CANNABIS, CONNECTIVITY, AND COMING OF AGE: ASSOCIATIONS BETWEEN CANNABIS USE, ANTERIOR CINGULATE CORTEX CONNECTIVITY, AND PSYCHOSOCIAL ADJUSTMENT DURING THE TRANSITION TO ADULTHOOD

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Cannabis use is common among adolescents and emerging adults and is associated with significant adverse consequences for a subset of users. Rates of cannabis use peak between the ages of 18-25, yet the neurobiological consequences for neural systems that are actively developing during this time remain poorly understood. In particular, cannabis exposure may interfere with adaptive development of white matter pathways underlying connectivity of the anterior cingulate cortex (ACC), including the cingulum and anterior thalamic radiations (ATR), which are vital to mature cognitive, affective, and social functioning and continue to mature throughout the third decade of life. The current study examined the effects of cannabis use on white matter microstructure of the cingulum and ATR among 158 subjects enrolled in the Pitt Mother & Child Project, a prospective, longitudinal study of risk and resilience among men of low socioeconomic status. Participants were recruited in infancy, completed follow-up assessments throughout childhood and adolescence, and underwent diffusion imaging at age 20 and 22. At age 20, moderate adolescent cannabis use was associated with higher fractional anisotropy (FA) and mean diffusivity (MD) of the cingulum and ATR. Longitudinally, cannabis exposure predicted altered white matter maturation in both the cingulum and ATR from age 20 to 22. Furthermore, microstructural changes in the cingulum

pathway mediated the positive association between cannabis use and antisocial behavior at age 23, even when accounting for earlier antisocial behavior, suggesting that cannabis effects on ACC connectivity may impact later externalizing behavior. These results demonstrate that cannabis exposure can have important effects on neurodevelopment during late adolescence and the transition to adulthood that may impact their functioning later in development.

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AUTHOR NOTE

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1.0 INTRODUCTION

1.1 CANNABIS USE DURING ADOLESCENCE AND THE TRANSITION TO ADULTHOOD

Cannabis is currently the most widely used drug of abuse, with 44% of individuals in the United States reporting use during their lifetime (SAMHSA, 2016). Cannabis use typically begins by mid-adolescence (SAMHSA, 2013), and rates of use peak between ages 18-25 when approximately 20% of individuals report use of cannabis in the last 30 days (SAMHSA, 2016). Furthermore, epidemiological studies generally suggest that rates of use are highest among male individuals of low socioeconomic status (SES; Johnston, O'Malley, Bachman, & Schulenberg, 2012). In fact, recent data shows that the gender gap in use has increased in recent years, due to a rise in use among low income men (Carliner et al., 2017). Additionally, there has been a recent trend of increasing adolescent use in parallel with a concurrent decline in adolescents' perceived risk of cannabis use (Johnston, O'Malley, Bachman, Schulenberg, & Miech, 2014).

1.1.1 Negative consequences of cannabis use

Despite growing public perception of cannabis as benign (Johnston et al., 2014), there is evidence to suggest that cannabis use can have significant deleterious consequences for a subset of users,

including substance dependence, mental health problems, and poor lifetime achievement (Volkow, Baler, Compton, & Weiss, 2014). National survey data suggest that up to 19% of current cannabis users between the ages of 18-25 meet criteria for cannabis use disorder (CUD; Wu, Zhu, Mannelli, & Swartz, 2017), characterized by symptoms including increased use over time, unsuccessful efforts to moderate use, tolerance, withdrawal, craving, and/or interference with major role obligations that lead to clinically significant impairment or distress (American Psychiatric Association, 2013). In fact, CUD is the most common illicit substance use disorder in the United states, with an estimated 4.2 million Americans above the age of 12 meeting criteria in the last year (Wu, Zhu, & Swartz, 2016). CUD is associated with a range of physical and mental health problems including respiratory, cardiovascular, and psychiatric disorders (Melis et al., 2017). However, despite the availability of multiple evidence-based treatments (Walther, Gantner, Heinz, & Majic, 2016), only 7.8% of adults with CUD seek cannabis-specific treatment (Wu et al., 2017). Low rates of treatment utilization may be due to a low perceived need for cannabis use treatment, as well as an absence of substance use screening and intervention in general medical settings (Wu et al., 2017). Improving public awareness about the risks associated with cannabis use may facilitate earlier intervention and higher rates of cannabis use treatment for individuals with CUD and improve physical and mental health outcomes for affected individuals.

In addition to CUD and other substance use disorders, cannabis use has also been linked to both internalizing (Marmorstein & Iacono, 2011) and externalizing psychopathology (Krueger, Markon, Patrick, Benning, & Kramer, 2007; McGee, Williams, Poulton, & Moffitt, 2000). Cannabis abuse has been shown to prospectively predict the development of depressive symptoms, both among individuals with (Womack, Shaw, Weaver, & Forbes, 2016) and without depressive symptoms at baseline (Bovasso, 2001). Similarly, cannabis use has been associated with higher

risk for later violent behavior (Schoeler et al., 2016), as well as conduct and antisocial personality disorders (McGee et al., 2000) among men. Associations between cannabis use and later psychopathology may be attributable to shared genetic, neurobiological, and environmental risk factors (Fu et al., 2002; Krueger et al., 2007), and/or cannabis effects on neural functioning that contribute to risk for other psychiatric disorders (Lichenstein, Musselman, Shaw, Sitnick, & Forbes, in press; Schoeler et al., 2016). Identifying individuals who are at the highest risk to suffer these negative consequences could facilitate the development of targeted prevention and intervention programs designed to mitigate cannabis' long-term deleterious impact. Therefore, it is imperative to develop a better understanding of the mechanisms underlying cannabis effects in order to predict when cannabis use is most likely to lead to negative long term outcomes.

1.1.1.1 Transition to adulthood as a sensitive period for negative cannabis effects

Adolescence has been hypothesized to represent a period when individuals are particularly vulnerable to negative effects of cannabis use due to ongoing neurobiological and psychosocial developmental processes (Volkow et al., 2014). Indeed, more significant changes in brain structure (Gruber, Dahlgren, Sagar, Gonenc, & Lukas, 2014) and cognitive and behavioral functioning have been observed following cannabis exposure during adolescence relative to adulthood (Lisdahl, Gilbart, Wright, & Shollenbarger, 2013), and the risk for developing cannabis dependence is approximately twice as high for individuals who initiate use in adolescence (Volkow et al., 2014; see definition of adolescence below). The majority of research on adolescent cannabis use has focused on early adolescence (i.e. before age 17), whereas few studies have focused on the effects of cannabis exposure during late adolescence and the transition to adulthood. Nonetheless, cannabis use (SAMHSA, 2014), abuse, and dependence (Delker, Brown, & Hasin, 2015) peak

between the ages of 18-25. Therefore, it is vital to establish a better understanding of cannabis effects during this later period to elucidate the temporal specificity of cannabis effects, identify risks associated with cannabis use during late adolescence and the transition to adulthood, and guide treatment recommendations for individuals with cannabis use problems.

1.1.2 Cannabis legislation in the United States

Dramatic changes in cannabis policy have taken place over the last 15 years such that 28 states and the District of Columbia have now legalized the use of cannabis for medicinal purposes, and 9 states have legalized recreational cannabis use among individuals over the age of 21 (McGinty, Niederdeppe, Heley, & Barry, 2017). Decriminalization and legalization of cannabis increase access for late adolescents/early adults in their early 20s and contribute to declining perceptions of its potential harm. Nonetheless, cannabis effects on brain, behavior, and long-term psychosocial development remain poorly understood (Batalla et al., 2013). Therefore, there is a critical need to improve our understanding of the correlates and consequences of cannabis use in order to guide ongoing policy decisions and inform public opinion about the risks and potential benefits of cannabis use.

1.2 ADOLESCENT DEVELOPMENT AND THE TRANSITION TO ADULTHOOD

Adolescence is broadly defined as the transitional period between childhood and adulthood (Crone & Dahl, 2012), but the precise onset and offset of adolescence are difficult to delineate (Spear,

2000). Traditionally, this period is thought to begin around the time of pubertal onset, roughly age 9-12 (Crone & Dahl, 2012) and end with relative independence from the parents (Casey, 2014) during the early- to mid-20s (Crews, He, & Hodge, 2007; Spear, 2000). Therefore, educational and occupational attainment are key factors that take place during the transition to adulthood during the early 20s and lay the groundwork for future success by providing the necessary skills to enter the workforce and facilitating financial independence.

1.2.1.1 Key developmental changes in adolescence and the transition to adulthood

Adolescence is marked by accelerated changes in multiple domains that aid in the pursuit of long-term goals, the establishment of peer and romantic relationships, and improvements in academic and occupational performance. Improved cognitive (i.e. working memory, performance monitoring, and cognitive control (Luna, Marek, Larsen, Tervo-Clemmens, & Chahal, 2015)), affective (i.e. emotional reactivity (Casey, 2014) and sensation seeking (Smith, Chein, & Steinberg, 2013)), and social (i.e. mentalizing, perspective taking (Crone & Dahl, 2012)) functioning are all known to take place during this developmental period. In particular, enhanced integration among these functional domains is hypothesized to facilitate goal-directed rather than affect-driven behavior, and enable young people to successfully navigate the tasks and challenges of adulthood (Crone & Dahl, 2012).

1.2.2 The neural basis of adolescent development and the transition to adulthood

Widespread developmental changes in neural structure and function are thought to underlie improvements in cognitive, affective, and social domains across adolescence (Andersen, 2003). These include changes in gray matter volume and cortical thickness, patterns of task-related and

resting-state neural activation, and maturation of white matter microstructure that influences the strength of connections with regions involved in higher-order functions, such as the anterior cingulate cortex. This process creates a period of plasticity, whereby environmental interactions shape the course of brain development, promoting specialization of neural networks to meet the demands of individuals' environments (Luna et al., 2015). Various neural systems follow distinct courses of development throughout this period, creating cascading points of vulnerability when environmental insults can have a disproportionately profound effect on the long term development of different systems throughout the brain (Bossong & Niesink, 2010).

A large proportion of existing research on adolescent brain development and substance use has focused on mid-adolescence as a time of heightened risk for use and increased vulnerability to the effects of exposure. Indeed, early-onset cannabis use has been associated with long-term deficits in neural structure and function, as well as cognitive and intellectual performance (Lisdahl et al., 2013). Nonetheless, the rate and quantity of cannabis use peaks beyond mid-adolescence during the transition to adulthood in the early-20s, yet very little research has focused on this time point. Therefore, it is also necessary to examine later neurodevelopmental processes that may be particularly vulnerable to the cannabis effects during this later period of peak cannabis use.

In particular, white matter pathways show protracted trajectories of microstructural development that continue throughout adolescence and into adulthood and support ongoing integration of cognitive, affective and social neural networks (Lichenstein, Verstynen, & Forbes, 2016; Yap et al., 2013). Furthermore, different white matter tracts display distinct temporal patterns of development, with sensorimotor tracts maturing earlier, and tracts implicated in higher-order functions maturing later (Simmonds, Hallquist, Asato, & Luna, 2014). Additionally, white matter microstructure has been found to be sensitive to the effects of cannabis exposure (Lisdahl

et al., 2013; see **1.3.3 Effects of cannabis use on the cingulum and ATR** section below for a review of relevant studies). Therefore, cannabis effects on late-developing white matter pathways may represent a potential mechanism whereby cannabis use during the transition to adulthood can impact neural development, interfere with ongoing adaptive changes in cognitive, affective, and social functioning, and negatively impact long-term functioning and educational and professional achievement for affected individuals (Volkow et al., 2014).

1.2.3 Cellular basis of white matter development

Changes in white matter microstructure across adolescence and young adulthood are thought to reflect a variety of developmental changes at the cellular level, including greater axonal directional coherence, reduction in the number of crossing fibers, increased axon diameter, and myelination (Paus, 2010). These changes all improve the conduction velocity of neural signals, increase the signal-to-noise ratio, and impact the synchronicity of distal synaptic events (Luna et al., 2015; Paus, 2010). Additionally, glial cells (i.e. oligodendrocytes and astrocytes) account for approximately half the signal in white matter neuroimaging voxels (Walhovd, Johansen-Berg, & Karadottir, 2014). New glia cells are generated throughout development and play an important role in driving neural plasticity in adolescence and adulthood (Wang & Young, 2014). A wide variety of environmental influences has been shown to impact the number and morphology of glial cells, suggesting that glial changes may represent a critical mechanism of experience-dependent white matter plasticity (Wang & Young, 2014). Therefore, changes in axonal structure and organization, myelination, and glia composition are all likely to contribute to age-related changes in microstructural measurements (Walhovd et al., 2014).

