baseline, might be alleviated with cannabis use. This framework also accommodates the dose-response relationship outlined in Chapter 3.1, as individuals with minor distress
only required low levels of cannabis, whereas high distress necessitated frequent use. This line of reasoning also supports the downward shift in amygdala reactivity as repeated cannabis use may have lowered distress levels and amygdala reactivity.

Translating the findings of Chapters 2 & 3 into practice is difficult. As discussed in Chapter 3, common antianxiety and antidepressant medications have been effective in attenuating amygdala reactivity to angry faces. Therefore, pharmaceutical intervention might be considered only for teens with especially high risk. On the other hand, it is challenging to justify a treatment approach relative to the risks of cannabis use. If schizophrenia is taken as the most functionally impairing or clinically relevant outcome associated with adolescent use, it is worth noting that the lifetime prevalence rates of schizophrenia is nearly 1% of the population (Simeone et al., 2015). And although a recent meta-analyses indicated adolescent use of cannabis is associated with a four-fold increase in the odds of receiving a schizophrenia or psychotic disorder diagnosis (Marconi et al., 2016), those results should be interpreted in light of the very low base rate. Moreover, the stepwise regression models indicated the right amygdala effect only explained an extra 1% of the variance in cannabis use levels at age 19. Therefore, it is unlikely that a clinically meaningful effect (i.e., lowering cannabis use) might be achieved by pharmacologically targeting this biomarker.

*Chapters 4 & 5 Findings*

Chapters 4 & 5 outlined a series of sex-specific machine learning analyses that uncovered risk profiles comprised of predictors from all data domains. The cross-
validated ROC AUCs indicated the models predicted future cannabis use well above chance levels, with some caveats to be addressed.

Analyses failed to predict better than chance only for males when baseline cigarettes were excluded for the differential prediction (Chapter 5), and predicting cannabis initiation by age 19 (Chapter 4.2). These findings suggest the female prediction analyses were more resilient to differences. This also highlights a theme throughout this dissertation that females exhibit a more distinct predictive profile for cannabis use relative to their male peers. Indeed, prediction accuracies for females were higher across all prediction analyses regardless of the comparison sample (controls or binge drinkers), machine learning algorithm (logistic regression or random forest), and age of initiation (16 or 19). This finding was encouraging as it signaled that females are more accurately predicted and may therefore be targeted more reliably for interventions than males. Despite the finding that substance use disorders are generally higher in males (Kuhn, 2015), the superior prediction for females is consequential as the literature indicates that females are more vulnerable to drug initiation (Anker and Carroll, 2010) and advance to substance use disorders faster than males (Hernandez-Avila et al., 2004).

From Chapter 4.1, the predictive profiles contained six psychosocial predictors that were shared across the sexes. Three of those predictors were related to other drugs, namely higher baseline cigarette and alcohol use, and parental cannabis use. Of the remaining three shared predictors, less negative feelings towards deviant behaviors, and higher novelty seeking and disorderly personality were identified.
Personality measures of the parent and child were frequently identified in predicting cannabis use. Despite the differences in the exact personality measure uncovered, all generally characterized a novelty seeking personality (see Figure 4.1.3 for exact personality measures). Moreover, novelty seeking personality was the most robust predictor of cannabis use by age 19 for females, and generalized to males in a post-hoc fashion (Chapter 4.2). Therefore, this measure may be considered a trait-like feature that predicts cannabis use regardless of the age of initiation or sex.

The identification of novelty seeking personality is consistent with the adolescent and substance use literature. Researchers previously implicated this personality trait in predicting cannabis use (Bidwell et al., 2015; Dugas et al., 2018), cigarette use (Hu et al., 2008) and alcohol abuse (Boson et al., 2019). Animal studies concluded that animals displaying more novelty seeking behaviors self-administered drugs like alcohol and psychomotor stimulants at a higher rate than animals that did not display similar behaviors (Belin and Deroche-Gamonet, 2012; Nadal et al., 2002; Suto et al., 2001). Furthermore, animal (Adriani et al., 1998), and human studies (Spear, 2000b) reported the novelty seeking personality phenotype peaks during adolescence. Together, these findings suggest adolescents with higher novelty seeking personality traits than their peers are at an even greater likelihood of drug initiation. This risk phenotype is therefore an important target for intervention and will be discussed later.

Less negative feelings towards deviant behaviors was identified as a shared predictor for males and females in Chapter 4.1, and also a shared differential predictor in Chapter 5 (although from different algorithms). This feature asked the child to report how
they would feel about engaging in deviant behaviors, so it was unclear if they reported based on personal experience or a hypothetical cognitive appraisal. And although IMAGEN did not collect measures on peer relationships, the literature suggests adolescents are more likely to engage in delinquent behaviors with their peers (Haynie and Osgood, 2005). Moreover, Van Ryzin & Dishion reported that cannabis use in young adults is predicted by deviant peer group membership (Van Ryzin and Dishion, 2014). Therefore, this effect might be characterizing the adolescents’ social network, although the lack of other corroborating data does not support this interpretation.

Machine learning analyses also discovered many novel brain predictors of cannabis use in adolescence. Starting with 1,946 brain measures, there were 22 brain predictors identified for females (17 in Chapter 4, and five in Chapter 5), and nine brain predictors identified for males (eight in Chapter 4, and one in Chapter 5). Despite this profound data reduction, the results were complex given the differences in modality and localization uncovered for each region of interest (ROI).

The brain predictors for males were related to the stop signal task and gray matter volume, whereas all task modalities were identified for females. The presence of the stop signal task ROIs highlights the utility of this task in capturing individual differences related to cannabis use. Appendix 2 contains a review paper that highlights how hypoactivations observed during this task relate to addictive behaviors (Spechler et al., 2016). The findings reported here are in line with that pattern as the majority of the stop task predictors for both sexes were hypoactivations relative to comparison samples (Figure 4.1.3 & Figure 5.3).
The anatomical locations of these hypoactivations are mostly in regions previously implicated in response inhibition tasks. For instance, regions supporting motor functioning like the cerebellum and thalamus (Ide and Li, 2011), and prefrontal regions supporting executive control functioning like the inferior frontal gyrus (Garavan et al., 1999) and orbital frontal cortex (OFC) (Whelan et al., 2012), were identified. A higher impulsive personality was also identified as a female-specific psychosocial predictor (Figure 4.1.3). Higher impulsivity and hypoactivations in the OFC have been reported to predict drug use in adolescence (Whelan et al., 2012). All together, these results suggested that deficits in the neurobiological systems supporting cognitive control mechanisms confer an increase in risk for cannabis use in adolescence.

Work by Tapert and colleagues previously reported that cannabis users demonstrated higher activations across prefrontal regions relative to controls following one month monitored abstinence (Tapert et al., 2007b). As no behavioral differences were observed, that study indicated more prefrontal processing resources are required for cannabis users to execute inhibitory control behaviors at the same level as non-users. Therefore, the predominant hypoactivations reported here may have facilitated drug taking behaviors (Nigg et al., 2006). And while the Tapert report was cross-sectional, those data contrast with the findings here and might imply prefrontal hyperactivations arise following cannabis use.

Relative to the other tasks, the reward task was infrequently identified and was also female-specific. Relative to controls, higher reward anticipation in the left middle frontal gyrus was identified in Chapter 4.1, and higher reward outcome activation in the
left superior cerebellum was identified in Chapter 5. The evident hyperactivations during anticipation might signal a propensity to over-evaluate the incentive salience of rewards, which generally aligns with the “wanting” component of the “wanting vs. liking” model of addiction (Berridge and Robinson, 2016).

Finally, a set of face processing predictors was identified for males and females. Lower neutral face reactivity appeared to be shared across the sexes, although in different regions for males and females (Figure 4.1.3). Higher activation for angry faces was identified for females only in the right ventromedial prefrontal cortex and possibly signaled an emotion regulation strategy (Urry et al., 2006).

The face processing amygdala effects reported in Chapters 2 & 3 were not reproduced in any of the data-driven analyses in Chapters 4 & 5. Reasons for this discrepancy are likely due to differences in measurement and modeling. For measurement, the ROI containing the amygdala in Chapters 2 & 3 were different from that used in Chapters 4 & 5. The parcellation atlas used in Chapters 4 & 5 was constructed using functional connectivity from resting state fMRI (Shen et al., 2013). This atlas did not contain an ROI as specific to the amygdala as the anatomically defined ROI used in Chapters 2 & 3. Upon visual inspection, the ROI covering the amygdala used in Chapters 4 & 5 contained voxels from the anterior temporal lobe and hippocampus. For modeling differences, Chapter 3 identified the right amygdala within the context of a relatively large sample (n=525) collapsed across sex. This large sample may have contributed to the statistical power necessary to detect a predictor that explained 1% of the variance in future cannabis use. Chapters 4 & 5 analyses were
executed separately for each sex for theoretical reasons, and also because sex was consistently the most robust predictor when running preliminary analyses collapsed across sex. Together, the reduction of statistical power and lack of amygdala ROI specificity reduced the likelihood of the amygdala being reproduced in Chapters 4 & 5.

These fMRI results from Chapters 4 & 5 add to the very sparse literature using fMRI to predict cannabis use in adolescence. There appeared to be only one study using fMRI to predict cannabis use in adolescence. In that study, Tervo-Clemmens reported on a visuospatial working memory task (Tervo-Clemmens et al., 2018). The results reported here are not comparable to the Tervo-Clemmens data given the inconsistent task modalities. Nonetheless, the results reported here add three more fMRI task modalities related to different psychological constructs to the literature on cannabis prediction.

Lastly, the GMV results were modestly male-specific, although differences were observed for females. Furthermore, the directions and locations were inconsistent within each sex. For females, greater GMV in the right pre-SMA, and less GMV in the right middle frontal gyrus (MFG) were identified (see Chapter 4.1). For males, greater GMV in the right inferior temporal lobe was the sole brain predictor uncovered in Chapter 5, whereas greater GMV in the right medial prefrontal cortex and less GMV in the left midcingulate cortex was uncovered in Chapter 4.1.

Medial temporal lobe differences were entirely absent from these structural results despite previous literature pointing to an inverse relationship between cannabis use and hippocampal and amygdalar brain volumes (Ashtari et al., 2011; Cousijn et al., 2012; Yucel et al., 2008). However, those studies were cross-sectional, and might be more
reflective of consequences of use given the animal studies that reported neurotoxicity of cannabinoids on those structures (Lawston et al., 2000; Quinn et al., 2008b).

Two prospective structural studies by Cheetham and colleagues, and Jacobus and colleagues, reported less brain volumes relative to controls across many prefrontal regions like the OFC (Cheetham et al., 2012b) and precentral gyri (Jacobus et al., 2016). Of note, Jacobus also reported less thickness in the right MFG predicted cannabis use, which was reproduced here for females only. Therefore, these results add to the literature by affirming less GMV in prefrontal regions predicts use, while the medial temporal lobe regions are more likely to reflect differences following use.

*Chapters 4 & 5 Implications*

Chapters 4 & 5 demonstrate that a machine learning approach yielded strong predictions and made novel discoveries that related the neurobiology to future cannabis use in adolescence. These chapters advance the field of psychiatry more generally by identifying sex- and drug-specific predictive biomarkers for cannabis use. Psychosocial predictors were also identified and can be used to tailor intervention strategies. As large neuroimaging studies like IMAGEN become more common, these approaches help maximize the information gained from large datasets.