Diffusion tensor imaging (DTI) is often used to quantify these developmental changes in white matter microstructure. DTI is a neuroimaging method that measures structural connectivity by determining the direction and magnitude of water diffusion in neural tissue (Dell'Acqua & Catani, 2012; Hagmann et al., 2006). Axonal structure and organization, myelination, and glia composition place constraints upon water movement within white matter pathways such that water diffuses more freely along the length of axons than in the direction perpendicular to them. Several metrics can be calculated using DTI that reflect the nature of water diffusion in the brain, including mean diffusivity (MD; average diffusion in all directions), axial diffusivity (AD; diffusion along the primary diffusion direction), radial diffusivity (RD; average diffusion perpendicular to the primary diffusion direction), and most commonly fractional anisotropy (FA), which represents the 'shape' of water diffusion in each voxel and is assumed to reflect the overall 'integrity' of white matter tracts (Jones, Knosche, & Turner, 2013; see Figure 1).

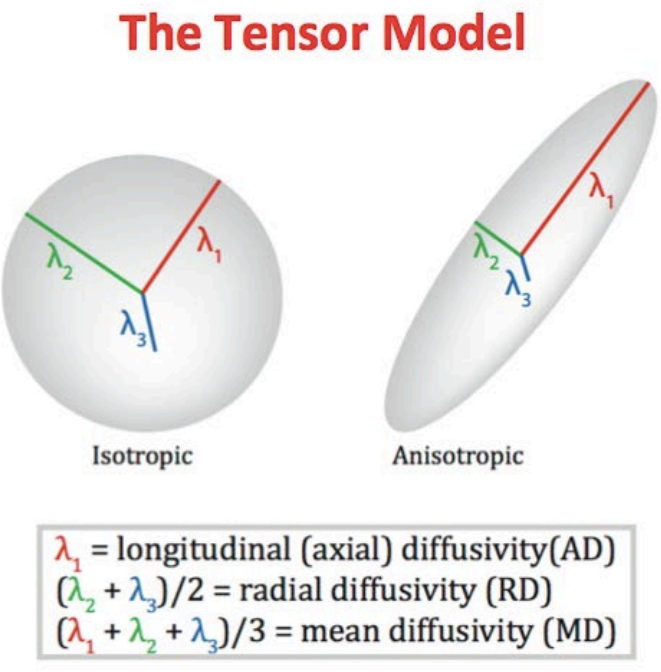

Figure 1. Illustration of the tensor model. Fractional anisotropy estimates the degree of directional dependence, or anisotropy, of the diffusion signal at each voxel. Axial diffusivity measures the primary direction of diffusion. Radial diffusivity is equivalent to the mean of the 2 eigenvalues perpendicular to the primary diffusion direction. Mean diffusivity is calculated from the mean of all 3 eigenvalues. Figure from Verstynen, T. (2014, June). Of microtubules & water molecules: Principles and applications of diffusion weighted imaging. Lecture presented at the Multimodal Neuroimaging Training Program Summer Workshop in Neuroimaging, Pittsburgh, PA.

1.3 ANTERIOR CINGULATE CORTEX CONNECTIVITY

White matter pathways that support connectivity of the anterior cingulate cortex (ACC) may be critical targets of cannabis effects during the transition to adulthood. The ACC is a region that plays an important role in integrating cognitive, affective, and social neural networks to guide behavior (Fossella et al., 2008), which has been hypothesized to function as a hub for internetwork connectivity (Luna et al., 2015). Functionally, the ACC is hypothesized to iteratively process task requirements, monitor performance, and drive activation in other brain regions necessary to optimize functioning (Weston, 2012), and age-related changes in ACC activation have been directly linked to age-related improvements in inhibitory control perfomance across adolescence and into adulthood (Ordaz, Foran, Velanova, & Luna, 2013). This region is able to support such a broad array of functions by virtue of its robust connecitivity with a variety of different brain regions, including prefrontal and motor cortical regions, as well as limbic and subcortical structures (Luna et al., 2015). Although the basic architecture of ACC connectivity remains stable from childhood, changes in the strength of various white matter pathways facilitate specialization and integration of neural networks across adolescence (Luna et al., 2015). In particular, the

cingulum bundle and anterior thalamic radiations are the primary white matter pathways linking the ACC with its distributed cortical and subcortical targets.

1.3.1 Cingulum

The cingulum bundle is a major white matter pathway that runs along the anterior-posterior axis of each hemisphere and facilitates communication between frontal, parietal, and temporal cortices as well as subcortical structures, including the striatum and hippocampus (see Figure 2). Based on the topography of cingulum fibers, this pathway can be divided into subgenual, dorsal, and parahippocampal/temporal sections that differ with regard to their spatial location, microstructural characteristics, and connectivity profiles (Jones, Christiansen, Chapman, & Aggleton, 2013). The subgenual and dorsal divisions contain fibers that connect subgenual and dorsal regions of the ACC to other anterior and posterior cingulate regions, prefrontal, motor, temporal, and parietal cortical areas, as well as subcortical structures including the amygdala, hippocampus, striatum, and hypothalamus (Beckmann, Johansen-Berg, & Rushworth, 2009; Heilbronner & Haber, 2014; Jones, Christiansen, et al., 2013). In contrast, the hippocampal/temporal section is composed of primarily posterior cingulate fibers that project to parietal and temporal cortical areas as well as the hippocampus (Beckmann et al., 2009; Heilbronner & Haber, 2014; Jones, Christiansen, et al., 2013). Given the current focus on ACC connectivity, the present study will include the anterior division of the cingulum. Functionally, white matter integrity of the cingulum has been associated with verbal intellectual abilities (Tamnes et al., 2010) cognitive inhibition performance (Treit, Chen, Rasmussen, & Beaulieu, 2014), social competence (De Pisapia et al., 2014), and has been found to mediate the positive relationship between age and executive functioning across development (Peters et al., 2014). Additionally, alterations in cingulum microstructure have been

identified among individuals with depression (Yang et al., 2017; Hayakawa et al., 2013) and antisocial behavior (Waller, Dotterer, Murray, Maxwell, & Hyde, 2017).

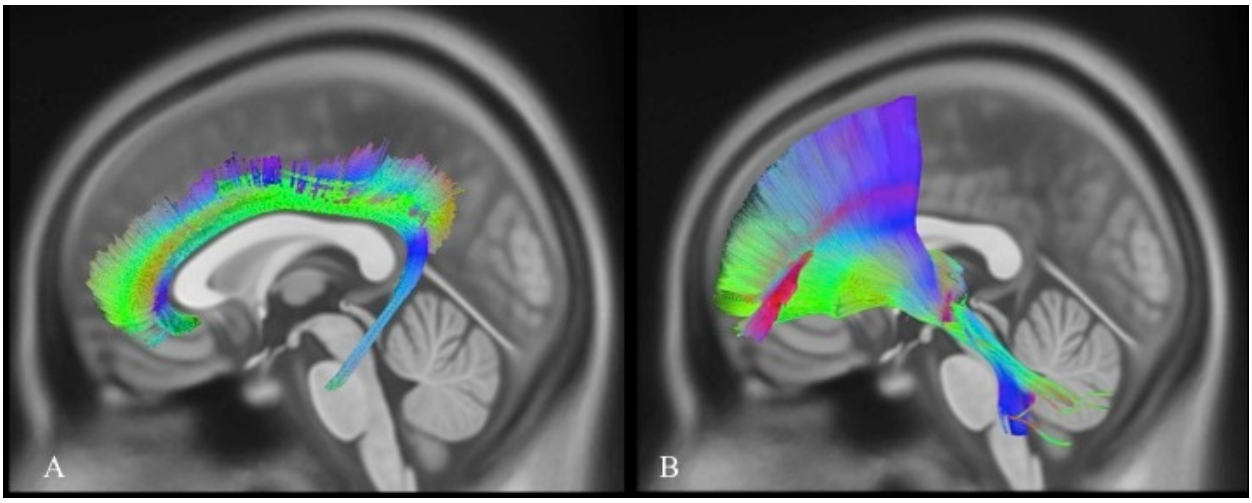

Figure 2. Illustration of the (A) cingulum and (B) anterior thalamic radiations (ATR). Figure adapted from Lichenstein, S. D., Verstynen, T., & Forbes, E. E. (2016). Adolescent brain development and psychopathology: a case for connectivity of the anterior cingulate cortex in affective and substance use. *Neuroscience and Biobehavioral Reviews, 70*, 271-287.

The cingulum pathway is among the latest developing white matter tracts (Grieve, Korgaonkar, Clark, & Williams, 2011; Imperati et al., 2011; Kochunov et al., 2012; Lebel & Beaulieu, 2011; Lebel et al., 2012; Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008; Peters et al., 2014; Tamnes et al., 2010; Trivedi et al., 2009; Westlye et al., 2010; Yu et al., 2014). Although various studies report subtly different trajectories of cingulum development, they consistently report increased FA throughout adolescence until age 27-42 (with peak timing varying across reports), followed by later decline. A recent meta-analysis of studies reporting developmental changes in cingulum

microstructure confirmed that cingulum FA increases throughout adolescence and into adulthood, reaching an estimated peak at age 34 (Lichenstein et al., 2016; see Figure 3). Given the array of higher order functions sub-served by this pathway and its protracted developmental trajectory, the cingulum is hypothesized to play a key role in supporting the integration of cognitive, affective, and social functions that facilitates a successful transition to adulthood and may also represent an important target for cannabis effects during this period.

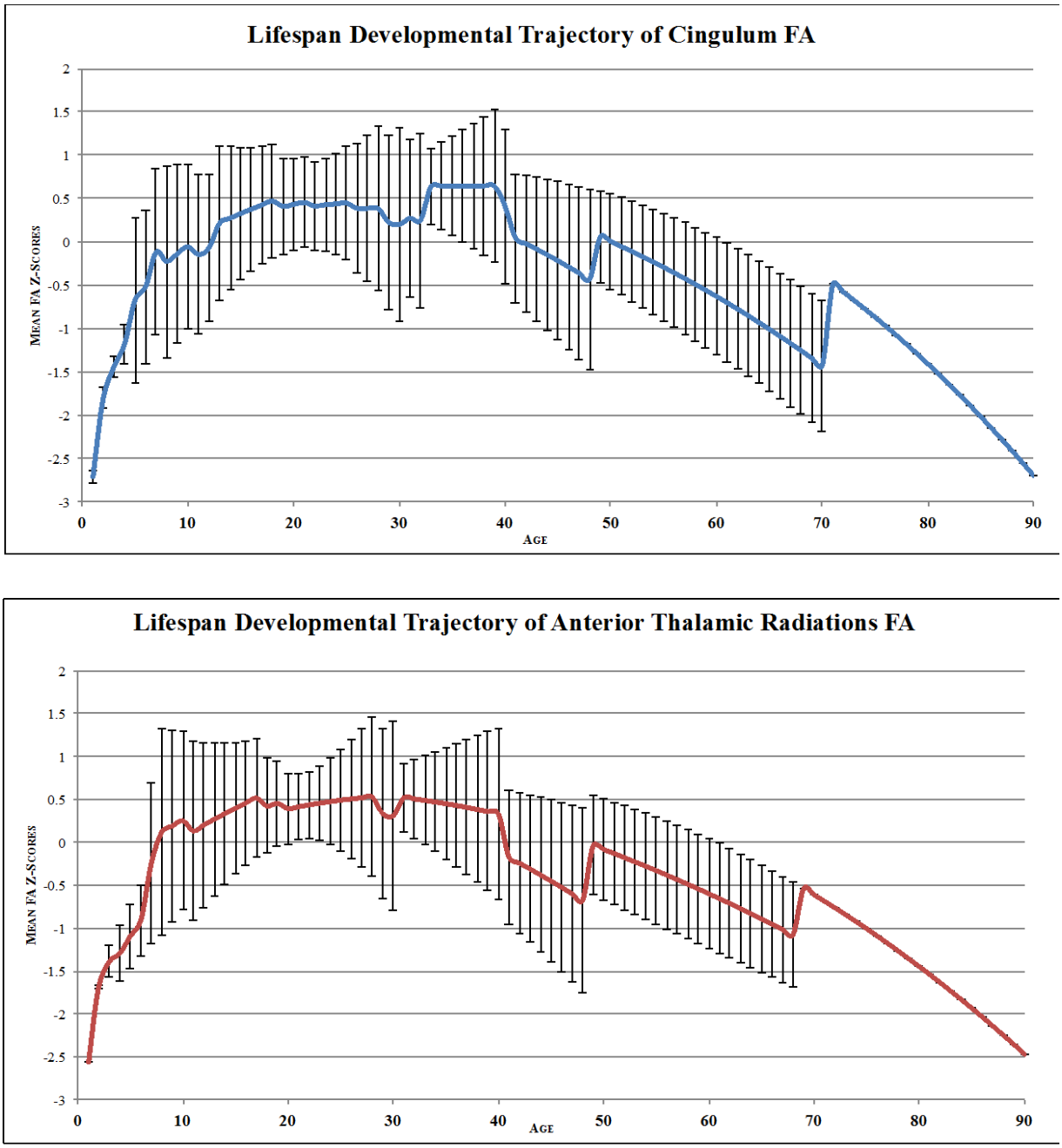

Figure 3. Meta-analytic results for developmental trajectories of cingulum and anterior thalamic radiations (ATR) fractional anisotropy (FA) across the lifespan. Regression models reported in published studies of white matter development (N=10 studies for cingulum, N=7 studies for the ATR) were used to simulate FA for each age within the range included in each study. FA values were then converted to z-scores in order to standardize across studies and produce estimates of the predicted direction and magnitude of change in FA at each age for each pathway. Blue and red lines represent mean FA z-scores from all models included for the cingulum and ATR,

respectively (N=13 models of cingulum development, N=9 models of ATR development). Error bars depict standard deviations. Figure adapted from Lichenstein, S.D. (2015). Adolescent development of structural anterior cingulate cortex connectivity: Implications for functional connectivity and risk for substance use disorders (Specialty Paper), University of Pittsburgh.

1.3.2 Anterior thalamic radiations (ATR)

The ATR is the primary conduit for communication between the ACC and subcortical limbic structures. This pathway projects from the anterior thalamic nuclei to the ACC and medial frontal regions via the anterior limb of the internal capsule (ALIC) and anterior corona radiata (ACR; Catani & Thiebaut de Schotten, 2012; Lobel et al., 2009; see Figure 2). The anterior thalamic nuclei are considered to be part of the limbic system as they receive input from the hippocampus and hypothalamic mammillary bodies (Catani & Thiebaut de Schotten, 2012). Therefore, the ATR facilitates the integration of emotional and visceral information with other cognitive functions of the ACC. White matter microstructure within the ATR has been linked to attention (Niogi, Mukherjee, Ghajar, & McCandliss, 2010), inhibition (Treit et al., 2014), task switching (Seghete, Herting, & Nagel, 2013), verbal intellectual ability (Tamnes et al., 2010), empathic concern (Parkinson & Wheatley, 2014), and delay discounting (Olson et al., 2009), as well as major depression (Yang et al., 2017) and antisocial behavior (Waller et al., 2017)

Prior studies of ATR microstructure have yielded mixed findings regarding the time course of ATR development. Nonetheless, the majority of studies support the hypothesis that ATR FA increases throughout adolescence and peaks between ages 27-32 (Imperati et al., 2011; Kochunov et al., 2012; Lebel et al., 2012; Westlye et al., 2010). Similarly, meta-analytic results also indicate

that ATR microstructure continues to mature throughout adolescence and young adulthood and reaches an estimated peak at age 28 (Lichenstein et al., 2016; see Figure 3). Given the evidence that the ATR is linked to many of the ACC's higher-order functions and displays developmental changes throughout the transition into adulthood, this pathway may also play a pivotal role in adaptive maturation during this period and be vulnerable to the negative effects of cannabis exposure at this time.

1.3.3 Effects of cannabis use on the cingulum and ATR

Abnormal white matter microstructure has been reported among cannabis users relative to control subjects, but there is considerable variation in the tracts implicated by different studies as well as the patterns of substance use among study samples (Baker, Yucel, Fornito, Allen, & Lubman, 2013). One recent report used graph theoretical analysis to quantify differences in structural network characteristics among late adolescent/young adult cannabis users relative to controls (Kim et al., 2011). The results demonstrated less efficient and more locally segregated network organization among cannabis users, generally considered an immature pattern, characterized by less integration between structural networks. Furthermore, altered local network organization of the cingulate cortex was also evident among cannabis-using subjects, indicating that cingulate connectivity may be particularly sensitive to the effects of cannabis exposure during the transition into adulthood. However, cross-sectional studies have yielded mixed results regarding cannabis effects on the cingulum and ATR. Both higher (Bava et al., 2009; Delisi et al., 2006; Jakabek, Yucel, Lorenzetti, & Solowij, 2016) and lower (Gruber et al., 2014; Gruber, Silveri, Dahlgren, & Yurgelun-Todd, 2011; Jacobus, Thayer, et al., 2013; Jakabek et al., 2016; Shollenbarger, Price, Wieser, & Lisdahl, 2015) FA have been reported among cannabis users relative to controls, and

several studies have failed to detect any significant effect of cannabis use in these tracts (Ashtari et al., 2009; Clark, Chung, Thatcher, Pajtek, & Long, 2012; Gruber & Yurgelun-Todd, 2005; Jacobus et al., 2009; Jacobus et al., 2009; Thatcher, Pajtek, Chung, Terwilliger, & Clark, 2010; Yucel et al., 2010).

Longitudinal studies have yielded more consistent evidence for reduced FA of the cingulum (Becker, Collins, Lim, Muetzel, & Luciana, 2015) and ATR (Bava, Jacobus, Thayer, & Tapert, 2013; Becker et al., 2015; Jacobus, Squeglia, Bava, & Tapert, 2013; Jacobus, Squeglia, Infante, Bava, & Tapert, 2013) across 2-3 years of cannabis use. A series of reports comparing mid-adolescents with comorbid alcohol and cannabis use to adolescent binge drinkers with no co-occurring cannabis use and non-substance using adolescents has consistently reported reduced FA of the ATR among cannabis using teens (Bava et al., 2013; Jacobus, Squeglia, Bava, et al., 2013; Jacobus, Squeglia, Infante, et al., 2013). Congruently, Becker et al. (2015) reported decreased white matter maturation of the cingulum and ATR among heavy cannabis users relative to controls during the transition to adulthood (age 20-22), whereas Epstein et al. (2015) failed to find any difference in cingulum microstructural development across 18 months among individuals (age 10-23) with cannabis use disorder relative to controls. Collectively, the longitudinal literature lends strong support for cannabis effects on developing structural ACC connectivity. However, existing studies are limited based on small sample sizes (maximum N=48 to date), comorbid alcohol use, and inconsistency in the age range studied. Future longitudinal studies with larger samples and well-defined follow-up intervals are necessary to clarify the effects of cannabis use on cingulum and ATR maturation.

1.4 POTENTIAL MECHANISMS OF CANNABIS EFFECTS ON ACC CONNECTIVITY

1.4.1 Endocannabinoid system

The endocannabinoid system is a neuromodulatory lipid system that is comprised of two cannabinoid receptor types, CB1 and CB2 receptors, and two major endogenous ligands, anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) (Morena, Patel, Bains, & Hill, 2015). CB1 receptors are the most abundant receptors in the brain and are widely expressed in the frontal cortex, medial temporal lobes, basal ganglia and cerebellum (Kim et al., 2011), with highest concentrations in the cingulate cortex (Khani et al., 2015). These receptors are expressed on glutamatergic, GABAergic, serotonergic, noradrenergic, and dopaminergic axon terminals and exert an autoregulatory influence by inhibiting neurotransmitter release from the presynaptic cell (Morena et al., 2015). Collectively, activation of the endocannabinoid system serves a neuromodulatory function by dampening presynaptic neurotransmitter release.

Although there is a paucity of research on adolescent development of the endocannabinoid system, preliminary evidence suggests that this system undergoes dynamic changes throughout adolescence and into adulthood (Rubino & Parolaro, 2015). However, the temporal and spatial specificity of adolescent endocannabinoid system changes remain poorly understood. Specifically, CB1 receptors become substantially more abundant between adolescence and young adulthood when other neurotransmitter systems have already begun pruning (Renard, Krebs, Le Pen, & Jay, 2014; Rubino & Parolaro, 2015). AEA levels have also been reported to increase throughout adolescence, exhibiting a 3-fold increase across adolescence (Rubino & Parolaro, 2015). Furthermore, there is also evidence to suggest that CB1 receptor activity regulates glutamatergic

pruning in the adolescent PFC, indicating a critical role for the endocannabinoid system in broader processes of cortical remodeling during adolescence (Rubino & Parolaro, 2015).

1.4.2 Effects of cannabis use on the endocannabinoid system

CB1 receptors are the primary target for delta-9-tetrahydrocannabinol (THC), the principal psychoactive ingredient in cannabis (Morena et al., 2015). Acute cannabis exposure activates CB1 receptors and typically produces feelings of relaxation, reduced anxiety, and less perceived stress, suggesting an important role for the endocannabinoid system in regulating stress responses (Morena et al., 2015). Chronic cannabis use has been associated with a robust downregulation and desensitization of CB1 receptors (Rubino & Parolaro, 2015). Specifically, a significant decrease in CB1 receptor density has been reported in the ACC and neocortex of chronic cannabis users relative to controls, whereas no difference in receptor density was observed in several subcortical brain regions (Hirvonen et al., 2012). Therefore, sustained cannabis use results in decreased cortical CB1 receptors, which may interfere with the endocannabinoid system's roles in modulating cortical plasticity and regulating neural responses to stress.

1.4.3 Endocannabinoid system and white matter

There are several mechanisms whereby endocannabinoid system changes may impact white matter integrity, including altered regulation of neural stress responses, decreased myelination and reduced oligodendrocyte survival. Normative endocannabinoid signaling is thought to function as a buffer against the negative effects of stress on the brain (Morena et al., 2015) and inhibit inflammatory processes (Molina-Holgado et al., 2002). Thus, alterations in endocannabinoid

functioning may produce heightened vulnerability to the deleterious effects of stress. Indeed, CB1 receptor antagonism has been found to increase activation of the hypothalamic-pituitary-adrenal (HPA) axis and interfere with typical HPA axis recovery following stress exposure (Morena et al., 2015). Therefore, a pervasive downregulation of CB1 receptors, as observed following chronic cannabis exposure, may create a state of chronic stress and increased pro-inflammatory cytokine production (Tian, Hou, Li, & Yuan, 2014). Neuroinflammation is hypothesized to negatively impact myelin morphology (Verstynen et al., 2013) and lead to poorer white matter integrity (Bettcher et al., 2015), suggesting one potential mechanism for cannabis effects on white matter microstructure.

Cannabis exposure could also lead to white matter changes through direct effects on oligodendrocyte survival and myelination. Oligodendrocytes are a class of glial cells whose processes comprise the myelin sheath for neurons throughout the central nervous system (Mitew et al., 2014). CB1 receptors have been identified on oligodendrocytes (Molina-Holgado et al., 2002) and agonism of the endocannabinoid system in adolescence has been shown to promote oligodendrocyte survival in prefrontal cortical regions (Bortolato et al., 2014). Additionally, chronic exposure to cannabinoids during adolescence has been associated with altered expression of the myelin basic protein gene and myelin proteolipid protein, two important components of the myelin sheath (an effect that does not occur following adult exposure; Lubman, Cheetham, & Yucel, 2015). Therefore, downregulation/desensitization of CB1 receptors among adolescent/young adult cannabis users could interfere with normative white matter development via reduced oligodendrocyte survival and decreased myelination.

1.5 PSYCHOSOCIAL IMPLICATIONS OF CANNABIS USE DURING THE TRANSITION TO ADULTHOOD

Altered development of neural connectivity, or "developmental miswiring" can have a myriad of damaging consequences for individuals' long term functioning (Di Martino et al., 2014). In particular, cannabis effects on ACC connectivity during the transition to adulthood may have particularly deleterious effects on long-term psychosocial adjustment due to the critical developmental tasks of this period. Key developmental milestones of this period include completing primary and/or secondary education and entering the workforce, and the degree to which individuals achieve success in these domains has significant implications for their long-term trajectories of accomplishment and well-being (Lui, Chung, Wallace, & Aneshensel, 2014). Indeed, higher educational attainment is associated with better health, economic productivity and social status (Lui et al., 2014; Silins et al., 2015), and is an important marker of upward social mobility among low-SES populations (Forrest, Hodgson, Parker, & Pearce, 2011).

Adolescent cannabis use has been associated with deficits in multiple domains of cognitive performance (Medina et al., 2007), lower grade point average, and more behavioral problems at school, even after controlling for alcohol use (Medina et al., 2007). Furthermore, cannabis users are more likely to discontinue school early (Lynskey & Hall, 2000; Silins et al., 2015), and greater exposure to cannabis has been linked to lower levels of degree attainment by age 25 (Fergusson & Boden, 2008; Maggs et al., 2015; Silins et al., 2015). Poor educational attainment during the transition to adulthood can have profound implications for individuals' occupational opportunities. Congruently, adolescent cannabis use has also been linked to lower income at age 25, higher use of welfare, higher rates of unemployment, as well as lower life satisfaction (Fergusson & Boden,

2008), and school dropout has been identified as a mediator of the negative association between cannabis use and later income (Green, Doherty, & Ensminger, 2016).

1.5.1 Atypical development of ACC connectivity as a potential mediator of negative consequences of cannabis use

White matter microstructure of the cingulum and ATR is one factor that may mediate cannabis effects on psychosocial adjustment. Based on the cognitive, affective, and social functions these pathways are thought to support (Lichenstein et al., 2016; Luna et al., 2015), interference with the typical developmental trajectory of ACC connectivity may have meaningful consequences for individuals' academic, occupational, and interpersonal functioning during the transition to adulthood. Furthermore, altered white matter integrity of the cingulum and ATR has also been implicated in both internalizing (Yang et al., 2017; Hayakawa et al., 2013) and externalizing (Waller et al., 2017) disorders among adults. Therefore, cannabis effects on these tracts may also contribute to the association between cannabis use and elevated risk for later psychopathology (Bovasso, 2011; Marmostein & Iocono, 2011; McGee et al., 2000; Schoeler et al., 2016; Womack et al., 2016). However, cannabis exposure effects during the transition to adulthood have been relatively understudied. Elucidating the mechanisms whereby cannabis use during this transitional period can impact long-term trajectories of psychosocial adjustment is critical to guide the identification of individuals at highest risk and to facilitate more targeted prevention and intervention efforts.