Starting with the prediction accuracies, the generally high ROC AUCs highlighted the generalizability of the models. It is important to stress again that these prediction models were all estimated within a cross-validation scheme and therefore addressed some of the issues related to reproducibility in neuroimaging research (Poldrack et al., 2017).
However, the gold standard remains to replicate findings in a completely external dataset. Therefore, these prediction models could be tested on the forthcoming data releases of the ABCD study, which is a population-level longitudinal study of development from childhood to adulthood (www.abcdstudy.org).

Returning to the idea of risk-stratification, the post-hoc regressions from Chapters 4 & 5 demonstrate that the addition of predictors from a different feature domain improves prediction accuracy. This finding is generally in line with prior “big data” prediction studies that reported improvements with the addition of new modalities (Jacobus et al., 2016; Whelan et al., 2014b). Therefore, adolescents can first be stratified by how well they align with the psychosocial predictors. Following identification of these profiles, the adolescent might be referred for a functional neuroimaging assessment. Those who exhibit the structural and/or functional neurobiological differences could be stratified again given their alignment with the neurobiological risk profiles. Although, at this point in time, this scenario is likely not justifiable in light of the amount of time and resources involved with functional neuroimaging assessments. Replication studies are needed to ensure the brain predictors reported here generalize to other datasets. Also, intervention studies informed by the neurobiological predictors are needed.

In terms of informing treatment interventions, it is challenging to recommend how the brain findings inform interventions. This challenge is underscored by the quantity of brain predictors uncovered from different modalities. To start, the functional tasks broadly informed the psychological constructs that influenced cannabis use in adolescence. For instance, exaggerated social threat processing can be inferred from
Chapters 2 & 3. As inferred from Chapters 4 & 5, heightened reward evaluations might be a female-specific construct, whereas poor response inhibition is a shared construct across the sexes. Rather than directly targeting each brain predictor per se, targeting these constructs might be a useful first step as a global approach.

Targeting a reduction in cigarette use is hypothesized to be most effective in reducing cannabis use. In light of the clear predictive relationship between cigarette and cannabis use reproduced here, special attention must be given to cigarette reduction efforts as they are hypothesized to lower cannabis initiation rates. This idea is supported by an epidemiological study that demonstrated a recent reduction in cigarette use likely protected against an increase in cannabis use in youths (Miech et al., 2017). Nicotine is also strongly implicated in alcohol use behaviors as both animal (Lê et al., 2003) and human studies (Barrett et al., 2006) demonstrated that nicotine facilitated alcohol consumption. In considering this pattern, a recent population level study indicated that smoking cessation predicted a reduction in alcohol use (Brown et al., 2016). Given this cross-drug reduction, and the pattern reported by Miech and colleagues, it is very likely that cannabis use will decline by removing the influence of cigarettes.

Targeting personality features to inform substance use interventions is an ongoing line of research by Conrod and colleagues (Conrod, 2016). As discussed in Chapter 4.2, personality-targeted intervention programs have successfully lowered cannabis use in adolescence (Mahu et al., 2015). The Mahu and colleagues intervention demonstrated specific effects on cannabis use levels by targeting a sensation seeking personality phenotype. Therefore, those findings are highly applicable to the novelty seeking
personality elevations reported here. Altering the novelty seeking personality phenotype, as demonstrated by Mahu, will likely be effective in lowering cannabis use.

A final set of psychosocial predictors to be considered is the influence of the parent. The most robust predictor of use identified in Chapter 4 was parental cannabis use (Figure 4.1.3). There are currently ten states in the US that legalized recreational cannabis use, with commercial distribution approved in all but Vermont and the District of Columbia (as of April 2019). Therefore, parents/guardians living in those states who use legally (and other parents/guardians with illicit use) should be informed about the influence their use might have on their child initiating cannabis (O’Loughlin et al., 2018; Sokol et al., 2018), and the risks of adolescent use per se (Gobbi et al., 2019; Volkow et al., 2016). On the other hand, the measure used here was for lifetime cannabis use, therefore it is unknown if parental cannabis use was at all concurrent with the life of the child. Nonetheless, it will be important to monitor how cannabis use patterns may change in adolescents in states permitting recreational cannabis use for adults. Finally, as parental personality measures also predicted use in the child (Figure 4.1.3), these findings demonstrated that the parent should also be considered during risk-stratification and possibly incorporated into interventions for their child when necessary.

Genetic Results

The genetic data used here were generally less predictive of cannabis use in adolescence than data from other domains. Chapter 4 described how it was necessary to collapse across sex to increase statistical power for the detection of eight predictive
SNPs. And although Chapter 5 identified a single SNP implicated in the differential prediction for females, it was determined that the frequency of the high-risk allele was higher in the binge drinking only sample. This finding therefore may better characterize the binge drinking only sample rather than the cannabis+binge drinking sample.

These findings add to the sparse literature relating genetic data to cannabis use. And while most GWAS studies reported on clinical levels of cannabis use in adults (Agrawal et al., 2011; Sherva et al., 2016), this study was unique in predicting cannabis initiation in adolescence. The candidate gene approach used here helped conceptualize neurobiological vulnerabilities to cannabis initiation as only SNPs related to neurotransmitter receptors and cannabinoid pharmacology were included.

Genetic variation on genes coding for norepinephrine, dopamine, and opioid (ADRA1b, ADRB2, DRD1, OPRM1) neurotransmitter receptors partially predicted cannabis use in adolescence. The mechanisms by which these variations conferred risk need to be studied further. Nonetheless, these findings are in line with previous studies. Animal models have implicated the role of norepinephrine and opioid pharmacology in cannabis use. Work by Oropeza and colleagues, and Page and colleagues, demonstrated that relative to vehicle, a cannabinoid agonist precipitated norepinephrine release in the prefrontal cortex of rats (Oropeza et al., 2005; Page et al., 2008). Thus, individuals with norepinephrine receptor variants might be more sensitive to the effects of cannabis due to prefrontal activation differences. Ghozland and colleagues demonstrated specificity of the mu-opioid receptor in THC-induced conditioned place preference (Ghozland et al., 2002) thus implicating these receptors in the reinforcement properties of cannabis. And for
dopamine, a human study by Ferri and colleagues reported more DRD1 risk alleles were found in cigarette+cannabis smokers relative to cigarette only smokers (Ferri et al., 2009). Therefore, the findings reported here are situated in the ongoing literature relating these neurotransmitter systems to cannabis use.

The findings across all results suggest genetic variation on the SNPs used here were modestly related to cannabis initiation. Instead, the psychosocial and brain predictors were better suited to predict initiation and use levels. These findings motivate the hypothesis that the SNPs are more relevant to the maintenance or escalation of cannabis use following initiation. For instance, genetic variation on genes related to the pharmacodynamic (CB1 receptors) and pharmacokinetic (FAAH) properties of cannabis might only exert their influence on use behaviors following cannabis exposure. Future directions testing these hypotheses are outlined below.

6.3: Limitations & Future Directions

This dissertation contained several important limitations to be discussed. To begin, the IMAGEN study employed a convenient community sampling method (Schumann et al., 2010). While the study contained a very large sample for a neuroimaging project, the dataset is unable to make population level inferences. The predominantly white European sampling also raises questions related to the generalizability of these findings to other populations and settings. Cultural differences were also apparent within the IMAGEN study. The Paris site covariate typically emerged as a predictor of cannabis use due to the higher prevalence of cigarette use at that site.
The IMAGEN study is also limited by the ESPAD drug use questionnaire. This instrument contained poor drug use level quantification as exhibited by the ordinal level (e.g., level 5=20-39x). It was also collected with poor temporal precision (age 14, 16, 19). Additionally, there is no information about the route of administration (inhalation, ingestion), drug preparation (plant matter, oils, etc.), or potency (THC composition). These features may have different abuse liabilities and functional outcomes. Additionally, the IMAGEN study did not collect any measures on social networks, peer drug use or other peer influences. As these measures have previously predicted use (Ali et al., 2011), their inclusion might have altered the prediction analyses reported here.

As a final limitation related to the data used here, Chapter 4.2 attempted to predict the initiation of cannabis use by age 19 from data collected at age 14. Future studies could incorporate measures collected at the age 16 assessment. The inclusion of data that were more proximal in time to the outcome measure might improve prediction. Moreover, the age 16 data could be used in reference to the age 14 data to determine if any changes between the time points might improve the prediction of age 19 use.

**Future Studies**

The majority of the findings were discussed in their ability to inform treatment interventions designed to alter a risk phenotype. Therefore, studies are needed to demonstrate how effective those interventions might be on yielding reductions in cannabis use. In doing so, those studies would also provide supporting evidence that the predictors were causally related to cannabis use.
As noted in discussion above related to the SNP results, genetic variation might be related to cannabis maintenance or escalation, rather than initiation. Therefore, future studies could test for linear associations (rather than the binary initiation association) between the SNPs and future cannabis use level. Random forest analyses might also be useful in identifying interactions between SNPs without having to explicitly model an excessive number of interaction terms in a linear regression model. Alternatively, the nine predictive SNPs from Chapters 4 & 5 could be selected to build interaction terms with any combination of predictors. Along these lines, the nine SNPs could also be selected for mediation analyses investigating how genetic variation relates to the neurobiology. For example, modeling the SNPs as a mediator between cannabis use and the brain data is hypothesized to inform the biological mechanisms of cannabis-precipitated brain changes.

The hypothesis-driven studies in Chapter 2 & 3 targeted the amygdala, in part, due to the high density of CB1 receptors. Future studies could focus on other regions with high CB1 densities like the hippocampus and cerebellum (Kawamura, 2006). Chapter 3 was also unique as it tested for consequences arising from cannabis use. As only the amygdala was considered, future analyses could explore functional differences at the whole-brain level. Moreover, all the brain data were analyzed within a mass univariate framework. That is, each brain predictor reflected the mean activation level (or total GMV) contained at that ROI. All ROIs were then modeled together as a collection of independent variables. Moving forward, functional connectivity or graph theoretical measures could be examined. Those measures, by design, estimate the inherent network
structure of the neurobiology and might better characterize the relationship between the brain and cannabis use beyond a mass univariate approach.

Connectivity measures have been previously used in addiction research to characterize neurobiological differences related to drug cue-reactivity (Janes et al., 2012) and craving (Janes et al., 2014). Previous studies have also successfully incorporated functional connectivity metrics with machine learning algorithms to classify cigarette smokers (Pariyadath et al., 2014), and predict treatment outcomes in individuals with substance use disorders (Steele et al., 2018). Therefore, applying these techniques would further illuminate the neurobiological mechanisms associated with cannabis use in adolescence.

Finally, the IMAGEN study is just starting to release the age 23 assessments of roughly 800 individuals. Future analyses examining how neurobiological trajectories from early adolescence to early adulthood either predicts cannabis use or changes as a consequence of use could be explored. Additionally, more clinically relevant prediction analyses could be conducted on the individuals who might have met diagnostic criteria for cannabis use disorder (DSM-5). A differential prediction analysis comparing individuals with cannabis use vs. cannabis use disorder would better characterize pathological use and stratify risk. A final clinical outcome to be predicted in these samples is schizophrenia as this disorder is typically not diagnosed until late adolescence into early adulthood (Gogtay et al., 2011). Those analyses would contribute to the debate on cannabis-precipitated psychotic disorders (Volkow et al., 2016).