1.6 CURRENT STUDY AIMS

Based on the protracted course of cingulum and ATR development (Lichenstein et al., 2016), high rates of cannabis use from age 18-25 (SAMHSA, 2016), evidence for altered network topology in the cingulate cortex (Kim et al., 2011) and altered microstructural development of the cingulum and ATR (Becker et al., 2015) among cannabis users in this age range, as well as poorer educational, occupational, and mental health outcomes among users (Fergusson & Boden, 2008; Lynskey & Hall, 2000; Maggs et al., 2015; Silins et al., 2015), there is strong evidence to suggest that cannabis use impacts developing ACC connectivity, and that this effect has potential implications for long term psychosocial functioning. However, previous studies of cannabis effects on white matter microstructure have been limited by small sample sizes, cross-sectional research designs, and insufficient attention to cannabis use during the early 20s.

The current study will build upon previous literature by utilizing a large sample of low income, urban men, who have particularly high rates of cannabis use in the general population. Additionally, we will analyze DTI data across 2 time points, allowing us to look at longitudinal cannabis effects on developing white matter. Finally, the current analyses will target the transition to adulthood, examining DTI data from age 20 and 22. Cannabis use has recently become legal in many states for medicinal and/or recreational purposes among individuals above the age of 21, yet very few previous studies have examined cannabis effects at this time. Furthermore, by looking specifically at change in white matter microstructure from age 20 to 22, we aim to replicate recent findings of lesser cingulum and ATR maturation during this time interval by Becker et al. (2015), and to extend their results by examining the implications of these cannabis effects for academic, occupational, and psychological functioning. Given that white matter pathways underlying ACC connectivity continue to undergo significant developmental changes into young adulthood, as well

as the critical developmental tasks of this period, it is crucial to examine cannabis effects on neurodevelopment and psychosocial adjustment during the transition to adulthood in order to anticipate the effects of recent legislative changes, guide ongoing policy, prevention, and treatment decisions, and inform public perceptions about the risks associated with cannabis use.

Aim 1. Characterize cross-sectional differences in ACC connectivity at age 20 based on adolescent cannabis exposure.

Hypothesis 1. Adolescent cannabis use (age 12-19) will predict poorer white matter integrity at age 20 (i.e. lower FA).

Aim 2. Examine longitudinal cannabis effects on developing ACC connectivity.

Hypothesis 2. Cannabis use across adolescence and the transition to adulthood (age 12-21) will be associated with lesser maturation of white matter microstructure in the cingulum and ATR, i.e. less positive change in FA from age 20 to 22 (see Figure 4).

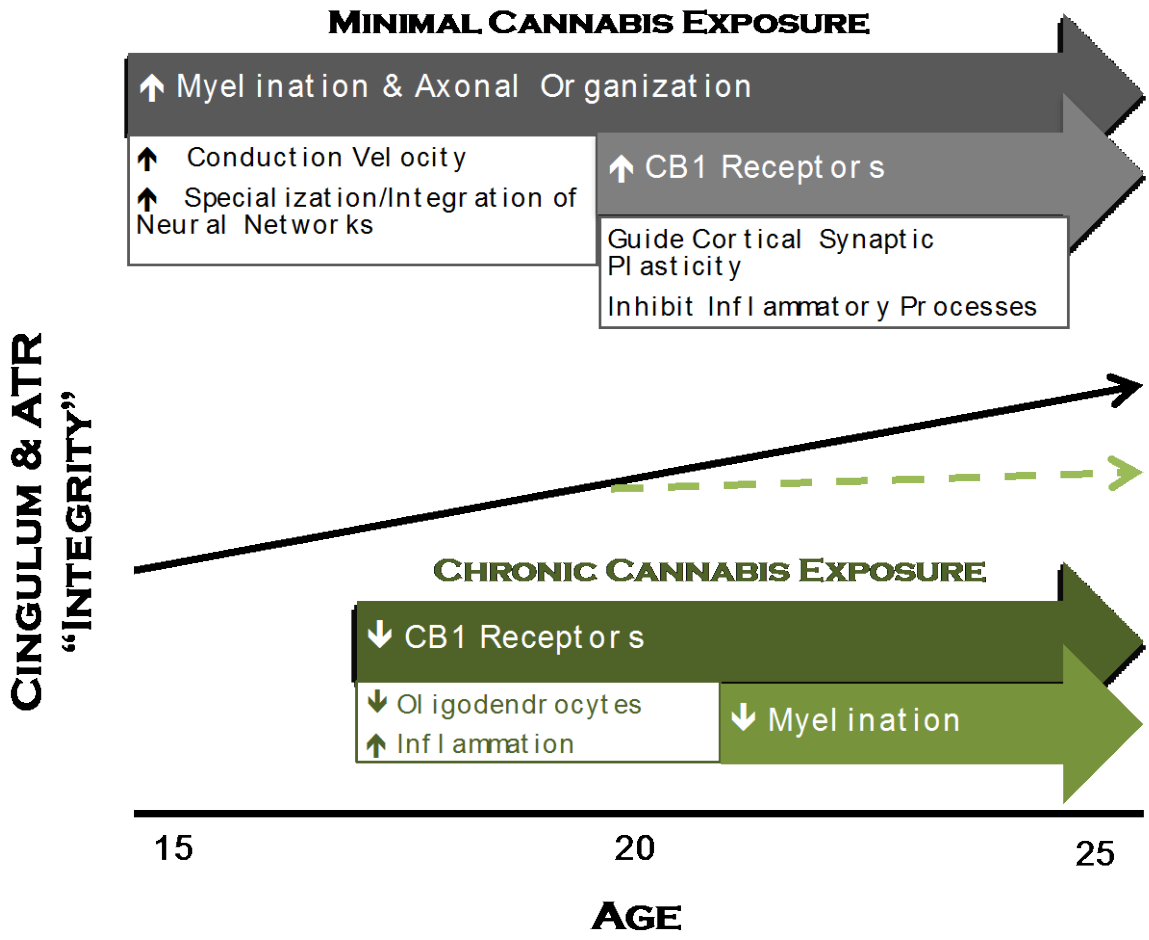

Figure 4. Hypothesized effects of cannabis exposure on anterior cingulate cortex connectivity during the transition to adulthood. The black line represents the typical trajectory of increased white matter integrity throughout adolescence and into adulthood. The dashed green line represents the current hypothesis that greater frequency of cannabis use from age 20-22 will predict less positive change in FA and less negative change in MD, AD, and RD from age 20 to 22.

Aim 3. Assess whether white matter microstructure mediates the association between cannabis use and poor psychosocial adjustment in young adulthood.

Hypothesis 3a. Greater frequency of cannabis use across adolescence and emerging adulthood will predict poorer psychosocial adjustment in young adulthood (i.e. lower educational and occupational attainment and greater internalizing and externalizing symptomatology).

Hypothesis 3b. White matter integrity of the cingulum and ATR will mediate the negative association between cannabis use and psychosocial adjustment.

2.0 METHOD

2.1 PITT MOTHER AND CHILD PROJECT (PMCP; SHAW, GILLIOM, INGOLDSBY, & NAGIN, 2003)

To test the current hypotheses, longitudinal clinical, neuroimaging, and psychosocial data from the Pitt Mother & Child Project was used. The Pitt Mother & Child Project is a longitudinal study of risk and resilience among men from low-SES families who have been followed prospectively for over 20 years. A total of 310 mother-son dyads were recruited from Women, Infant, and Children (WIC) nutritional supplement program clinics in the Pittsburgh area when subjects were 6-17 months old, and they have been followed throughout childhood, adolescence, and into young adulthood (age 1.5, 2, 3.5, 5, 5.5, 6, 8, 10, 11, 12, 15, 17, 20, 22, and 23). The full sample is 53% European American, 36% African American, 6% biracial, and 6% other races. At the time of enrollment, the mean income was $2892 per year and the mean Hollingshead SES score was 24.5, which corresponds to impoverished or working class status (Shaw et al., 2003).

Rates of cannabis use are particularly high among males (Swendsen et al., 2012) in urban settings (Johnston et al., 2012), and individuals from low SES (Reinherz, Giaconia, Hauf, Wasserman, & Paradis, 2000). In fact, rates of cannabis use increased 6% from 2007 to 2014 among men in households earning less than $20,000 (Carliner et al., 2017). Therefore, the PMCP sample is ideally suited to examine longitudinal correlates and consequences of cannabis use. Indeed, prior findings from this study already have shed light on how individual (i.e. externalizing problems, educational aspirations), familial (i.e. maternal depression, parenting characteristics, parental knowledge), and peer factors (i.e. peer substance use, perception of peers' substance use)

interact to impact risk and resiliency for adolescent substance use (Martin et al., 2015; Sitnick, Shaw, & Hyde, 2014). The current study aims to build upon previous work with this sample by incorporating longitudinal diffusion imaging data acquired at ages 20 and 22 to examine cannabis effects on developing white matter microstructure in the cingulum and ATR and to evaluate whether these effects mediate the link between cannabis exposure and poor psychosocial outcomes during the transition to adulthood.

2.1.1 Current study participants

A subset of 158 male PMCP participants were included in the current analyses for whom diffusion tensor imaging data was acquired at both age 20 and 22. Subjects were excluded from the MRI portion of the study if they endorsed any standard MRI contraindications, including prior head injury or concussion, metal in their body, pregnancy, or claustrophobia. Out of the full sample (*N*=310), *n*=186 completed an MRI scan at age 20 (*n*=31 declined MRI, *n*=25 prior head injury/concussion, *n*=17 could not be contacted, *n*=15 bullets/metal fragments, *n*=10 currently incarcerated, *n*=8 claustrophobic, *n*=5 out of the area, *n*=5 in the military, *n*=4 too ill to participate, *n*=2 braces, *n*=1 too large for scanner, *n*=1 on drugs, *n*=1 deceased). Twenty-eight subjects did not complete a second DTI scan, resulting in *n*=158 with longitudinal DTI data.

2.2 MEASURES

2.2.1 Cannabis use

Lifetime cannabis use was assessed with the Lifetime History of Drug Use and Drug Consumption (LHDU) semi-structured interview, which has been found to be psychometrically sound (Day et al., 2008; Skinner, 1982). Participants who endorsed a positive lifetime history of cannabis use (at least 3 times in one year) reported their age of cannabis use onset, annual frequency of use (days/month and quantity/day), and their greatest use in one day (maximum quantity of use and number of days using that quantity) for each year since their first use. This measure was administered at laboratory follow-up visits at age 20 and 22. In the event that their report differed between age 20 and 22, the earlier age of onset and higher amount was retained.

Adolescent cannabis use was measured by calculating the sum of participants' average days/month using cannabis at each time point from age 12-19. A sum was chosen instead of a mean because the latter measure may be misleading for subjects who escalated or decreased their use over time, which is typical during this age range. Because participants were scanned around the time of their 20th birthday, age 19 represents the year preceding their baseline DTI scan. Because these data were not normally distributed and contained a significant proportion of zero values, participants were categorized into groups based on the distribution of cannabis use frequency (see Figure 5). The sample was divided into terciles based on total frequency of use from age 12-19: minimal adolescent cannabis users (n=56; sum of age 12-19 average days/month of use ≤1), moderate adolescent cannabis users (n=49; sum of age 12-19 average days/month of use=1.5-44, i.e. approximately weekly use), and heavy adolescent cannabis users (n=53; sum of age 12-19 average days/month of use =45-217, i.e. multiple uses/week).

Cumulative cannabis use across adolescence and the transition to adulthood was measured by calculating the sum of participants' average days/month using cannabis at each time point from age 12-21. Because participants were scanned around the time of their 22nd birthday, age 21 represents the year preceding their follow-up DTI scan. Again, participants were split into terciles to account for the non-normal distribution of data and significant proportion of zero values (see Figure 5). Fifty-three participants were characterized by minimal cumulative use (≤ 2.48), *n*=52 were considered moderate users (2.49-60, i.e. up to 1.5 times/week), and *n*=53 were classified as heavy users (>60, i.e. multiple uses/week). Classifications were similar for adolescent and cumulative cannabis use frequency. All 53 participants categorized as minimal adolescent users were also classified as minimal users during adolescence and the transition to adulthood. However, several participants classified as moderate or heavy users in adolescence were reclassified into the heavy or moderate cumulative cannabis exposure group based on their pattern of use from age 20-21 (*n*=11).