Seeking as a Phenotypic Marker of Adolescent Substance Use. Subst. Abuse Res. Treat. 9s1, SART S22440.


gene (CNR1) and cannabis dependence symptoms in adolescents and young adults. Drug Alcohol Depend. 104, 11–16.


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APPENDIX 1: LIST OF CONTRIBUTING AUTHOR PUBLICATIONS DURING GRADUATE SCHOOL

*Empirical Papers (Reverse Chronological Order)*


*Shared co-first author publication


**Book Chapter**

This Chapter has been previously published in the following format:


**Abstract**

Historically, neuroscientific research into addiction has emphasized affective and reinforcement mechanisms as the essential elements underlying the pursuit of drugs, their abuse, and difficulties associated with abstinence. However, research over the last decade or so has shown that cognitive control systems, associated largely but not exclusively with the frontal lobes, are also important contributors to drug use behaviors. Here, we focus on inhibitory control and its contribution to both current use and abstinence. A body of evidence points to impaired inhibitory abilities across a range of drugs of abuse. Typically, studies suggest that substance-abusing individuals are characterized by relative hypoactivity in brain systems underlying inhibitory control. In contrast, abstinent users tend to show either normal or supernormal levels of activity in the same systems attesting to the importance of inhibitory control in suppressing the drug use urges that plague attempts at abstinence. In this chapter, the brain and behavioral basis of response inhibition will be reviewed, with a focus on neuroimaging studies of response inhibition in current and abstinent drug abusers.

**INTRODUCTION**

A defining characteristic of addiction is the loss of control over one’s behavior. It
is central to the diagnosis of a substance use disorder, it is characteristic of the all-too common relapses of abstinent users attempting to stay clean, and it is apparent when initial intentions to have just one drink escalate into a binge drinking session. Although hedonic processes such as liking and craving may form the core motivation to consume drugs, certain cognitive processes, such as attention and memory, likely contribute to these drives whereas others, such as response inhibition, likely contribute to the individual’s efforts to resist these drives. For instance, Bechara’s cognitive theory of addiction posits that the augmented bottom-up signal of appetitive salience for drugs, in part, attenuates top-down inhibitory control (Bechara, 2005).

Cognitive control processes, also commonly referred to as executive functions, are attentionally demanding, and consciously available, volitional processes that initiate a certain action or interrupt ongoing actions (Atkinson and Shiffrin, 1968; Schneider and Shiffrin, 1977; Shiffrin and Schneider, 1977). Cognitive control takes on many forms, including, but not limited to, attentional control and inhibitory control. Attentional control involves the interaction between perceiving environmental cues and the allocation of perceptual processing resources (Norman and Shallice, 1985) whereas inhibitory control broadly refers to counteracting behaviors preceding, accompanying, or resulting from cues. With regard to addiction, initiation of drug cravings may involve mechanisms by which stimuli associated with previous drug use are detected and processed (Grant et al., 1996; Hester et al., 2006), while the inhibition of behavior may involve mechanisms related to monitoring and regulation (Forman et al., 2004; Kaufman et al., 2003). This chapter will focus on inhibitory control, largely operationalized as response inhibition,
and its contribution to substance abuse. Specifically, response inhibition will be considered as a means to characterize substance use, abstinence, and recovery in substance-dependent individuals.

**RESPONSE INHIBITION TASKS**

Inhibitory control is broadly conceptualized as the ability to suppress or countermand a thought, action, or feeling. Many investigators study inhibitory control using carefully designed tasks like the stop-signal task, or the go/no-go task, that measure an individual’s ability to suppress a prepotent motor response. During the stop-signal task, subjects perform a primary task such as identifying with button-press responses if a visually presented arrow (the target stimulus) points to the left or the right. On a minority of trials, often one quarter of trials, a unique auditory or visual stimulus (the stop-signal) follows the target and instructs the subject to countermand their response. Task difficulty is manipulated by varying the delay between the target and stop stimulus, such that the longer the delay the more difficult it is to inhibit the response. By calculating how fast subjects respond on trials without a stop-signal and the average stop-signal delay on trials in which they successful inhibit 50% of the time, one can estimate the speed of the response inhibition process known as the stop signal reaction time (SSRT) (Logan and Cowan, 1984). During go/no-go tasks, subjects are presented with a continuous stream of stimuli, the majority of which require a button-press response (go trial), and a minority requiring no response (no-go trial) with the inhibitory demand being induced through the prepotency to respond even on no-go trials. While both tasks are arguably very rudimentary examples of inhibitory control, there is evidence, outlined below, that the
neural circuitry subserving response inhibition is also involved in other types of cognitive and emotional inhibition, thereby indicating that they may serve as reasonable probes for more complex inhibitory demands, including those related to resisting drugs. As the neural circuitry of response inhibition is relatively well understood and yields reliable and sensitive behavioral measures of inhibitory ability, it has generated a significant number of studies focused on the role of response inhibition in addiction (Luijten et al., 2014; Smith et al., 2014).

THE NEUROBIOLOGY OF CONTROL

Neuroimaging research has identified the dorsolateral prefrontal cortex (dlPFC) as a brain region critical for cognitive control. Evidence suggests the dlPFC is implicated during dual-task coordination (D’Esposito et al., 1995; Mansouri et al., 2009), task switching (Badre and Wagner, 2006; Dove et al., 2000; Sohn et al., 2000), memory updating (Edin et al., 2009; Salmon et al., 1996), and response sequencing, monitoring, and manipulation (Kim et al., 2013; Owen et al., 1996). This is consistent with the human lesion literature implicating the frontal lobes in organizing, regulating, and producing coherent behavior (Luria and Pribram, 1973; Stuss and Frank Benson, 1987). Frontal lobe-damaged patients appear to lose important aspects of autonomous cognitive control as evidenced by the loss of behavioral control to environmental contingencies (e.g., capture errors and utilization behaviors; Lhermitte, 1986). Although the focus of this review will be on prefrontal systems mediating control, these systems operate in conjunction with extensive parietal, premotor, cingulate, subcortical, and cerebellar networks. Further, despite the evidence implicating the frontal lobes in cognitive control,
the assignment of specific frontal loci to specific functions is far from resolved due, perhaps, to one of the defining characteristics of the frontal lobes being their ability to flexibly adapt to task demands. Dosenbach and colleagues suggest that different brain networks are involved in distinct aspects of control with the frontoparietal cortex implicated in initiating and adapting behavior, while sustained stable task performance is associated with the anterior cingulate cortex (ACC), anterior insula, frontal operculum, and anterior prefrontal cortex (Dosenbach et al., 2007).

With a specific focus on inhibitory control, a body of research (lesion, transcranial magnetic stimulation (TMS), and fMRI methodologies) implicates the right inferior frontal cortex (rIFC) in motor response inhibition (Aron et al., 2003; Chambers et al., 2006; Garavan et al., 1999, 2006). More broadly, the rIFC is one node of a motor inhibition network which also includes the pre-supplementary motor area (pre-SMA), and subthalamic nucleus (STN) (Aron et al., 2014). It is unclear about the exact causal pathways of these regions (Duann et al., 2009; Neubert et al., 2010; Swann et al., 2012), but research proposes that the STN receives input from both the rIFC and pre-SMA, and the STN inhibits motor activity at the basal ganglia (Aron and Poldrack, 2006; Schmidt et al., 2013). Figure A2.1 shows a number of the main cortical areas activated during response inhibition from the largest neuroimaging study of the STOP task (Whelan et al., 2012). Human lesion studies provide converging evidence that lesions in the right pre-SMA (Floden and Stuss, 2006; Nachev et al., 2007) and the right inferior frontal gyrus (IFG) subregion pars opercularis impair response inhibition (Aron et al., 2003, 2004, 2014). The first study using TMS found that temporary deactivation of the right IFG pars
opercularis selectively impaired the ability to stop an already initiated action, whereas the
deactivation of the same region did not affect physiological arousal or the ability to execute responses, confirming the important role of the IFG in the regulation of response inhibition (Chambers et al., 2006). In addition, Cai and colleagues showed that stimulation of the right pre-SMA slowed the implementation of stopping (measured via SSRT) but had no influence on modulation of response tendencies and suggested that this region impairs stopping behavior through a specific disruption of response inhibition (Cai et al., 2012). These studies are supported by the temporal and spatial precision afforded by electrocorticography studies, which have found the rIFC responds prior to successful inhibition (Swann et al., 2009, 2012). Recent studies suggest that this may reflect a broader role for this region in detecting attentionally salient events (Hampshire et al., 2010), although it may be the case that in order to evoke right IFG activity, the salience of these events must be relevant to response control (Dodds et al., 2011).

Although typically not activated in imaging studies of motor response inhibition, there is considerable evidence of a role for the orbitofrontal cortex (OFC) in impulse control. For example, OFC damage in a rodent model increases SSRT (Eagle et al., 2008), while patients with lesion damage to the OFC show increased self-report and cognitive measures of impulsivity and altered time perception relative to healthy controls and non-OFC lesioned patients (Berlin et al., 2004). That said, many behaviors that appear impulsive might not be driven by a deficit in impulsivity per se. For example, Torregrossa and colleagues argue that the most robust deficit in OFC damaged animals is in reversal learning. Seemingly impulsive behaviors, such as perseverative responding,
and failure to alter responding when rewards for a learned behavior are devalued, may in fact reflect impairment in the ability to update the value of an outcome, especially under changing circumstances (Torregrossa et al., 2008).

There is evidence that regions implicated in motor inhibition and, in particular, right frontal cortex, are involved in aspects of inhibitory control beyond response inhibition. This includes the suppression of drug cravings elicited by a cocaine video: brain activation in the rIFC was increased when inhibiting a craving response and was negatively coupled with activation levels in the right nucleus accumbens (Volkow et al., 2010). In a think/no-think paradigm, in which paired associates are actively suppressed, activation in rIFC was associated with suppressing the sensory components of memories (Depue et al., 2007). de Fockert and colleagues showed that increasing working memory load increased activity levels in bilateral inferior and middle frontal gyri while simultaneously increasing the distraction caused by (and sensory processing of) irrelevant faces (de Fockert et al., 2001). Hester and colleagues modified this paradigm to show that irrelevant drug stimuli produced heightened activity in visual cortex in cocaine users relative to drug-naïve controls (Hester and Garavan, 2009). Critically, those users with the greatest levels of activity in right prefrontal cortex showed the smallest behavioral interference caused by the distracting drug stimuli. In a similar manner, a study of the ability to ignore ecstasy-related stimuli produced greater occipital activation but reduced right prefrontal activation in ecstasy users relative to controls (Roberts and Garavan, 2013). Tabibnia and colleagues identified the rIFC in a number of inhibitory deficits of methamphetamine-dependent subjects (Tabibnia et al., 2011). Results indicated lower
gray matter in the rIFC in dependent subjects relative to controls, and gray matter in this region was correlated with drug craving, response inhibition performance, and a test of affect regulation. Finally, Behan and colleagues have recently shown that the rIFC is more active when subjects suppress reward anticipation (Behan et al., 2015). Here, a novel task required subjects to prepare for either a target to which they must respond as fast as possible to receive a reward, or, a stop-signal indicating they should make no response. A psychophysiological interaction analysis suggested the possibility of having to inhibit, rather than respond quickly, produced activity increases in the rIFC, which were correlated with activity decreases in the ventral striatum. Further, the rIFC activity was adjacent to a distinct rIFC region associated with motor inhibition. Combined, this brief review suggests that the rIFC may have a broad role in inhibitory processes that extend beyond motor inhibition. That said, there remains a lack of a comprehensive theory relating the similarities and differences between the various types of inhibitory control to their neurobiological and psychological overlap. Further research probing the multiple types of inhibitory control in the same sample may be a valuable advance.