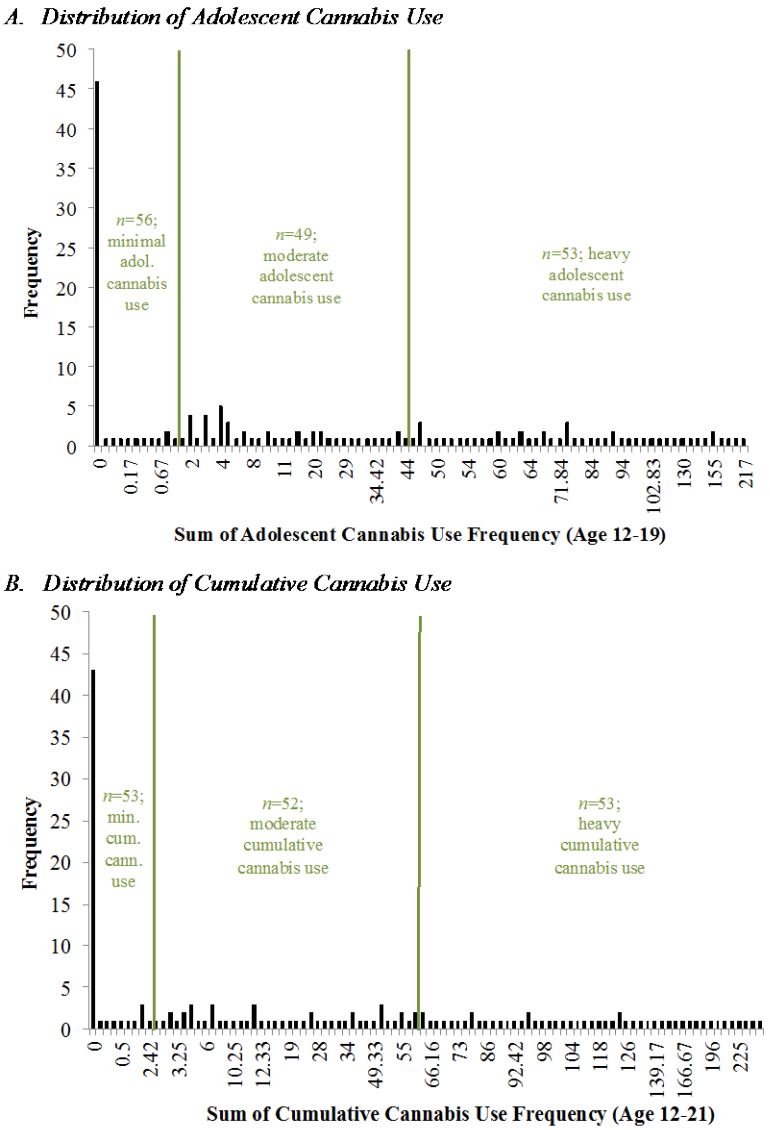

Figure 5. Distribution of cannabis use and group classification. Panel A illustrates the distribution of adolescent cannabis use frequency (age 12-19). Participants were divided into terciles and classified as having minimal (*n*=56), moderate (*n*=49), or heavy (*n*=53) adolescent cannabis use. Panel B illustrates the distribution of cumulative cannabis use frequency (age 12-21). Again, participants were divided into terciles and classified as having minimal (*n*=53), moderate (*n*=52), or heavy (*n*=53) cumulative cannabis exposure.

2.2.2 Covariates

A wide variety of sociodemographic and mental health characteristics has been shown to influence white matter microstructure in addition to cannabis exposure during adolescence and the transition to adulthood. In particular, alcohol use, tobacco use, IQ, SES, and psychopathology have all been linked to measures of white matter integrity. Therefore, these variables were considered as covariates for the current analyses. Additionally, head motion during scanning has also been found to influence DTI metrics, so head motion was included as a covariate in all DTI analyses.

2.2.2.1 Alcohol use.

Alcohol use in adolescence has been found to have neurotoxic effects on white matter microstructure (Elofson, Gongvatana, & Carey, 2013) and previous studies have found cannabis effects on brain structure to be attenuated when alcohol use is accounted for (Weiland et al., 2015). Therefore, it was of interest to control for adolescent alcohol exposure in the current analyses. Alcohol use was assessed with the Lifetime History of Alcohol Use and Alcohol Consumption semi-structured interview (Skinner, 1982). At the age 20 follow-up visit, participants reported their frequency of alcohol consumption (days/month) and their average number of drinks per occasion for each year since their first alcohol use. Measures of alcohol use frequency and number of drinks were multiplied in order to obtain a measure of overall quantity of alcohol exposure for each year. Alcohol exposure for each year from age 13-19 was summed to create a measure of lifetime alcohol exposure, and a log transformation was used to account for a positive skew in the data.

2.2.2.2 Tobacco use.

Regular tobacco use was also considered as a covariate because smoking status has been linked to poor whole-brain white matter integrity (Gianaros, Marsland, Sheu, Erickson, & Verstynen, 2013; Gons et al., 2011). Each participant was classified based on whether or not they reported daily use of tobacco during the last year on The Alcohol and Drug Consumption Questionnaire (ADCQ; Cahalan, Cisin, & Crossley) at age 20.

2.2.2.3 IQ.

IQ was also considered as a covariate based on evidence that full scale and performance IQ are associated with greater white matter integrity of the cingulum, corona radiata and internal capsule (Chiang et al., 2009). Prorated full Scale IQ (FSIQ) scores were derived from participants' performance on a short form of the Wechsler Intelligence Scale for Children (WISC-III; Wechsler, 1991) at age 11. The WISC-III is a common measure of child cognitive abilities, and prorated FSIQ scores were calculated by converting raw scores to scaled scores for each subtest.

2.2.2.4 Socioeconomic status (SES).

Lower SES has been linked to decreased white matter integrity across the whole brain and in the cingulum, anterior corona radiata, and anterior internal capsule, specifically (Gianaros et al., 2013). Socioeconomic status is a multifaceted concept, and both limited household resources and contextual neighborhood factors can have a significant impact on child development (Shaw, Hyde, & Brennan, 2012). Therefore, the current study used a composite measure including both familial income and neighborhood impoverishment. Familial income was assessed by mother report, and the mean value from the first 3 assessments was calculated (age 1.5, 2, and 3.5). Neighborhood impoverishment was quantified by combining several block level variables from census data

collected in 1990 (Shaw et al., 2012). Variables included in the overall neighborhood impoverishment factor score were: median family income, percent families below poverty level, percent on public assistance, percent unemployed, percent single-mother households, percent African-American, and percent Bachelor's degree or higher (see Shaw et al., 2012). As with family income, the average neighborhood impoverishment score for the block group in which each participant lived during the first 3 assessments was averaged. Both mean family income and mean neighborhood impoverishment were converted to Z-scores, and the mean of these standardized scores was used as the composite measure of early SES.

2.2.2.5 Psychopathology.

Both internalizing and externalizing psychopathology have been linked to aberrant white matter microstructure (Bracht, Linden, & Keedwell, 2015; Haney-Caron, Caprihan, & Stevens, 2014) and poor academic performance (Moilanen, Shaw, & Maxwell, 2010). However, cannabis use often co-occurs with various psychiatric disorders, and may even play a causal role in the development of certain conditions (Bovasso, 2001; Patton et al., 2002). As a result, concurrent measures of psychopathology in late adolescence may be confounded by cannabis exposure effects. Therefore, internalizing and externalizing scores from the parent report form of the Child Behavior Checklist (Achenbach & Edelbrock, 1983) were averaged across ages 10-12 because these assessments precede the onset of cannabis use. Both variables were log transformed to account for positive skew in the data.

2.2.2.6 Race.

Self-report of race at age 20 was used.

2.2.2.7 Head motion.

Head motion can have a significant impact on estimates of white matter microstructure derived from DTI data (Yendiki, Koldewyn, Kakunoori, Kanwisher, & Fischl, 2014). Therefore, mean head displacement was calculated for each participant for each DTI scan, and included as a covariate in all analyses examining white matter microstructure.

2.2.3 Psychosocial adjustment measures

2.2.3.1 Educational attainment.

At age 22, participants reported the highest level of education they had completed on a 13-point scale, ranging from 1 – below grade 9, to 13 – completion of graduate degree.

2.2.3.2 Occupational attainment.

Occupational attainment was assessed based on data from the Revised Work Characteristics and Unemployment Measure (Conger, 1988), which was administered to participants over the phone at age 23. Outcome variables included participants' employment status at age 23, i.e. currently employed/student vs. unemployed during the last year, and their self-reported job satisfaction ("How happy are you with this job?"), rated on a 5-point scale (1=very happy, 5=very unhappy).

2.2.3.3 Interpersonal functioning.

Participants also completed the Revised Booth Marital Distress Measure (Booth, Johnson, & Edwards, 1983) during their age 23 phone visit, which is a 5-item questionnaire that assesses romantic partner instability among married and dating couples. The total score (sum of items all items) was use.

2.2.3.4 Depressive symptoms.

The Beck Depression Inventory was administered at age 22, a 21-item self-report measure that commonly used to quantify depressive symptoms (Beck, Steer, & Garbin, 1988). The total score (sum of all items) was used.

2.2.3.5 Externalizing behavior.

Participants completed the Self-Report of Delinquency (Elliot, Huizinga, & Ageton, 1985) during their age 23 phone visit, in which they report on the frequency with which they have engaged in a variety of antisocial behaviors during the prior year on a 3-point scale. The total score (sum of all items) was used in the current analyses.

2.2.4 Diffusion Tensor Imaging (DTI)

Participants underwent DTI scanning as part of their follow-up study visits at age 20 and 22. Subjects were scheduled for their study visit as close to their birthday as possible, and completed a battery of interviews and questionnaires, as well as structural MRI, functional MRI and DTI scanning.

2.2.4.1 DTI acquisition.

Diffusion imaging data were acquired on a 3T Siemens Tim Trio scanner at the University of Pittsburgh MR Research Center. Two axial 2D diffusion tensor imaging (DTI) bipolar scans were acquired using identical parameters at both ages. DTI parameters were: time-to-repetition (TR)=8400 ms; time-to-echo (TE)=91ms; field of view=256x256; frequency=96; phase=96; 64 slices of 2mm thickness were acquired for a total scan time=9 min and 56 s. Diffusion-sensitizing

gradient encoding was applied in 61 uniform angular directions with a diffusion weighting of b=1000 s/mm^2. Seven reference images with no diffusion gradient (b=0) were also acquired.

2.2.4.2 DTI preprocessing.

Preprocessing was carried out using the Oxford Centre for Functional MRI of the Brain (FMRIB) Software Library (FSL; Smith et al., 2004). Raw dicom files were converted to nifti, .bvec, and .bval files using *dcm2nii* DICOM to NIFTI conversion. Subsequently, *fslroi* was used to extract a B0 image from each subject's nifti file and then the brain was segmented from the skull and other extracranial structures using brain extraction (*bet*; Smith, 2002). Diffusion data were eddy current corrected using *eddy_correct*, and then *dtifit* was used to fit a tensor model at each voxel. This command outputs images with all 3 eigenvectors and eigenvalues and a raw T2 image for each subject, as well as calculating fractional anisotropy, mean diffusivity, and the mode of the anisotropy. The first eigenvalue provides an estimate of axial diffusivity in each voxel, and *fslmaths* was used to calculate radial diffusivity by taking the mean of the 2nd and 3rd eigenvalues.

2.2.4.3 Tract-Based Spatial Statistics (TBSS).

Statistical analysis of the DTI data was conducted using tract-based spatial statistics (TBSS; Smith et al., 2006), part of the Oxford Centre for Functional MRI of the Brain (FMRIB) Software Library (FSL; Smith et al., 2004). TBSS is an approach that creates a sample-wise white matter skeleton to isolate the core of each major fiber pathway in order to minimize several common diffusion imaging confounds (Smith et al., 2006). This method reduces bias from partial volume effects by discarding voxels on the edge of each pathway adjacent to gray matter and is less susceptible to subtle inconsistencies or inaccuracies in registration because of its focus on the core regions of each tract. This method also provides good sensitivity for detecting white matter changes by

reducing the total number of voxels included and demanding less stringent correction for multiple comparisons relative to whole-brain voxelwise approaches.

All subjects' FA data were eroded and end slices were removed to eliminate likely outliers. Next, all FA images were aligned into a common space using the nonlinear registration tool (FNIRT; Andersson, Jenkinson, & Smith, 2010; Rueckert et al., 1999). A mean FA image was then created and thinned to create a mean FA skeleton which represents the centers of all tracts common to entire sample. The mean FA skeleton was then thresholded at 0.2 to create a binary skeleton mask onto which each subject's aligned FA data was then projected onto. For MD, AD, and RD measures, these images are registered to the FA skeleton. The Johns Hopkins University White Matter Tractography Atlas (Wakana et al., 2007) was used to identify the right and left cingulum (cingulate gyrus) and anterior thalamic radiations as regions of interests (ROIs). Finally, *fslmaths* was used to calculate the mean FA for each subject within each ROI of the skeletonized data. This procedure was repeated for MD, AD, and RD data, and mean values were extracted to SPSS for further analysis.