RESPONSE INHIBITION AND DRUGS OF ABUSE

Substance using populations are characterized by deficits in response inhibition. A recent meta-analysis (Smith et al., 2014) of 97 studies found evidence for impaired response inhibition among those dependent on alcohol, cocaine, methamphetamine, tobacco, and MDMA.

*Nicotine*
Although findings in the literature are mixed, a recent meta-analysis found a small but significant effect relating cigarette smoking to response inhibition deficits (Smith et al., 2014). Results from neuroimaging investigations have generally found alterations in the neural circuitry associated with response inhibition in smokers compared to nonsmoking controls (de Ruiter et al., 2012; Luijten et al., 2013; Nestor et al., 2011; but see Galván et al., 2011). For example, Nestor et al. (2011) found that smokers showed reduced activation compared to nonsmokers in a widely distributed network including the ACC, left IFG, bilateral inferior parietal lobules, and bilateral insula. This is similar to the findings of de Ruiter et al. (2012) who found reduced activation of the rostral ACC during inhibition in smokers.

One interesting line of research has examined the relationship between neural activity during successful response inhibition and craving for cigarettes. Berkman et al. (2011) demonstrated that subjects with greater task-related neural activity in nodes of the response inhibition network (bilateral inferior frontal gyrus, SMA, putamen, and left caudate) smoked less in response to subsequent, naturally occurring occasions of cigarette craving. These results suggest that functioning in the circuitry underlying motor inhibition translated to greater behavioral control in response to craving. Further, these investigators found an inverse relationship between amygdala activation during response inhibition and behavior, such that subjects with greater amygdala activation had a stronger positive relationship between craving and smoking behavior. These findings link altered patterns of neural activation with behavioral constructs known to be critical in addiction. Further, as studies have reported hypoactivation in the neural circuitry for
response inhibition without differences in task performance, this study underscores the potential utility of neuroimaging as a sensitive measure of neurobiological alterations related to impulsive behavior. Finally, there is considerable value in studies that link lab-based measures of neurobiological function with assessments of inhibitory control in the real world. Real-world behaviors as assessed, for example, by mobile technologies, open up valuable opportunities to relate the neurobiology of inhibitory control to avoid drug use in the natural environment, which in many cases is laden with cues to use.

Alcohol

Alcohol abusers have increased commission error rates compared to nondrinkers or social drinkers on go/no-go tasks (Kamarajan et al., 2005; Murphy and Garavan, 2011), and longer SSRTs on the stop-signal task compared to controls (e.g., Goudriaan et al., 2011; Lawrence et al., 2009; Rubio et al., 2007). However, mixed results have been reported with a number of studies showing no difference in response inhibition related to alcohol consumption (Li et al., 2009; Papachristou et al., 2013; Schmaal et al., 2013). It has been suggested (Smith and Mattick, 2013) that this may relate to sex differences, based on evidence that heavy drinking may be preferentially associated with impaired response inhibition in females (Nederkoorn et al., 2009; Smith and Mattick, 2013; Townshend and Duka, 2005). That said, few studies have been sufficiently powered to specifically examine sex differences in response inhibition related to alcohol consumption. Nonetheless, Smith and colleagues reported an overall impairment in response inhibition in their meta-analysis and suggested that a dose response relationship
may exist between impaired response inhibition and drinking patterns (Smith et al., 2014). However, there have been no systematic studies addressing this possibility.

Studies using functional neuroimaging to examine response inhibition in problem drinkers are limited. Li and colleagues found no performance differences on SSRT but lower activation in left dIPFC in alcohol-dependent patients (Li et al., 2009). However, these subjects were all successfully abstinent in alcohol treatment at the time of scanning, making it difficult to determine if activation patterns were related to alcohol withdrawal or early recovery from alcohol dependence. Recent findings have shown that alcohol-use disorders are associated with lower activation in the IFG, insula, inferior parietal lobule, and ACC compared to controls (Claus et al., 2013). When comparing heavy to light alcohol consumption in college drinkers, the heavy drinkers showed impaired performance and altered patterns of neural activity during response inhibition in areas including the ACC, portions of the frontal lobe, hippocampus, and thalamus (Ahmadi et al., 2013). Structural neuroimaging experiments have suggested that chronic alcohol abuse is associated with global volume reduction, cortical and subcortical gray matter reductions, and enlargement of the ventricles. The volume loss in frontal, cerebellar, and subcortical regions are believed to play a critical role in individual differences related to task performance (Chanraud et al., 2007; Scheurich, 2005; Sullivan, 2003). Therefore, as the neural architecture supporting response inhibition deteriorates, behavioral inhibition capacity is likely to suffer.

Cannabis
Studies in both adolescent and adult cannabis users have found little evidence for disrupted cognitive performance (Grant et al., 2012; Jager et al., 2010; Schweinsburg et al., 2010; Tapert et al., 2007); however, see Moreno et al. (2012). Interestingly, several studies have demonstrated that while there are inconsistent effects of cannabis use on inhibitory performance, there are neural differences that can be detected via fMRI (Behan et al., 2014; Hester et al., 2009; Roberts and Garavan, 2010; Schweinsburg et al., 2008; Tapert et al., 2007). For example, Roberts and Garavan investigated neural activity using fMRI during response inhibition in adolescent cannabis users and nondrug using controls. While users had equal performance to control subjects, the users had increased activation in frontal and parietal regions during successful inhibitions. This pattern of activation was interpreted to indicate increased neural resources required of the users to achieve performance levels comparable to controls (Roberts and Garavan, 2010). Similar results were found in a study of college students (cannabis users compared to nondrug users) where there was equal task performance but increased activation in the right inferior parietal lobule, the right putamen, and the supplementary motor area in the users (Hester et al., 2009).

It is notable that the pattern of effects in cannabis users (comparable performance but greater activation relative to controls) differs from the hypoactivity associated with other drugs of abuse. Some evidence suggests that heavier use, earlier onset, and greater cumulative cannabis consumption is associated with smaller increases in activation in frontal and parietal regions compared to lighter users or those who begin using later (Schweinsburg et al., 2008, 2010). Such findings indicate that there may be an interaction
of brain development and cannabis exposure on brain function and may additionally suggest a compensatory mechanism in heavy cannabis users (Jacobus et al., 2009). Another possibility is that the increased activation of cannabis users may compensate for altered functional connectivity between regions. Recently, Orr and colleagues showed increased intrahemispheric and decreased interhemispheric resting-state connectivity in adolescent heavy cannabis users (Orr et al., 2013). The same sample of adolescent users, when performing a go/no-go task showed impaired performance but no regional activation differences relative to controls. Instead, the users showed increased connectivity during the task between bilateral parietal lobes and left cerebellum, and these same regions showed increased resting-state connectivity (Behan et al., 2014). Although these results may suggest that atypical patterns of activation in cannabis users may be related to differences in inter- and intrahemispheric connectivity, the full set of results fails to offer a straightforward message. As cannabis is the most commonly used illicit drug and the onset of use is common during the sensitive adolescent neurodevelopmental period, it is important that the effects of cannabis on neurocognitive function vis-à-vis inhibitory control be the subject of further inquiry.

**Cocaine**

There is strong evidence that cocaine users have poorer response inhibition than nonusers. This is observed in studies using the stop-signal task (Colzato et al., 2007; Fillmore and Craig, 2002; Li et al., 2006; Morie et al., 2014; but see Vonmoos et al., 2013) and in go/no-go tasks (Fernández-Serrano et al., 2011; Hester and Garavan, 2004;
Hester et al., 2007; Kaufman et al., 2003; Lane et al., 2007). A review by Spronk and colleagues calculated pooled effect sizes for both SSRT on the stop-signal task and errors of commission on go/no-go tasks and found a moderate pooled effect size (0.50) of cocaine user status on the stop-signal task and a moderate to large (0.64) pooled effect size for errors of commission on the go/no-go task (Spronk et al., 2013). fMRI studies have generally shown reduced neural activity in the PFC including rostral ACC and SMA (Hester and Garavan, 2004; Kaufman et al., 2003; Li et al., 2007).

Using independent component analysis on a stop-signal task, Elton and colleagues discriminated cocaine users from nonusers based on activity patterns decomposed into 11 components. Two of these components were specifically related to stop-signal success, and cocaine users exhibited decreased activation in these networks compared to controls. One network comprised the bilateral IFG, angular gyri, middle temporal, and posterior parietal gyri, and the other network comprised the dlPFC, ventrolateral PFC, dorsomedial PFC, anterior insula, and middle temporal gyrus (Elton et al., 2014).

**MDMA/Ecstasy**

A meta-analysis found that overall there is a small effect size on inhibitory errors in heavy MDMA users compared to controls (Smith et al., 2014). Among individual studies, there are several that reported no behavioral performance differences (von Geusau et al. 2004; Quednow et al., 2006; Roberts et al., 2013; Roberts and Garavan, 2010). However, two of these studies used neuroimaging and found altered neural processing in MDMA users. For example, Roberts and colleagues found that
ecstasy/polydrug users showed altered EEG patterns suggestive of attentional or inhibitory deficits (Roberts et al., 2013). Similarly, Roberts and Garavan (2010) found intact performance but increased activation in the response inhibition network (right DLPFC, inferior frontal gyrus, and parietal lobule) in recreational ecstasy users. Other studies of current MDMA users have reported moderately impaired behavioral performance in response inhibition (Hoshi et al., 2007). Taken together, the available literature suggests a small impairment in response inhibition associated with MDMA use and altered neural processing in users with intact behavioral performance.

**RESPONSE INHIBITION AND ABSTINENCE**

Relapse is, in many regards, a defining characteristic of drug dependence. Successful abstinence might be viewed within a framework whereby prefrontal cognitive systems seek to control biased attention and pathological behaviors. Hence, successful abstinence may rest on the outcome of the antagonism between drug-wanting systems driven, for example, by ventral striatally mediated salience attribution systems (Robinson and Berridge, 2003), and drug-denying systems governed by the prefrontal cortex (Goldstein and Volkow, 2002; Figure A2.2).

There is, however, relatively little empirical data on the neurobiology of successful abstinence despite its potential value for informing therapeutic interventions. The extant literature has typically investigated short-term abstinence and has revealed many persistent deficits, which, for example, for cocaine users, are more pronounced in heavy users in lateral and medial prefrontal regions associated with cognitive control (Bolla et al., 2003, 2004). Abstinent cannabis users show a similar pattern of lateral and
medial hypoactivity but have also been reported to show bilateral hippocampal hyperactivity (Eldreth et al., 2004). There is, however, evidence to suggest that prolonged abstinence will correct the general pattern of prefrontal hypoactivity in users (see below) with, for example, cocaine abstinence reducing high-risk responses on a gambling task (Bartzokis et al., 2000). Structural MRI studies have found reduced gray matter volume in prefrontal, orbitofrontal, and cingulate regions in cocaine abstinent individuals (Fein et al., 2002; Matochik et al., 2003), which, some argue, can last even with prolonged abstinence (Tanabe et al., 2009). Interestingly, Connolly and colleagues found in a cross-sectional analysis that cocaine abstinent individuals reached control-like levels of gray matter volumes in the cingulate, insula, and dlPFC by 35 weeks of abstinence (Connolly et al., 2013).

During abstinence, impulse control might be important for suppressing drug-seeking behaviors and drug cravings. Although subjective reports of craving often prove to be poor predictors of subsequent abstinence, cognitive and neuroimaging measures can sometimes do better (Grüsser et al., 2004; Kosten et al., 2005). For example, higher scores on a self-report measure of impulsivity (the Barratt Impulsiveness Scale) have been shown to predict poorer treatment outcome (Moeller et al., 2001; Patkar et al., 2004). With regard to brain predictors, unfortunately, the neuroimaging literature on predicting relapse is small and has employed a variety of tasks that were not necessarily designed to induce a craving response or to assess the user’s ability to exercise inhibitory control over that response. Nonetheless, the existing results do identify prefrontal systems, among other regions, as effective predictors of treatment outcome. For example,
using a two-button prediction task, Paulus and colleagues showed activation levels in prefrontal, temporal, and posterior cingulate regions early in abstinence to predict subsequent relapse for methamphetamine users (Paulus et al., 2005). Grüsser et al. (2004) found that activity in response to alcohol-related stimuli in the putamen, ACC, and medial prefrontal cortex predicted relapse. In cocaine treatment-seeking individuals, fMRI error-related processing (stop-error vs. stop-success) revealed blunted activity in the dorsal ACC predicted relapse in both sexes, while females exhibited reduced thalamic activity, and males exhibited reduced insular activity (Luo et al., 2013). Although it does not follow from these findings that behavioral measures of impulse control should also predict abstinence, the predictive value of prefrontal cortex suggests that regulatory processes may be involved.

Given the important role that cognitive processes may play in avoiding relapse in drug users and gamblers (Cox et al., 2002; Goudriaan et al., 2008; Passetti et al., 2008; Waters et al., 2003), it may be the case that the best predictors of treatment outcome are those that reflect cognitive control over drug urges rather than the drug urges themselves. This is supported by a study by Brewer et al. (2008) who identified cognitive control prefrontal regions, in addition to other subcortical and posterior cingulate regions, as being the best predictors of treatment outcome in a treatment-receiving sample of cocaine users. Further evidence for the assertion that impulse control might contribute to successful abstinence arises from cross-sectional research of abstinent former users using a go/no-go task. These studies show an apparent reversal in activation patterns, such that prefrontal hypoactivity in current users is paired with prefrontal hyperactivity in abstinent
users. For example, Connolly et al. (2012) showed that both short-term abistent cocaine users (1–5 weeks) and long-term abistent users (4–24 months) present with fMRI hyperactivity in cognitive control regions relative to drug-naïve controls. That is, the brain regions involved in impulse control (e.g., right middle and rIFC), which are consistently shown to be hypoactive in current users, show elevated activity in former users compared to drug-naïve controls. Subsequent studies have shown former users to be either comparable in performance, fMRI activation levels, and motor-inhibition-related ERP components to controls (Bell et al., 2014; Morie et al., 2014) or to show elevated activation associated with successful inhibitions (Hester et al., 2013). The latter study also revealed blunted activation in response to errors and punishments in the former users suggesting some deficits may persist longer into abstinence.

Evidence for enhanced cognitive control contributing to successful abstinence is also observed in former cigarette smokers (abstinent for 2 years). Using a go/no-go task, current smokers showed reduced activity relative to controls in the dlPFC and the ACC while the former smokers revealed greater inhibition and error-related activation in the ACC relative to the current smokers (Nestor et al., 2011). A recent study in cigarette smokers highlighted behavioral effects of practicing self-control (i.e., small acts of impulse control such as avoiding sweets were practiced over 2 weeks before quitting) which significantly improved abstinence rates; 27% in the self-control group, relative to 12% in a control condition, were still abstinent 1 month after quitting (Muraven, 2010).

TMS and tDCS have shown some efficacy in enhancing cognitive control. Jacobson and colleagues demonstrated faster SSRTs while stimulating the right inferior
frontal gyrus (Jacobson et al., 2011). Applying this technique to substance-abusing individuals may prove fruitful. One study in alcohol detoxification found a single session of TMS over the rIFC facilitated cognitive control performance a week later (Herremans et al., 2013). Similarly, pharmacological interventions targeting cognitive enhancement in cigarette smokers have provided some support for the facilitation of abstinence. Focusing on studies directly assessing response inhibition performance, galantamine, a cholinesterase inhibitor, has reduced subjective craving for cigarettes, while improving performance on a go/no-go task (Sofuoglu et al., 2012). Another study suggests that the use of an NMDA partial agonist, D-cycloserine, attenuates subjective ratings of cigarette “stimulation” and “relaxation,” while improving performance on a go/no-go task (Nesic et al., 2011). Lastly, in a combined fMRI-pharmacological study of guanfacine, a noradrenergic agonist, smokers exhibited reduced cigarette consumption. While no effect was found on task performance, the fMRI results indicate guanfacine attenuated dlPFC responses. The authors interpret this finding as a possible guanfacine-related facilitation of cognitive efficiency.

In summary, the extant literature suggests compromised inhibitory control in active users and normalized or enhanced control in abstinent users. If inhibitory control is shown to be an important contributor to abstinence then this raises exciting possibilities for pharmacological or behavioral interventions. In time, neuroimaging measures may enable us to predict who is most likely to abstain (e.g., related to the integrity of the circuitry underlying inhibitory control) and, by tracking recovery in this circuitry, give guidance on who is most at risk for subsequent relapse.
CONCLUSION AND FUTURE DIRECTIONS

The preceding review suggests that deficits in inhibitory control characterize substance dependence. There are, however, drug-specific effects that require further elaboration (e.g., the mixed findings in cannabis users). The integration of functional activation, functional connectivity, and brain structural data is important, but so too is a much richer phenotypic characterization of the users including their drug use histories (age of onset, polydrug use), mental health comorbidities, family and environmental influences, and so on. In reviewing the literature, there persists a lack of a comprehensive understanding on how the various types of inhibitory control relate to one another, psychologically and neurobiologically. More assessments of drug use and other types of inhibitory control (e.g., delaying gratification) or inhibitory control in reward-related contexts may yield new insights. It is a conundrum that although different aspects of inhibitory control appear to be uncorrelated with one another (e.g., self-report personality measures, impulsive choice, and impulsive responding; Reynolds et al., 2006), drug users score highly impulsive on all. Combining this with the evidence that inhibitory control is related to reward processes such as drug-induced euphoria and drug self-administration (Cervantes et al., 2013; Weafer and de Wit, 2013), suggests that more conceptual work is required to integrate these constructs. Finally, as noted above, relating lab-based measures of inhibitory control to drug urges and craving in the natural environment is an important extension of the existing research.

With regard to abstinence, there are two questions of primary importance, and for both, inhibitory control appears to be a central construct. First, to what extent does
inhibitory control predicts abstinence? This is important clinically (i.e., identifying who is most likely to relapse can help in allocating interventions and additional services) and also theoretically (i.e., the predictors of relapse give good guidance on the mechanisms that may contribute in a causal manner to abstinence; Garavan et al., 2013). Second, what is the time-course of recovery of inhibitory control and other processes pertinent to addiction? One speculation is that certain processes (e.g., the incentive salience attributed to drugs and drug cues mediated by structures such as the ventral striatum) may persist long after the cessation of use and may underlie relapse risk. It may be the case that inhibitory control recovers to normal (or greater than normal) levels relatively early in abstinence, and while inhibitory control exercised over drug cravings and behaviors is essential to abstinence, relapse is highly likely when this regulatory function becomes disrupted as happens, for example, under stressful situations. Large sample, longitudinal studies of abstainers that assess multiple functions at multiple time-points are required to fully elaborate the role that inhibitory control contributes to avoiding relapse.
References


neural substrates which May promote nicotine abstinence through increased cognitive control. Neuroimage 56 (4), 2258–2275.


**Figures**

**Figure A2.1:** Response inhibition on the STOP task produces robust activation in parietal and frontal cortex, including bilateral inferior frontal gyrus.

![Brain image 1](image1.png)

**Figure A2.2:** Stop Task Related Activity Hypotheses

![Brain image 2](image2.png)

We hypothesize that abstinence relies upon recovery of prefrontal systems involved in inhibitory control (regions such as the right IFG and OFC shown on the left). Vulnerability to relapse may be reflected in reinforcement or salience systems (involving regions such as the ventral striatum shown on the right). We hypothesize that relapse may arise from lapses in the prefrontal regulatory systems.
APPENDIX 3: STRUCTURAL BRAIN CORRELATES OF ADOLESCENTS WITH BEHAVIORAL AND EMOTIONAL DYSREGULATION

This Chapter has been previously published in the following format:


Abstract

Objective: To characterize the structural and functional neurobiology of a large group of adolescents exhibiting a behaviorally and emotionally dysregulated phenotype.

Methods: Age 14 adolescents from the IMAGEN study were investigated. Latent class analysis (LCA) on the Strengths and Difficulties Questionnaire (SDQ) was used to identify a class of individuals with elevated behavioral and emotional difficulties (“dysregulated”; n=233) who were compared to a matched sample from a low symptom class (controls, n=233). Whole-brain gray matter volume (GMV) images were compared using a general linear model with 10,000 random label permutations. Regional GMV findings were then probed for functional differences from three fMRI tasks. Significant brain features then informed mediation path models linking the likelihood of psychiatric disorders (DSM-IV) with dysregulation.

Results: Whole-brain differences were found in the right orbitofrontal cortex (R.OFC; p<.05; k=48), with dysregulated individuals exhibiting lower GMV. The dysregulated group also exhibited higher activity in this region during successful inhibitory control (F_{1,429}=7.53, p<.05). Path analyses indicated significant direct effects between the likelihood of psychopathologies and dysregulation. Modeling the R.OFC as a mediator
returned modest partial effects, suggesting the path linking the likelihood of an anxiety or conduct disorder diagnoses to dysregulation is partially explained by this anatomical feature.

**Conclusion:** A large sample of dysregulated adolescents exhibited lower GMV in the R.OFC relative to controls. Dysregulated individuals also exhibited higher regional activations when exercising inhibitory control at performance levels comparable to controls. These findings suggest a neurobiological marker of dysregulation, and highlight the role of the R.OFC in impaired emotional and behavioral control.

**Introduction**

Adolescents exhibiting severe difficulties regulating behavior and emotion are commonly referred for psychiatric evaluation but are difficult to classify into discrete diagnostic categories, with “comorbidity” being the rule rather than the exception in child psychiatry. Previous labels for these dysregulated children included severe mood dysregulation (SMD) or irritability (Leibenluft, 2011) with the acknowledgement that these individuals will likely meet diagnostic criteria for other disorders. Recently, “disruptive mood dysregulation disorder” (DMDD) (American Psychiatric Association, 2013) was added to the DSM-5 to better classify dysregulated children. Research indicates the prevalence of dysregulation is between 0.8 and 3.3%, with particularly high co-occurrence with externalizing and internalizing disorders (Copeland et al., 2013), (Dougherty et al., 2014). As individuals with a singular diagnosis may be thought of as behaviorally or emotionally dysregulated, it is specifically the individuals with a set of difficulties spanning both behavioral and emotional domains who need to be studied.
further. Considering the addition of this disorder into the DSM, and research showing the functional outcomes of dysregulated youths are strikingly poor, (Copeland et al., 2014) it is imperative to identify the neurobiological correlates of dysregulation. Characterizing the pathophysiological substrates will help inform dysregulation nosology, provide diagnostic biomarkers, and help inform targeted treatment methods.