2.3 DATA ANALYSIS

2.3.1 Model selection

In order to determine the appropriate covariates to include in analyses of cannabis effects on microstructure of the cingulum and ATR, the Akaike Information criterion (AIC) was used to compare candidate models and assess whether each covariate improved the prediction of white matter microstructure. For aim 1, partial regression models were calculated predicting age 20 white

matter microstructure (FA and MD of the cingulum and ATR) based on adolescent cannabis exposure, controlling for head motion during the age 20 DTI scan. For aim 2, partial regression models were calculated predicting change in white matter microstructure from age 20 to 22 (FA and MD of the cingulum and ATR) based on cumulative cannabis exposure, controlling for head motion during the age 20 and 22 DTI scans. The AIC values for the partial models were then compared to AIC values for regression models including each of the potential covariates. AIC values from the models including control variables were subtracted from the AIC value of the original partial model. Smaller AIC values reflect better regression model fit and difference values greater than 2 were considered to reflect a substantial improvement in model fit. Any covariate that substantially improved the model fit was included in the final analyses. Because AD and RD are subcomponents of FA, covariates that improved the model fit for FA were also be included in analyses of AD and RD.

2.3.2 Aim 1. Characterize cross-sectional differences in ACC connectivity at age 20 based on adolescent cannabis use group

In order to evaluate whether cannabis use during adolescence (age 12-19) predicts baseline white matter microstructure at age 20, analysis of variance was used to determine whether microstructure of the cingulum and ATR differed between minimal, moderate, and heavy adolescent cannabis use groups. Separate models were constructed for FA, MD, AD, and RD of the cingulum and ATR. In addition to adolescent cannabis use, effects of hemisphere and head motion were also included, as well as any covariates identified by our model selection procedure (see above). Subject ID number was included in each model as a random effect variable.

An exploratory whole-brain analysis was also performed to assess the effects of adolescent cannabis exposure throughout the brain, in addition to the 2 tracts of interest. The *randomise* tool in FSL (Winkler, Ridgway, Webster, Smith, & Nichols, 2014) was used to conduct ANOVA analyses to determine whether adolescent cannabis use groups differed in FA or MD throughout the white matter skeleton, controlling for head motion during the age 20 scan. A voxel-based FWE-corrected significance threshold of $p<.01$ was used to evaluate results. Any additional regions identified in which age 20 white matter microstructure differed significantly based on adolescent cannabis use were also included in Aim 2 analyses to determine whether cumulative cannabis exposure predicts change in white matter microstructure from age 20 to 22.

2.3.3 Aim 2. Examine longitudinal cannabis effects on developing ACC connectivity

In order to assess longitudinal cannabis effects, analysis of variance was used to estimate whether change in microstructure of the cingulum or ATR from age 20-22 (i.e. difference in FA between age 20 and 22) varied between cumulative cannabis exposure groups. Separate models were constructed for change in FA, MD, AD, and RD of the cingulum and ATR. In addition to cumulative cannabis use, effects of hemisphere and head motion (at age 20 and 22) were also included, as well as any covariates that substantially improved the model fit (see above). Subject ID number was included in each model as a random effect variable.

2.3.4 Aim 3. Assess whether white matter microstructure mediates the association between cannabis use and poor psychosocial adjustment in young adulthood

2.3.4.1 Cannabis effects on psychosocial adjustment.

In order to examine whether psychosocial adjustment in young adulthood varied among cannabis use groups, analysis of variance was used to test whether cumulative cannabis across adolescence and the transition to adulthood exposure predicted educational or occupational attainment, interpersonal functioning, internalizing symptoms or externalizing behavior in young adulthood.

2.3.4.2 ACC connectivity and psychosocial adjustment.

In order to evaluate the implications of cingulum and ATR microstructure for psychosocial adjustment during the transition to adulthood, linear regression was used to test whether microstructure of the cingulum and ATR predict educational attainment, job satisfaction, interpersonal functioning, internalizing symptoms, or antisocial behavior in young adulthood. Binary logistic regression was used to assess whether cingulum or ATR microstructure predicted employment status at age 23.

2.3.4.3 Mediation of cannabis effects by white matter microstructure.

Finally, the PROCESS macro (Version 2.13; Hayes, 2013) for SPSS (Version 21) was used to formally test whether microstructure of the cingulum and ATR mediates the association between cumulative cannabis use and psychosocial adjustment in young adulthood. According to Hayes and Rockwood (2016), "by contemporary thinking, tests of significance for the individual paths *a* and *b* are not required to determine whether *M* mediates the effect of *X* on *Y*, contrary to the causal steps logic which requires that both a and b are statistically significant" (p. 5). Therefore, this

approach allows for the examination indirect effects, even when individual paths in a mediation model are not significant (Hayes, 2013). A third variable (M) is considered eligible to be interpreted as a mediator of the relationship between X and Y if there is temporal precedence of predictor variables, i.e. X precedes M (Kraemer, Kiernan, Essex, & Kupfer, 2008). In this instance, cannabis exposure precedes white matter development during the transition to adulthood, so change white matter microstructural development of the cingulum and ATR from age 20 to 22 can be evaluated as a potential mediator of cannabis effects on academic, occupational, interpersonal, and mental health outcomes in young adulthood.

3.0 RESULTS

3.1 SUBJECT CHARACTERISTICS

The current sample (*n*=158) is 51.3% Caucasian, 41.1% African American and 7.6% other races (see Table 1 for subject characteristics). Overall, participants were characterized by low family income in early childhood (*M*=$1208.18/month across the first 3 assessments, *SD*=669.9), internalizing (*M*=5.28, *SD*=4.99) and externalizing symptoms (*M*=8.65, SD=6.85) in the normal range in early adolescence (age 10-12), normal IQ (*M*=96.11, *SD*=18.39), and less than 14% reported a non-substance-related psychiatric disorder at age 20 or 22.

Table 1. Subject Demographic and Clinical characteristics**.** *p<.05 **p<.01. Superscript numbers in parentheses indicate which groups were significantly different from one another, based on pairwise Bonferroni-corrected post-hoc testing or pairwise $\chi 2$ tests, as applicable (1=Minimal/No Cannabis Exposure Group, 2=Moderate Cannabis Exposure Group, 3=Heavy Cannabis Exposure Group). Internalizing and externalizing symptoms were measured using the Child Behavior Checklist (CBCL) (Achenbach & Edelbrock, 1983), parent report, at child age 10, 11, and 12. IQ was assessed using a short form of the Wechsler Intelligence Scale for Children (WISC-III) (Wechsler, 1991). Mood Disorder includes Major Depressive Disorder (MDD; age 20 n=15, age 22 n=20), Bipolar Disorder (age 20 n=2, age 22 n=1), and dysthymia (age 20 n=3, age 22 n=5). Anxiety Disorder includes Panic Disorder (age 20 n=2, age 22 n=3), Social Phobia (age 20 n=12, age 22 n=9), Specific Phobia (age 20 n=5, age 22 n=8), Obsessive Compulsive Disorder (OCD; age 20 n=4, age 22 n=3), Post-Traumatic Stress Disorder (PTSD; age 20 n=3, age 22 n=2), and Generalized Anxiety Disorder (GAD; age 20 n=1, age 22 n=2).

	Full Sample (n=158)		Minimal/No Cannabis Exposure (n=53)		Moderate Cannabis Exposure (n=52)		Heavy Cannabis Exposure (n=53)		Group Comparison	
Race	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	χ^2	*p*
White	81	51.3	32	60.4	24	46.2	25	47.2	5.87	0.437
Black	65	41.1	16	30.2	24	46.2	25	47.2		
Biracial	8	5.1	3	5.7	2	3.8	3	5.7		
Other	4	2.53	2	3.8	2	3.8	0	0		
SES	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*F*	*p*
Family Income (per month) (Mean first 3 assessments)	1208.18	669.9	1236.88	594.24	1409.28 (3)	768.12	982.16 (2)	574.26	5.74	0.004**
Neighborhood Risk Score (Mean first 3 assessments)	0.37	1.12	0.03 (3)	0.81	0.45	1.25	0.63 (1)	1.2	4.06	0.019*
Childhood Clinical and Cognitive Assessments										
Internalizing Symptoms (Mean Age 10-12)	5.28	4.99	5.38	5.14	4.95	4.82	5.49	5.07	0.55	0.581
Externalizing Symptoms (Mean Age 10-12)	8.65	6.85	7.98	6.51	7.77	6.22	10.09	7.55	0.15	0.865
IQ (Age 11)	96.11	18.39	97.89	20.82	96.61	19.94	93.86	13.94	1.68	0.19
Age 20 Psychopathology	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	χ^2	*p*
Mood Disorder	18	11.39	5	3.16	4	2.53	9	5.7	2.49	0.287
Anxiety Disorder	21	13.29	6	3.8	5	3.16	10	6.33	2.17	0.339
Psychosis	3	1.9	2	1.27	0	0	1	0.63	2.05	0.358
Antisocial Personality Disorder	12	7.6	0 (3)	0	0 (3)	0	12 (1,2)	22.64	25.5	<.001**
Age 22 Psychopathology										
Mood Disorder	22	13.9	8	15.1	7	13.5	7	13.2	0.106	0.948
Anxiety Disorder	20	12.7	3	5.7	5	9.6	12	22.6	7.69	0.021
Psychosis	3	1.9	1	1.9	1	1.9	1	1.9	0	1
Antisocial Personality Disorder	17	10.8	1 (3)	1.9	0 (3)	0	16 (1,2)	30.2	31.82	<.001**

3.1.1 Cannabis use

Seventy-nine percent of participants (*n*=124) reported a lifetime history of cannabis use. As expected, the prevalence of cannabis use in the current sample is notably higher than nationally-representative samples of 18-25 year olds, in which 52.7% of individuals report a positive lifetime history of cannabis use (SAMHSA, 2016). No subjects reported regular use prior to age 12. Overall, participants initiated cannabis use around age 16 (*M*=15.74, *SD*=2.16) and reported an average of 4 and a half years of use (*M*=4.44, *SD*=2.75; see Table 2). Rates of use increased with age through age 20, then decreased from age 20 to 21. The frequency of cannabis use reported by the current sample was similar to frequency estimates reported for cannabis users between the ages of 18-25 at the national level (SAMHSA, 2016). According to the National Survey on Drug Use and Health, among current cannabis users between the ages of 18-25 in 2015, 21.5% reported using 1-2 days/month, 14.1% reported using 3-5 days/month, 20% reported using 6-19 days/month, and 44.4% reported using 20+ days/month (SAMHSA, 2016), compared to the current sample in which 21% reported using 1-2 days/month of use, 13.6% reported using 3-5 days/month, 25.9% reported using 6-19 days/month, and 39.5% reported using 20+ days/month at age 21. Twenty-five percent of participants met DSM-IV criteria for substance abuse and 8.9% met criteria for substance dependence at age 22, with cannabis use disorders accounting for the vast majority of substance use diagnoses (100% for substance abuse and 92.8 for substance dependence).

Table 2. Cannabis Use Characteristics . *p<.05 **p<.01. Superscript numbers in parentheses indicate which groups were significantly different from one another, based on pairwise Bonferroni-corrected post-hoc testing or pairwise $\chi 2$ tests, as applicable (1=Minimal/No Cannabis Exposure Group, 2=Moderate Cannabis Exposure Group, 3=Heavy Cannabis Exposure Group). Duration of use reflects the number of years (from age 12-21) that participants reported cannabis use frequency ≥ 1x/week. Substance use disorder diagnoses determined based on the Structured Clinical Interview for DSM-IV (SCID), administered at age 20 and 22 study visits. Substance Abuse: Includes Cannabis Abuse (age 20 n=31, age 22 n=39), Sedative Abuse (age 20 n=0, age 22 n=4), Stimulant Abuse (age 20 n=0, age 22 n=1), Opioid Abuse (age 20 n=2, age 22 n=3), Cocaine Abuse (age 20 n=2, age 22 n=2), Hallucinogen/PCP Abuse (age 20 n=2, age 22 n=2), and Other Substance Abuse (age 20 n=1, age 22 n=1). Substance Dependence: Includes Cannabis Dependence (age 20 n=18, age 22 n=13), Sedative Dependence (age 20 n=1, age 22 n=2), Opioid Dependence (age 20 n=1, age 22 n=3), Cocaine Dependence (age 20 n=1, age 22 n=22), Hallucinogen/PCP Dependence (age 20 n=0, age 22 n=1), and Other Substance Dependence (age 20 n=1, age 22 n=0).