The NIMH recently advocated the Research Domain Criteria (RDoc), which hypothesizes psychiatric problems coexist on a spectrum of severity with symptoms that cut across discrete diagnostic categories. Therefore, in this report using a large dataset of adolescents (the IMAGEN study), (Schumann et al., 2010) we adopted a latent class analysis (LCA) approach to the Strengths and Difficulties Questionnaire (SDQ)(Goodman, 1997) to identify groups of individuals endorsing similar patterns of behavioral and emotional problems. The result of an SDQ-LCA provides class groupings, as well as dimensional characteristics of emotional and behavioral problems hypothesized to contain varying patterns of symptomatology that resist discrete categorization. One class is specifically hypothesized to comprise a profile analogous to DMDD. In other words, in the absence of DMDD diagnoses, we hypothesized a class of individuals exhibiting a profile in line with a dysregulation phenotype. Although measurement of a dysregulation profile is a major challenge in the field, (Althoff et al., 2010a; Deutz et al., 2016, 2018) the intent of our investigation is to characterize the neural correlates of dysregulation as defined by one measurement method (among a suite of others). (Althoff et al., 2010b; Jordan et al., 2016)
Structural neuroimaging, and specifically, voxel-based morphometry (VBM), has been used to study many psychiatric constructs across stages of development. VBM allows the researcher to measure the volumes of the major tissue types of the brain, (Ashburner and Friston, 2000) thus providing a neurobiological framework to closely study a behavioral profile of interest. VBM has informed many adolescent psychiatric disorders related to dysregulation including anxiety, (Radua et al., 2010) depression, (Bora et al., 2012) and conduct disorder. (Fairchild et al., 2011) Regarding previous structural neuroimaging studies of dysregulation, Adleman and colleagues used VBM to uncover differences among children with SMD, bipolar disorder (BP), and controls, with the SMD group exhibiting the lowest gray matter volume (GMV) in bilateral pre-supplemental motor area, right insula and dorsolateral prefrontal cortex. (Adleman et al., 2012) Additionally, Gold and colleagues used VBM to study youths with anxiety, BP, ADHD, and DMDD, compared to controls. Gold found GMV differences specific to, and across psychiatric disorders, with dysregulated participants exhibiting lower GMV in the right dorsolateral and superior frontal cortex. (Gold et al., 2016) Therefore, for our primary analysis using whole-brain VBM data, we hypothesized dysregulated individuals would exhibit lower GMV relative to controls in cortical regions implicated in regulatory processes such as the bilateral insula, right-sided dorsolateral prefrontal cortex, and ventromedial/orbitofrontal cortex. (Adleman et al., 2012; Rogers and De Brito, 2016)

Regions uncovered from the primary anatomical analysis can be used as regions of interest in post-hoc analyses on the fMRI data from the IMAGEN study. These post-
hypothesis that differences in brain structure yields differences in brain function. Interrogating both structure and function with the same dataset maximizes the information gained about the neurobiological characteristics of dysregulation, and captures the brain’s trait-like features measured via structural neuroimaging, and state-like features measured during functional task demands. Follow-up analyses on neuroimaging data can also be used to explain the relationship between candidate comorbidity diagnoses (Copeland et al., 2013) and dysregulation. For instance, an identified neurobiological correlate of dysregulation can be modeled as a mediator in a path analysis linking the likelihood of a psychiatric disorder to dysregulation. In doing so, we test the hypothesis that the brain mediates the relationship between a disorder and dysregulation in some linear fashion. As we only probe data from the age 14 assessment of the IMAGEN study, these mediation models infer correlation and not causation.

Methods

Participants were drawn from the IMAGEN study of adolescent development. (Schumann et al., 2010) Comprehensive study details are available in the online Standard Operating Procedures (https://imagen-europe.com/). The IMAGEN study conformed to the ethical standards outlined by the Declaration of Helsinki and was approved by ethics committees at each site including King’s College, London; Central Institute of Mental Health, Mannheim; Charite, Universitatsmedizin Berlin; University Medical Center Hamburg-Eppendorf; University of Nottingham; Trinity College Dublin; Institut National de la Sante et de la Recherche Medicale, Orsay. After description of the study to
participants and their parents, written informed consent was obtained. Individuals who provided assent were assessed at age 14. For this report, all data were taken from the baseline assessment only (age 14). Participants with SDQ data (N=2,126) were used as the starting sample of the analysis (Age $M=14.56$, $SD=.44$; Females=1,081, 51%). Selected participants from the LCA analysis were then drawn from the sample who received an anatomical scan with GMV images passing quality control (N=2,024).

**Strengths and Difficulties Questionnaire**

The SDQ is a 25-item instrument designed to characterize children across five domains including emotional symptoms, conduct problems, hyperactive behavior, peer problems, and prosocial behaviors. (Goodman, 1997) Hence, the SDQ is especially suited to capture both the behavioral and emotional features related to dysregulation. Furthermore, the SDQ is widely used and has been shown to predict psychiatric diagnoses later in life. (Goodman and Goodman, 2011) Data included in the analysis were from the parent reporting on their child’s behavior in the past six months. SDQ data from N=2,126 participants were used in the latent class analysis.

Each SDQ item is measured on an ordinal scale: 0=Not True, 1=Somewhat True, 2=Certainly True. While the majority of the instrument is negatively valenced (e.g., “Often unhappy”, “Often lies or cheats”), the few positively valenced items are reversed coded with the exception of the entire prosocial domain. Therefore, higher values within the emotional, conduct, hyperactive or peer domain reflect difficulties, whereas higher values within the prosocial domain reflect strengths. For the input to the latent class
analysis, positively valenced items from the prosocial domain were recoded to match the overall pattern of the instrument.

Previous investigators have reported using the SDQ-Dysregulation Profile (SDQ-DP) to measure the dysregulation construct based on the sum of five proposed items. (Holtmann et al., 2011) Rather than imposing the recommended SDQ-DP cutoff of scores $\geq 5$ as dysregulated, we used a data driven approach to characterize individuals based on patterns of similar problem behaviors. And while the SDQ-DP is based on five SDQ items spanning behavioral and affective problems, youths who score high on only the behavioral items may be categorized as dysregulated despite scoring low on the emotional items. The use of latent class analysis is hypothesized to overcome this limitation by identifying a class of individuals who are most likely to exhibit co-occurring behavioral and affective problems. Nonetheless, the SDQ-DP was calculated and compared to the class probabilities returned from the latent class analysis.

*Latent Class Analysis*

Latent class analysis (LCA) is an example of a mixture model used to estimate group membership of latent constructs. LCA is robust to the categorical data format of the SDQ and assigns probability scores to each participant reflecting the likelihood of class membership. Participants were categorized into the class with the highest probability score.

Latent class models were estimated using the software Mplus via an EM algorithm. Model comparison was performed on analyses returning 1-class through 7-
class solutions. The best-fitted model was identified using multiple measures of fit. The Bayesian Information Criterion (BIC) is a goodness-of-fit index that penalizes models with more classes. Lower BIC values indicate more parsimonious models. Because standard loglikelihood tests are biased in this analytic environment, two other examinations were used to compare a K class model to a K-1 class model, the Vuong-Lo-Mendell-Rubin likelihood ratio test (VLMRT) and the bootstrap likelihood ratio test (BLRT). In each case, significance comparing a K class model to a K-1 class model indicates additional information is provided. If it is not significant, then the K-1 class model can be accepted. In addition, models with higher entropy (closer to 1) indicate a clearer delineation of classes. (Celeux and Soromenho, 1996) In this analysis, all indices other than the BLRT (which was not discriminating) indicated a 5-class model fit (see Table A3.1). These classes were then used to identify two groups of interest, a dysregulated group, and a low symptom comparison group (controls). Group identification was determined based on their respective patterns of item endorsement as further explained below.

**Structural Neuroimaging Methods**

Across the eight acquisition sites, participants were scanned on 3T MRI scanners from various manufacturers (Phillips, General Electric, Bruker, and Siemens). Standardization and quality assurance efforts were made to insure all sites used the same MRI acquisition parameters and yielded comparable data. High-resolution anatomical magnetic resonance images were acquired, including a 3D T1-weighted magnetization
prepared gradient echo sequence based on the ADNI protocol (http://adni.loni.usc.edu). The structural image was collected for nine minutes using the following parameters: TR=2300ms; TE=2.8ms; flip angle=8o; matrix size=240x256; voxel resolution=1.1mm3; and 160 contiguous slices at a thickness of 1.1mm.

Whole-brain gray matter volume (GMV) images were generated using optimized voxel-based morphometry procedures in SPM8. (Ashburner and Friston, 2000) High-resolution anatomical magnetic resonance images were acquired, including a 3D T1-weighted magnetization prepared gradient echo sequence based on the ADNI protocol (http://adni.loni.usc.edu). Structural MRI preprocessing included segmenting and normalizing the images into Montreal Neurological Institute template space. The gray matter segmentation images were then modulated to obtain volumetric images, rather than tissue concentration images. N=2,024 participants received a structural MRI and had GMV images passing quality control.

In preparation for the between-group GMV comparison, variables potentially influencing adolescent GMV (age, sex, site of imaging acquisition, handedness, puberty status, (Carskadon and Acebo, 1993) verbal and performance IQ, (Wechsler, 2003) and total GMV) were partialled out of the images. To do so, all participants from the baseline IMAGEN sample with preprocessed GMV images (N=2,024) were submitted to a multiple regression with only the confounding variables included in the design matrix. The residual GMV image for each participant was then used in the permutation test described below. This procedure was used because including nuisance covariates in the permutation analysis prohibitively increased computation time.
In light of recent criticisms related to the proper correction for multiple comparisons in neuroimaging research, (Eklund et al., 2016) permutation analyses have been advocated as a non-parametric approach to closely control the number of false-positives in a statistical analysis. (Winkler et al., 2014) Here, we used a random label permutation test applied to the residual output of the aforementioned nuisance regression. Each participant’s group membership was randomly shuffled and a whole-brain two-group t-test using a general linear model was fitted to the residualized images. Random label shuffling was repeated 10,000 times, thus building a null distribution at each voxel, to which the originally labeled results were compared. Threshold-free cluster enhancement correction (TFCE)(Smith and Nichols, 2009) was then used to control the family-wise error rate for identifying clusters of residual gray matter that exhibit significant group differences. Regions of interest (ROI) surviving a TFCE corrected $\alpha < .05$ were then probed for fMRI group differences, as well as being modeled as the mediator in candidate path analyses linking the likelihood of psychopathology to dysregulation. Permutation analyses were conducted using FSL’s Permutation Analysis of Linear Models (Winkler et al., 2014) on the University of Vermont’s Advanced Computing Core.