	Full Sample (n=158)		Minimal/No Cannabis Exposure (n=53)		Moderate Cannabis Exposure (n=52)		Heavy Cannabis Exposure (n=53)		Group Comparison	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*F*	*p*
Age of Onset (n=124 Lifetime Users)	15.74	2.16	17.73$^{(2,3)}$	1.4	16.12$^{(1,3)}$	2.12	14.68$^{(1,2)}$	1.81	19.14	<.001**
Duration of Use (n=124 Lifetime Users)	4.44	2.75	0.26$^{(2,3)}$	0.56	3.77$^{(1,3)}$	1.96	6.58$^{(1,2)}$	1.57	109.83	<.001**
Average Frequency of Cannabis Use (days/month; n=124 Lifetime Users)										
Age 12	0.42	3.09	0	0	0.02	0.14	0.96	4.69	1.45	0.239
Age 13	0.84	3.77	0	0	0.12	0.51	1.87	5.65	3.47	.034*
Age 14	2.02	5.8	0$^{(3)}$	0	0.55$^{(3)}$	2.8	4.27$^{(1,3)}$	8.02	7.36	.001**
Age 15	3.96	8.42	0$^{(3)}$	0	1$^{(3)}$	3.17	8.29$^{(1,3)}$	11.14	15.192	<.001**
Age 16	6.73	10.46	0$^{(3)}$	0.02	3.74$^{(3)}$	7.47	12.29$^{(1,3)}$	12.25	16.68	<.001**
Age 17	8.06	11.28	0.07$^{(3)}$	0.23	3.13$^{(3)}$	6.5	16.07$^{(1,3)}$	12.23	35.52	<.001**
Age 18	10.64	12.29	0.06$^{(3)}$	0.1	4.52$^{(3)}$	7.14	20.63$^{(1,3)}$	11.35	60.63	<.001**
Age 19	11.28	12.43	0.09$^{(3)}$	0.24	4.02$^{(3)}$	5.18	22.42$^{(1,3)}$	10.68	96.95	<.001**
Age 20	11.68	12.69	0.14$^{(3)}$	0.33	3.84$^{(3)}$	5.29	22.97$^{(1,3)}$	10.39	101.95	<.001**
Age 21	9.57	11.67	0.07$^{(3)}$	0.23	3.39$^{(3)}$	4.52	18.93$^{(1,3)}$	11.84	59.95	<.001**
Substance Use Disorders at Age 20	*N*	%	*N*	%	*N*	%	*N*	%	χ^2	*p*
Substance Abuse	33	20.9	0$^{(2,3)}$	0	7$^{(1,3)}$	4.43	26$^{(1,2)}$	16.46	40.38	<.001**
Cannabis Abuse	31	19.6	0$^{(2,3)}$	0	5$^{(1,3)}$	3.16	26$^{(1,2)}$	16.46	45.36	<.001**
Substance Dependence	21	13.3	0$^{(2,3)}$	0	4$^{(1,3)}$	2.53	26$^{(1,2)}$	10.76	25.47	<.001**
Cannabis Dependence	18	11.4	0$^{(3)}$	0	2$^{(3)}$	1.27	26$^{(1,2)}$	10.13	28.3	<.001**
Substance Use Disorders at Age 22										
Substance Abuse	39	24.7	0$^{(2,3)}$	0	10$^{(1,3)}$	19.2	29$^{(1,2)}$	54.7	44.51	<.001**
Cannabis Abuse	39	24.7	0$^{(2,3)}$	0	11$^{(1,3)}$	21.2	28$^{(1,2)}$	52.8	40.62	<.001**
Substance Dependence	14	8.9	0$^{(3)}$	0	1$^{(3)}$	1.9	13$^{(1,2)}$	24.5	24.64	<.001**
Cannabis Dependence	13	8.2	0$^{(3)}$	0	0$^{(3)}$	0	13$^{(1,2)}$	24.5	28.06	<.001**

As expected, patterns of cannabis use differed significantly between cumulative cannabis exposure groups. During the year preceding their follow up DTI scan (age 21), participants in the minimal/no cumulative cannabis exposure group (*n*=53) reported using 0.07 days/month, participants in the moderate cumulative cannabis exposure group (*n*=52) reported using 3.39 days/month (approximately once/week), and participants in the heavy cumulative cannabis exposure group (n=53) reported using cannabis 18.93 times/month (approximately 5 times/week). Participants in the heavy cumulative cannabis use group were also characterized by the lowest income and the highest neighborhood risk in early childhood, as well as the highest prevalence of antisocial personality disorder (see Table 1).

3.1.2 Alcohol use

Ninety-six percent of participants (*n*=151) reported a lifetime history of alcohol use. As expected, alcohol use increased across adolescence and the transition to adulthood. Annual alcohol use was assessed beginning at age 13 when participants reported using alcohol 0.04 days/month on average (*SD*=0.48), and consuming an average of .09 drinks/occasion (*SD*=1.12). These rates increased to 4.63 days/month at age 21 (*SD*=4.64), and an average of 4.5 drinks/occasion (*SD*=4.07). Cumulative alcohol exposure (based on average days/month drinking*average drinks/occasion for each time point, age 12-19) differed significantly among cannabis use groups (F=9.5, p<.001), such that participants' quantity of alcohol exposure was higher among those with higher rates of cannabis use (see Table 3).

Table 3. Alcohol and Other Substance Use Characteristics**.** *p<.05 **p<.01. Superscript numbers in parentheses indicate which groups were significantly different from one another, based on pairwise Bonferroni-corrected post-hoc testing or pairwise $\chi 2$ tests, as applicable (1=Minimal/No Cannabis Exposure Group, 2=Moderate Cannabis Exposure Group, 3=Heavy Cannabis Exposure Group). Cumulative alcohol exposure reflects the sum of participants' annual quantity of alcohol use (average days/month and average drinks/occasion were multiplied in order to obtain a measure of overall quantity of alcohol exposure for each year). Lifetime history of illicit substance use was assessed using the Lifetime History of Drug Use and Drug Consumption (LHDU) semi-structured interview; positive lifetime history was determined based by consensus from age 20 and age 22 study visits.

	Full Sample (n=158)		Minimal/No Cannabis Exposure (n=53)		Moderate Cannabis Exposure (n=52)		Heavy Cannabis Exposure (n=53)		Group Comparison	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*F*	*p*
Cumulative Alcohol Exposure	86.01	176.18	$24.76^{(3)}$	65.76	$68.57^{(3)}$	100.76	$1485.23^{(1,2)}$	262.65	9.5	<.001**
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	χ^2	*p*
Daily Smoker	44	27.8	$3^{(2,3)}$	5.7	$14^{(1,3)}$	26.9	$27^{(1,2)}$	50.9	25.76	<.001**
Lifetime History of Illicit Substance Use										
Cocaine/Crack	16	10.1	$0^{(2,3)}$	0	$4^{(1,3)}$	7.7	$12^{(1,2)}$	22.6	15.43	<.001**
Stimulants	13	8.2	1	1.9	5	9.6	7	13.2	4.7	0.096
Sedatives	20	12.7	$1^{(3)}$	1.9	$5^{(3)}$	9.6	$14^{(1,2)}$	26.4	15.07	.001**
Opioids	21	13.3	$1^{(3)}$	1.9	$5^{(3)}$	9.6	$15^{(1,2)}$	28.3	16.95	<.001**
Inhalants	5	3.2	0	0	1	1.9	4	7.5	5.32	0.07
Hallucinogens	19	12	$0^{(2,3)}$	0	$7^{(1)}$	13.5	$12^{(1)}$	22.6	12.99	0.002**
Ecstacy	22	13.9	$0^{(2,3)}$	0	$5^{(1,3)}$	9.6	$17^{(1,2)}$	32.1	23.95	<.001**

3.1.3 Other illicit substance use

Relative to cannabis and alcohol use, rates of other illicit substance use were low in the current sample. Less than 15% of participants reported lifetime use of any illicit drug other than cannabis (see Table 3). Lifetime history of illicit drug use differed among cannabis use groups, with significantly higher rates of use among heavy cannabis users relative to moderate and minimal/non-users for the majority of drug classes.

3.1.4 Psychosocial attainment

Approximately half of participants reported that they had completed some post-secondary education (*n*=82) at age 22 and 30% were currently employed at age 23 (see Table 4). Relationship distress and self-reported depressive symptoms did not differ significantly among cannabis exposure groups, whereas educational attainment and self-reported antisocial behavior did differ between groups, with heavy users reporting the lowest level of education and highest level of antisocial behavior.

Table 4. Psychosocial Adjustment. *p<.05 **p<.01. Superscript numbers in parentheses indicate which groups were significantly different from one another, based on pairwise Bonferroni-corrected post-hoc testing or pairwise $\chi 2$ tests, as applicable (1=Minimal/No Cannabis Exposure Group, 2=Moderate Cannabis Exposure Group, 3=Heavy Cannabis Exposure Group). Educational attainment was measured on a 13-point scale, ranging from 1 – below grade 9, to 13 – completion of graduate degree.

	Full Sample (n=158)		Minimal/No Cannabis Exposure (n=53)		Moderate Cannabis Exposure (n=52)		Heavy Cannabis Exposure (n=53)		Group Comparison	
Educational Attainment Age 22	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	χ^2	*p*
Completed HS/GED (or less)	76	48.1	22[3]	41.5	15[3]	28.8	39[1,2]	73.6	22.43	<.001**
Completed Any Post-Secondary Education	82	51.9	31[3]	58.5	37[3]	71.2	14[1,2]	26.4		
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*F*	*p*
Continuous Measure of Education	6.68	2.22	6.94[3]	2.32	7.38[3]	2.25	5.74[1,2]	1.72	8.58	<.001**
Occupational Attainment Age 23	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	χ^2	*p*
Currently Employed	47	29.7	12	22.6	17	32.7	18	34	3.19	0.202
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*F*	*p*
Job Satisfaction (Scale 1-5)	2.22	0.91	2.14	0.83	2.4	0.96	2.1	0.94	1.29	0.279
Other Adjustment Indices	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*F*	*p*
Self-Report of Delinquency (SRD; Age 23)	10.22	7.5	6.18[1,3]	6.65	11.56[1]	6.44	13.36[1]	7.54	13.86	<.001**
Beck Depression Inventory (BDI; Age 22)	4.81	6.15	3.25	3.87	5.83	7.6	5.37	6.19	2.65	0.074
Relationship Distress (Age 23)	3.28	0.86	3.38	0.72	3.07	0.99	3.38	0.84	1.16	0.319

3.2 AIM 1. CROSS-SECTIONAL CANNABIS EFFECTS ON ACC CONNECTIVITY AT AGE 20

Model selection procedures demonstrated that including race improved the model fit for age 20 cingulum FA (see Table 5). Therefore, reported models for FA, AD, and RD include movement during the age 20 scan, hemisphere, and race as covariates. For MD, model selection procedures demonstrated that none of the potential covariates improved the model fit for age 20 MD of the cingulum or ATR (see Table 5). Therefore, reported models for MD include only movement during the age 20 scan and hemisphere as covariates.

Table 5. Model Selection. Akaike Information Criterion (AIC) values were calculated for regression models predicting white matter microstructure (FA and MD of the right and left cingulum and ATR) based on adolescent cannabis exposure, controlling for head motion during DTI scanning. These were compared to AIC values for regression models including each of the potential covariates. Smaller AIC values reflect better regression model fit. Therefore, the AIC values from the models including our control variables were subtracted from the AIC value of the original model. Difference values greater than 2 reflect a substantial improvement in model fit. Race was the only control variable that improved the model fit for right cingulum FA at age 20. Therefore, race was included as a covariate in regression models predicting FA, AD, and RD at age 20.

Difference Between Partial Model AIC and AIC of Model including Control Variable							
	Race	SES	IQ	Internalizing	Externalizing	Tobacco	Alcohol
Age 20 FA							
Right Cingulum	7.53*	-14.04	-194.84	-112.96	-114.66	-13.23	-30.27
Left Cingulum	-0.28	-17.64	-203.79	-122.96	-123.48	-10.83	-37.36
Right ATR	-1.91	-23.42	-231.56	-142.25	-144.60	-19.54	-52.29
Left ATR	-1.99	-23.71	-231.80	-142.95	-144.56	-19.52	-48.74
Age 20 MD							
Right Cingulum	-1.99	-66.27	-614.94	-376.68	-376.59	-45.86	-129.58
Left Cingulum	-2.00	-68.38	-628.43	-386.33	-385.95	-40.82	-128.21
Right ATR	-1.78	-68.39	-618.56	-381.64	-381.62	-43.78	-132.75
Left ATR	-1.96	-65.39	-626.31	-387.38	-387.30	-40.30	-134.56
Change in FA from Age 20 to 22							
Right Cingulum	0.49	-27.33	-223.22	-141.17	-146.27	-17.00	-33.86
Left Cingulum	-2.00	-29.14	-242.91	-149.92	-151.55	-19.60	-44.24
Right ATR	-1.87	-30.24	-263.88	-156.36	-158.87	-20.48	-43.31
Left ATR	-1.79	-30.45	-247.19	-149.51	-151.76	-18.62	-43.17
Change in MD from Age 20 to 22							
Right Cingulum	-1.32	-63.90	-599.11	-373.19	-373.16	-37.97	-119.77
Left Cingulum	-1.33	-64.13	-607.49	-380.35	-380.59	-33.42	-123.36
Right ATR	-1.84	-64.64	-597.51	-371.26	-371.15	-35.13	-123.68
Left ATR	-1.49	-63.73	-594.29	-371.82	-371.82	-30.99	-126.71

3.2.1 Cingulum

3.2.1.1 FA.

Significant effects on cingulum FA were observed for adolescent cannabis exposure (age 12-19; F=2.96, p=.05; see Figure 6), hemisphere (F=12.67, p=.000), and race (F=4.85, p=.003) at age 20. Contrary to our hypothesis, individuals reporting moderate cannabis use in adolescence displayed

higher FA relative to individuals reporting low or no use and those reporting heavy adolescent use. However, none of the pairwise differences between cannabis exposure groups met the Bonferroni-corrected significance threshold. The effect of hemisphere was driven by greater FA in the left hemisphere. The effect of race reflects greater FA in the cingulum for African American followed by biracial subjects, Caucasian subjects, and participants of other races. Given prior evidence that accounting for alcohol use may attenuate cannabis effects on brain structure, we also evaluated alcohol use as a covariate. The effect of adolescent cannabis exposure (F=3.34, p=.037) remained significant when alcohol use (F=6.77, p=.01) was included as a covariate, and post-hoc pairwise comparisons demonstrated a significant difference between cingulum FA of moderate and low or no use groups (p=.04).