*Functional Neuroimaging Methods*

Three fMRI tasks commonly used in psychiatric neuroimaging were administered, including the stop signal task, monetary-incentive delay task, and a face-processing task. The stop signal (inhibitory control) task requires participants to inhibit a prepotent motor
response. (Rubia et al., 2007) Motor inhibitory control performance during this task is commonly measured using the stop signal reaction time (SSRT), an estimate of the speed of the inhibitory process, calculated from the average latency period between the “go” and “stop signal” during successful inhibition trials. (Logan and Cowan, 1984) The monetary-incentive delay task measures the processing of both anticipation and receipt of monetary rewards. (Knutson et al., 2000) The face-processing task involves the passive viewing of angry faces, neutral faces, and control images. (Grosbras, 2005) See Supplement 1, available online, for full details on the fMRI tasks.

All fMRI data were submitted to standard preprocessing methods and whole-brain contrast images specific to each task were estimated using a general linear model (see Supplement 1, available online, for fMRI processing details). Specifically, unsuccessful and successful inhibitory control, monetary reward anticipation and receipt, angry faces, neutral faces, and the differential activation for angry minus neutral faces, were each used to explore any functional differences between the groups. For each contrast image, the mean value within a region of interest (ROI) was extracted and analyzed using two-group ANCOVA models with a Bonferroni corrected alpha based on the number of contrasts tested for each task modality.

Likelihood of Psychiatric Diagnoses

Psychopathology was determined using the Developmental and Well-Being Assessment (DAWBA; http://www.dawba.info/a0.html), a set of computer-administered interviews, questionnaires, and ratings generating DSM-IV psychiatric diagnoses for ages
5-17. Based on the child and parent responses, a computer algorithm generates scores to predict the likelihood of meeting criteria for DSM-IV diagnoses (“band scores”). These band scores range from 1 to 5, representing a probability of <0.1% to >70%. DAWBA band scores have been shown to yield prevalence estimates that broadly compare to clinician-rated diagnoses. (Goodman and Goodman, 2011)

*Mediation Analyses*

Mediation was conducted in Mplus using a robust weighted least squares estimator to estimate direct and indirect effects, with bias-corrected 95% confidence intervals generated from 1000 bootstrapped samples. The use of bootstrapping the indirect effects is a more powerful method of inferring mediation compared to the traditional five-step procedure. (Hayes, 2009) The independent variables for the five separate mediation analyses included the full range of DAWBA band scores, representing the likelihood of receiving a DSM-IV diagnosis for anxiety, depression, conduct disorder (CD), oppositional defiance disorder (ODD), and attention deficit hyperactivity disorder (ADHD). These five constructs were informed by Copeland and colleagues who assessed the prevalence rates of DMDD comorbidity with these disorders. (Copeland et al., 2013) Furthermore, these disorders broadly capture the co-occurring internalizing and externalizing problems exhibited by dysregulated individuals.

The identified GMV features were modeled as a mediator between each band score and the binary dysregulation status as the dependent variable. Hence, models were constructed to test the hypothesis that the underlying neurobiology influences the
relationship between a related psychiatric disorder and the dysregulated phenotype. As
the initial neuroimaging analysis here tests for a biomarker of dysregulation in isolation,
follow up path analyses assessed the involvement of brain structure with dysregulation in
the context of affiliated psychiatric diagnoses reported by Copeland and colleagues. Any
significant indirect effects uncovered by these path models provides evidence indicating
the correlation between the likelihood of a related disorder and being dysregulated is
driven, in part, through changes in focal brain structure.

Results

Latent Class Analysis Results

The best-fitting LCA model returned a five-class solution (see Table A3.1 for fit
statistics). Here, we describe each class and offer a label to characterize their profile.
Class 1, the “defiant class” (18% of the sample), contained individuals with low prosocial
traits and slightly elevated conduct problems and hyperactivity. Class 2, the “emotional
difficulties” class (16% of the sample), contained individuals with the highest emotional
difficulties. Class 3, the “dysregulated class” (12% of the sample), contained individuals
with very high levels of difficulties across all five domains. Class 4, the “hyperactive
class” (25% of the sample), contained individuals with elevated hyperactivity. Class 5,
the “low symptom class” (29% of the sample), contained individuals with very low levels
of problem behaviors across all domains. And while class 5 is labeled “low symptoms”,
we note that the defiant, emotional difficulties, and hyperactive classes also exhibit low
symptoms on domains outside of their problem areas. These findings are consistent with
studies reporting high prevalence rates of any level of psychiatric symptomatology in adolescence. (Copeland et al., 2011) See Figure A3.1 for the average item-endorsement for each class and Table 2 for the five SDQ summary scores for each class.

While other classes exhibited elevations in a single domain (i.e., emotional difficulties class; hyperactive class), the dysregulated class distinctly exhibited co-occurring behavioral and affective problems. These individuals exhibited the highest probability of endorsing conduct problems, hyperactivity, peer problems, and the second highest probability of endorsing emotional problems (closely following the emotional difficulties class), and prosocial problems (closely following the defiant class). The low symptom class (the largest sample) was selected as the comparison group as they exhibited the lowest probability of endorsing all problematic behaviors. See Table S1, available online, for comparison of the dysregulated class to the full sample on descriptive characteristics.

Next, the SDQ-DP was calculated to compare to the LCA results using bivariate correlations between the SDQ-DP sum score and the probability of each class membership. Results indicated the SDQ-DP was most positively associated with the dysregulated class \(r_{2126}=.61, p<.001\) and most negatively associated with the low symptom class \(r_{2126}=-.44, p<.001\), thus providing support for the dysregulated phenotype captured by class 3 and the low symptom group captured by class 5. However, these correlations may be inflated as both measures were estimated from similar items on the same dataset. Nonetheless, Holtmann and colleagues report correlations between their SDQ-DP and Child Behavior Checklist-Dysregulation Profile
(CBCL-DP) binary score at $r=.45$ and CBCL-DP sum score at $r=.75$. Therefore, the LCA results reported here are in line with these other measurement instruments.

There were 184 participants included in the LCA who did not provide anatomical scan data (for reasons including failing quality control, MRI safety issues, etc.). However, chi-square tests indicated there was no difference in LCA class membership in this subsample relative to the larger sample with anatomical scan data ($\chi^2_{\text{no scan}}=2.2, p>.05$). Thus, we do not believe there was any skew in the LCA class assignment by the participants who did not provide anatomical scan data.

**Neuroimaging Sample Characteristics**

Of the 261 dysregulated and 613 low symptom comparison individuals identified from the LCA, 233 dysregulated and 564 comparison individuals provided useable GMV data. For the sample of 233 dysregulated individuals, an equal size subset of comparison individuals was selected from the low symptom class. This control group was pseudo-randomly selected so as to match to the dysregulated group by containing an equal number of males and females who showed no differences on total GMV, pubertal development, performance and verbal IQ, or age, and contained similar distributions for handedness and site of acquisition (see Table S2, available online, for group comparisons). And while site was included in the initial nuisance regression of the full IMAGEN dataset, it is difficult to precisely account for site when there are unequal representations at each site. Hence, a pseudo-random sampling of the two groups was performed to identify a subsample of individuals with equal representations at each site.
Results using this perfectly site-matched subsample were consistent with the main findings reported below. See Supplement 1, available online, for more information.

**Whole-brain Residual Gray Matter Volume Comparison**

After running a two-sample t-test using a general linear model with 10,000 random label permutations, a single cluster survived TFCE-correction for multiple comparisons ($P_{FWE-corr} < .05, k=48$ voxels). This cluster was found in the right orbitofrontal cortex (OFC), center of mass at (MNI: 24, 30, -16), spanning the orbital sulcus with extent into the posterior orbital gyrus. In this region, dysregulated individuals exhibited lower residual GMV relative to their peers with low symptoms (see Figure A3.2).

**Laterality Test**

As only one hemisphere survived strict correction, and there is growing interest in prefrontal asymmetry, a contralateral region of interest analysis was performed post-hoc. To perform this test, we translated the right-sided region of interest onto the left hemisphere and extracted regional GMV for all subjects. Two-sample t-tests indicated the left OFC ROI yielded significant differences (L.OFC: $t_{462} = -3.32, p < 1.0 \times 10^{-3}$), similar to the findings in the right OFC, albeit at a relatively lower magnitude of effect (R.OFC: $t_{462} = -4.40, p < 1.0 \times 10^{-4}$).

**fMRI Comparisons**
The identified sample for GMV analyses (n=466) was selected on the basis of the quality of their anatomical image, meaning some of these participants did not have fMRI data available. See Table S3 and Supplement 1, available online, for full details regarding these reduced samples, and reasons for missingness. In preparation for the ROI-level between-group comparisons using the fMRI data, we first examined the amount of head motion in the images. For each subject, the mean framewise displacement (mean FD) was calculated for each of the three fMRI tasks. Based on prior developmental neuroimaging studies,(Silvers et al., 2016) a head motion exclusionary criterion of mean FD > .9mm was used. For the stop signal task, 3 dysregulated participants were excluded. For the faces task, 1 dysregulated participant was excluded. For the MID task, 5 dysregulated and 1 low symptom participants were excluded. Importantly, these reduced samples for fMRI comparisons retained critical between-group similarities as the starting samples for anatomical comparisons. Chi-square (for categorical measures) and $t$-tests indicated that after excluding subjects, the reduced samples retained their best-matched characteristics and did not differ on age, sex, handedness, IQs, or total GMV ($p > .05$).

Data were then submitted to standard two-sample $t$-tests to determine any group differences in head motion for a given task. Results indicated that while mean FD did not exceed thresholds previously reported as problematic, (Power et al., 2012; Siegel et al., 2014) the dysregulated sample exhibited significantly more head motion during each fMRI acquisition (see Table S4, available online). Although participants’ head motion parameters were included in the design matrix during their fMRI contrast estimation, we also included mean FD as a covariate in the ROI-level between-group ANCOVA models.
For the stop signal task, results indicated a significant between-group difference during successful inhibitory control trials $F_{1,377}=5.61, p_{corr}<.05$, such that the dysregulated group showed higher activation ($n=186, M=0.15, SD=1.2$) than the low symptom group ($n=194, M=-0.09, SD=0.92$). To ensure these findings were not driven by the difference in head motion, similar ANCOVA models were estimated on 5,000 pseudo-random subsamples of the data matched on head motion. Results were consistent, leading to a mean $F_{1,307}=4.9, p<.05$, suggesting the between-group difference on successful inhibitory control activations were not driven by head motion. See Supplement 1, available online, for more information.

Due to problems with the behavioral task performance adaptive algorithm, stop signal reaction time (SSRT) scores were available on only a subset of participants. A between-group comparison on those individuals with useable SSRT behavioral data (Dysregulated $n=97$; Controls $n=107$) yielded no significant differences on SSRT ($t_{202}=0.38, p=.71$). Given the reduced sample sizes of participants with SSRT data, no imputations were performed for SSRT, and it is unknown the degree to which the effects might generalize to the starting samples. No between-group activation differences were detected for unsuccessful inhibitory control trials, or on any of the remaining fMRI contrasts (reward and face processing tasks).

**Mediation Analyses**

The likelihood of having any of the five psychopathologies exhibited a significant total and direct effect with dysregulation, substantiating their relationship with
dysregulation. (Copeland et al., 2013) Bias-corrected confidence intervals around the indirect effect of the right OFC GMV ROI indicated this region partially mediated the likelihood of an anxiety disorder diagnosis ($c=.023$, 95% CI [0.003, 0.043]) or conduct disorder diagnosis ($c=.018$, 95% CI [.003, .033]) to dysregulation status (see Figure A3.3). No significant indirect effects were detected to link the brain between the likelihood of depression, ODD, or ADHD with dysregulation. Additionally, regional fMRI brain activation during successful inhibitory control did not yield any significant mediation effects. See Table A3.3 for mediation model results.

**Discussion**

We report that emotionally and behaviorally dysregulated adolescents exhibited lower GMV in the right OFC relative to their non-dysregulated peers. These findings were identified by a conservative permutation analysis between two large samples of closely matched groups. Secondary analyses indicated that within this same region, the dysregulated group exhibited higher functional brain activation when executing successful inhibitory control behaviors. These fMRI results provide some specificity on the psychological correlates of the GMV effect, such that the anatomical difference associated with dysregulation was related to inhibitory control but not to face or reward processing. Taken together, these results suggest dysregulation is characterized by differences in cortical regions involved with executive functioning. Lastly, the volume of the right OFC region partially mediated relationships between the likelihoods of an anxiety disorder and a conduct disorder diagnosis and dysregulation.
It is interesting that the right OFC was uncovered from a conservative whole-brain analysis and also exhibited differences on the stop signal task, as there is a body of research implicating the OFC in behavioral and emotional regulation. For example, previous research on the IMAGEN sample identified this region as participating in a network of brain activity during successful inhibitory control trials. (Whelan et al., 2012) As the dysregulated and low symptoms groups exhibited similar task performance, the greater activity in the right OFC of the dysregulated group may reflect greater effort or cognitive resources needed to execute inhibitory behaviors equal to that of their peers. Therefore, dysregulation may be partly dependent on a neurobiological inhibitory control network compromised in its ability to efficiently regulate behavior.

The OFC is also putatively involved in integrating attention and emotion by assigning a signal of affective value to stimuli. Previous work using event-related potentials (ERP) during an affective go/no-go task was conducted on children with co-occurring internalizing and externalizing disorders. One set of results identified higher ventral prefrontal activations during inhibitory control trials in children with poor self-regulatory abilities as measured via parent-child observations. (Granic et al., 2012) In a related treatment study of similar children, treatment success was characterized by attenuation of activation levels in the ventral prefrontal region during inhibitory control trials. (Lewis et al., 2008) Hence, our findings are in line with these reports and suggest the OFC as both a potential biomarker and candidate region for targeted clinical interventions to help improve outcomes in children with dysregulated behavioral profiles.
In terms of the mediation results, the investigated psychopathologies all exhibited a significant and large total effect on dysregulation, indicating that the likelihood of having an internalizing or externalizing disorder was associated with an increased likelihood of being dysregulated. These findings are consistent with previous reports identifying similar patterns of comorbidity from three datasets of child psychopathology. (Copeland et al., 2013) Moreover, the direct effects were also large, accounting for nearly 98% of the total effect for all disorders (see Table A3.3). Given these relationships, the significant partial mediation results are notable as little variance is left to be explained by the indirect paths. Yet despite these relatively weak indirect effects, the significant findings highlight the transdiagnostic nature of the right OFC region insofar as it is a mediator to dysregulation for the likelihood of anxiety and conduct disorder. Although a significant mediation was not observed for depression, ODD, or ADHD it would be incautious to conclude that the mediation effect has specificity for anxiety and conduct disorder as effects in similar directions were observed for depression ($p=.065$) and ODD ($p=.070$; see Table A3.3). On the whole, the data suggest a small but potentially important role for the OFC in linking internalizing and externalizing disorder to dysregulation. Lastly, we reiterate the path models should not be misinterpreted as implying the likelihood of an anxiety, conduct disorder, or the brain feature caused dysregulation as the models are restricted to age 14 data only.

Limitations of this study include the lack of DSM-5 diagnostic measures, as it is unclear if the individuals contained in the dysregulation group meet DMDD diagnostic criteria. Future studies are needed to evaluate the degree to which the SDQ captures
individuals who receive a DSM-5 DMDD diagnosis following a clinical interview. Likewise, measurement studies are also needed to determine the correlation between popular measurement methods like SDQ-LCA, SDQ-DP and CBCL-DP, and their correlation with clinical ratings. Additionally, recent work taking a factor analytic approach to the SDQ has identified a dysregulation factor using just three of the five domains, omitting the prosocial and peer problem domains. (Deutz et al., 2018) However, given that elevations in the CBCL Social Problems domain frequently accompany the CBCL-DP, (Althoff et al., 2010a) this approach risks omitting relevant features of the dysregulation construct.

In considering dysregulation measurement inconsistencies, differences in the precise brain region uncovered here with the previous regions uncovered by Adleman and by Gold and colleagues are likely attributed to differing measurement approaches. Nonetheless, the right-sided prefrontal anatomical finding is broadly consistent with these prior results. Although earlier fMRI studies of inhibitory control in dysregulation failed to detect significant group differences (Deveney et al., 2012) this is likely due to our fMRI analysis, by design, being restricted to a single anatomically defined region of interest. Another important consideration in interpreting the present fMRI results and integrating them with past findings is the potential confounding role of head motion. Despite including mean framewise displacement as a covariate, ANCOVA models are generally unable to completely control for a significant between-group difference in that covariate. Confidence in our results comes from the 5,000 pseudo-random subsampling
procedure in which the group differences were recapitulated with subsamples chosen not to differ on head motion.

A caveat regarding the mediation results is that the path models were estimated using DAWBA band scores. As the DAWBA contains many skip rules leading some participants to “screen in” for extra items, these skips rules are sometimes related to high SDQ domain scores. Estimating paths between band scores and a binary dysregulation score determined via an SDQ-LCA consequently may contain a degree of circularity. Another limitation of the present study is the use of single informant data, although previous studies suggest agreements among multi-informants are generally low. (De Los Reyes and Kazdin, 2005) Finally, given the neurodevelopmental changes underway at age 14, it is unknown if the neuroanatomical difference identified here persists throughout the lifespan. Future longitudinal studies on dysregulated individuals are needed to determine the psychosocial and neurobiological antecedents of dysregulation, as well as the developmental effect of neural maturation on the persistence of dysregulation into late adolescence and adulthood.
References


Tables

Table A3.1: Latent Class Analysis Model Fit Statistics

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<tr>
<th>Number of Classes</th>
<th>-2 loglikelihood</th>
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<th>BLRT</th>
<th>Entropy</th>
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BIC = Bayesian Information Criterion; BLRT=Bootstrap Likelihood Ratio Test; NA=Not Applicable; VLMR=Vuong-Lo-Mendell-Rubin Likelihood Ratio.

Table A3.2: Strengths and Difficulties Questionnaire Summary Scores For Each Latent Class

<table>
<thead>
<tr>
<th>Class (N)</th>
<th>SDQ Summary Scores</th>
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<td>Emotional Symptoms (M, SD)</td>
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<tr>
<td>1 (373)</td>
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<tr>
<td>2 (340)</td>
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</tbody>
</table>

Class 3 comprised the “dysregulated” group who exhibited highest levels of impairment across all dimensions. Class 5 comprised the “low symptom” control group who exhibited the lowest levels of impairment. Summary scores were calculated by the sum of five items related to each dimension.(Goodman, 1997) Higher values signify more difficulty except within the prosocial domain. M=Mean; SD=Standard Deviation; SDQ = Strengths and Difficulties Questionnaire.
Table A3.3: Summary of Mediation Models

<table>
<thead>
<tr>
<th>Model</th>
<th>B</th>
<th>S.E.</th>
<th>p</th>
<th>95% Bootstrapped Confidence Interval</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Anxiety to Dysregulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Effect</td>
<td>.690</td>
<td>.055</td>
<td>.001</td>
<td>.582</td>
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<tr>
<td>Direct Effect</td>
<td>.667</td>
<td>.058</td>
<td>.001</td>
<td>.554</td>
</tr>
<tr>
<td>Indirect Effect</td>
<td>.023</td>
<td>.010</td>
<td>.025</td>
<td>.003</td>
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<tr>
<td>Depression to Dysregulation</td>
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<tr>
<td>Total Effect</td>
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<td>.613</td>
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<tr>
<td>Direct Effect</td>
<td>.683</td>
<td>.045</td>
<td>.001</td>
<td>.595</td>
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<tr>
<td>Indirect Effect</td>
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<td>.009</td>
<td>.065</td>
<td>-.001</td>
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<td>Conduct Disorder to Dysregulation</td>
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<tr>
<td>Total Effect</td>
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<td>.001</td>
<td>.712</td>
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<tr>
<td>Direct Effect</td>
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<td>.036</td>
<td>.001</td>
<td>.692</td>
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<tr>
<td>Indirect Effect</td>
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<td>.008</td>
<td>.022</td>
<td>.003</td>
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<td>ODD to Dysregulation</td>
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<td>.001</td>
<td>.794</td>
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<td>Direct Effect</td>
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<td>.025</td>
<td>.001</td>
<td>.776</td>
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<tr>
<td>Indirect Effect</td>
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<td>.008</td>
<td>.070</td>
<td>-.001</td>
</tr>
<tr>
<td>ADHD to Dysregulation</td>
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<td></td>
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<tr>
<td>Total Effect</td>
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<tr>
<td>Indirect Effect</td>
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<td>.006</td>
<td>.124</td>
<td>-.003</td>
</tr>
</tbody>
</table>

Total effects reflect association between the likelihood of disorder and dysregulation. Direct effects reflect the association between the likelihood of a disorder and dysregulation while accounting for the GMV region of interest (mediator). Indirect effects are the difference in betas, and reflect the magnitude of mediation through the region of interest. Significant indirect effects (95%CI >0) in bold. ADHD=Attention Deficit Hyperactivity Disorder; GMV=Gray Matter Volume; ODD=Oppositional Defiant Disorder.
Figures

Figure A3.1: Average Strengths and Difficulties Questionnaire Item Endorsement for Five Classes

Each SDQ item present on the x-axis, ordered by the five respective SDQ domains to aid in interpretability. Average item endorsement on y-axis, from 0-2 (Not true, somewhat true, certainly true). Items with asterisks indicate reverse coding. Dysregulated class (3) in green line; low symptom class (5) in black line. SDQ=Strengths and Difficulties Questionnaire.

Figure A3.2: Right Orbitofrontal Cortex Region of Interest

Cluster (k=48 voxels; center of mass MNI coordinates: 3), identified as passing TFCE-correction (p < .05) from a two-sample residual gray matter volume permutation analysis. This cluster was also present in a two-group permutation analysis estimated without residualized images or nuisance covariates. MNI=Montreal Neurological Institute; TFCE=Threshold-Free Cluster Enhancement.(Smith and Nichols, 2009)
Figure A3.3: Mediation Models with Significant Indirect Effects

Path models of the relationship between the likelihood of anxiety disorder (left), or, conduct disorder (right), to dysregulation, mediated by the right orbitofrontal cortex gray matter volume ROI. All coefficients are standardized and pass a null-hypothesis significance test at $p<.05$. The indirect effects (dotted line, c paths) reflect the magnitude of mediation through the ROI, with significance determined by 95% confidence intervals generated from 1000 bootstrapped samples (see Table 3). Total effects (c’ paths) reflect the bivariate correlation between a disorder and dysregulation when the mediator is excluded. The negative parameter estimates for the paths into and out of the ROI (a and b paths) are in line with the lower GMV exhibited by the dysregulated group. GMV=Gray Matter Volume; ROI=Region of Interest.