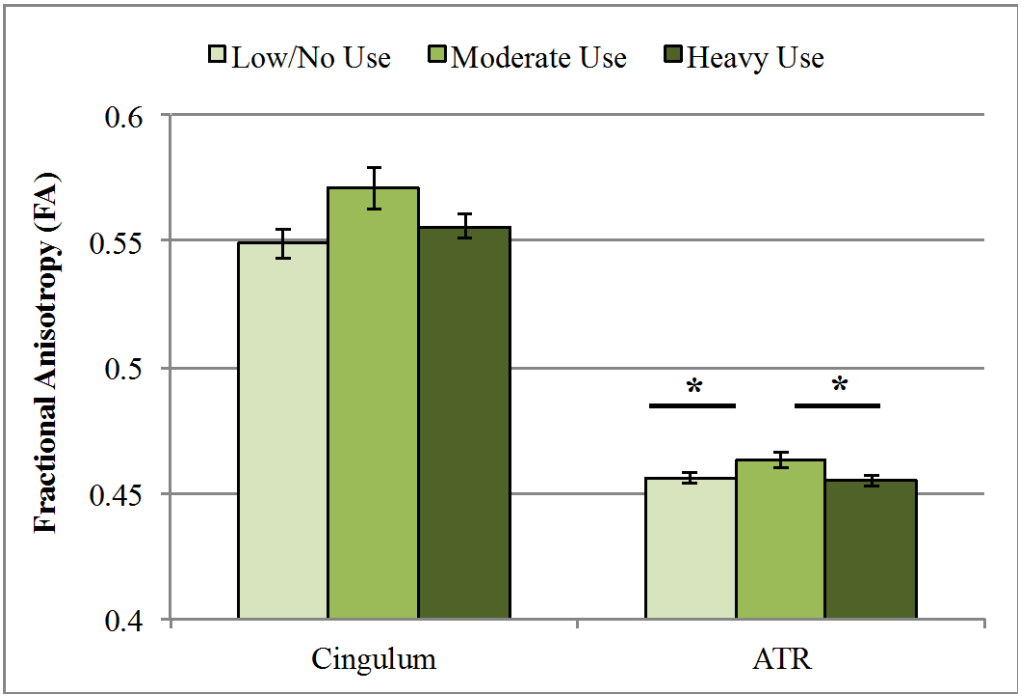

Figure 6. Main effect of adolescent cannabis exposure on cingulum and ATR FA at age 20. Significant main effects of adolescent cannabis exposure group were observed for both the cingulum (F=2.96, p=.05) and ATR

(F=3.48, p=.032). For the ATR, Bonferroni-corrected post-hoc tests demonstrated that the pairwise differences between the moderate use group and the low or no use group ($p_{corrected}$<.05), as well as the heavy use group ($p_{corrected}$<.01) were statistically significant.

A significant cannabis exposure by race interaction was found (F=2.82, p=.017; see Figure 7), such that among African American participants there was no significant effect of adolescent cannabis exposure (F=.58, p=.56) whereas among Caucasian participants, adolescent cannabis exposure had a significant effect on cingulum FA (F=11.01, p=.000). Among Caucasian participants, moderate cannabis use was linked to greater FA relative to low or no cannabis use ($p_{corrected}$=.048), whereas heavy adolescent cannabis use was associated with lower FA relative to both the low or no use group ($p_{corrected}$=.031) and the moderate use group ($p_{corrected}$=.00002). Within group associations were not examined for biracial subjects (n=8) and subjects of other races (n=4) due to insufficient sample sizes. No significant cannabis exposure by hemisphere interaction was present.

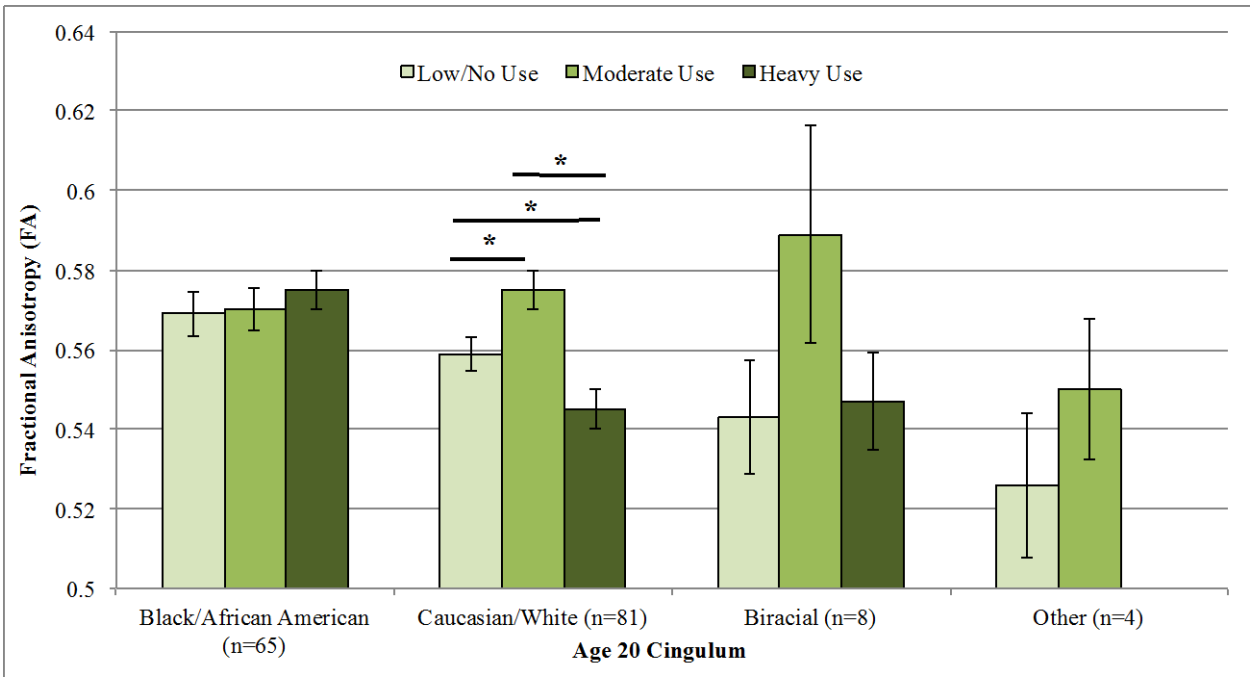

Figure 7. Interactive effect of cannabis exposure and participant race on cingulum FA at age 20. A significant cannabis exposure by race interaction was found (F=2.82, p=.017), such that among African American participants there was no significant effect of adolescent cannabis exposure (F=.58, p=.56) whereas among Caucasian participants, adolescent cannabis exposure had a significant main effect on cingulum FA (F=11.01, p=.000). Among Caucasian participants, moderate cannabis use was linked to greater FA relative to low or no cannabis use ($p_{corrected}$<.05), whereas heavy adolescent cannabis use was associated with lower FA relative to both the low or no use group ($p_{corrected}$<.05) and the moderate use group ($p_{corrected}$<.001). Within group associations were not examined for biracial subjects (n=8) and subjects of other races (n=4) due to insufficient sample sizes.

3.2.1.2 AD/RD.

Adolescent cannabis exposure did not have a significant effect on cingulum AD (F=2.57, p=.08) or RD (F=0.07, p=.93).

3.2.1.3 MD.

Significant effects on cingulum MD were observed for adolescent cannabis exposure (F=7.39, p=.001; see Figure 8) and hemisphere (F=12.72, p=.000). The moderate exposure group displayed higher MD than both other groups. Post-hoc tests revealed that the pairwise difference between cingulum MD of the moderate and heavy exposure groups was statistically significant ($p_{corrected}$=.0004). The effect of adolescent cannabis exposure (F=4.75, p=.009) remained significant when alcohol use (F=3.8, p=.052) was included as a covariate The main effect of hemisphere was driven by higher MD in the left cingulum. No significant interaction between cannabis exposure and hemisphere was found.

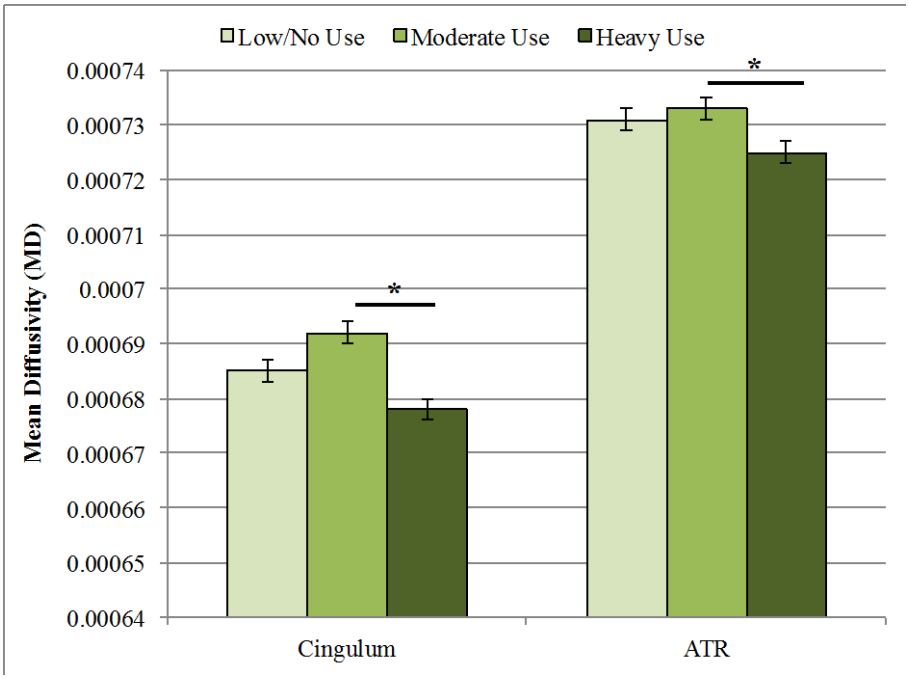

Figure 8. Main effect of adolescent cannabis exposure on cingulum and ATR MD at age 20. Significant main effects of cannabis exposure were observed for both the cingulum ($F=7.39$, $p=.001$) and ATR (F=6.29

p=.002). For both tracts, Bonferroni-corrected post-hoc tests demonstrated that the pairwise differences between the moderate use group and the heavy use group ($p_{corrected}$<.05) were statistically significant.

3.2.2 ATR

3.2.2.1 FA.

Significant effects on ATR FA were observed for adolescent cannabis exposure (F=6.29 p=.002; see Figure 6) and hemisphere (F=15.75, p=.000). Similar to the pattern observed for cingulum FA, the moderate use group displayed higher FA than both the low or no use and the heavy use groups. Bonferroni-corrected post-hoc tests demonstrated that the pairwise differences between the moderate use group and the low or no use group ($p_{corrected}$=.013) and the heavy use group ($p_{corrected=}$.004) were statistically significant, whereas the low or no use and the heavy use group were not significantly different. The effect of hemisphere was driven by greater FA in the right hemisphere. No significant cannabis exposure by hemisphere or race interactions were found for ATR FA at age 20. The effect of adolescent cannabis exposure (F=8.5, p=.0003) remained significant after controlling for alcohol use (F=1.1, p=.26), and the pattern of pairwise results remained consistent.

3.2.2.2 AD/RD.

A significant effect of adolescent cannabis exposure on ATR AD was observed (F=4.31, p=.014; see Figure 9). Consistent with the pattern observed for ATR FA, the moderate exposure group had higher AD than both other groups. Post-hoc tests demonstrated that the pairwise difference between the moderate and heavy exposure groups was statistically significant ($p_{corrected}$=.012). This

effect remained significant (F=4.28, p=.015) after controlling for alcohol exposure (F=6.4, p=.012), and post-hoc pairwise tests revealed a significant difference between the low/non-using group and the moderate use group ($p_{corrected}$=.02). Adolescent cannabis exposure did not have a significant effect on ATR RD at age 20 (F=2.4, p=.09).

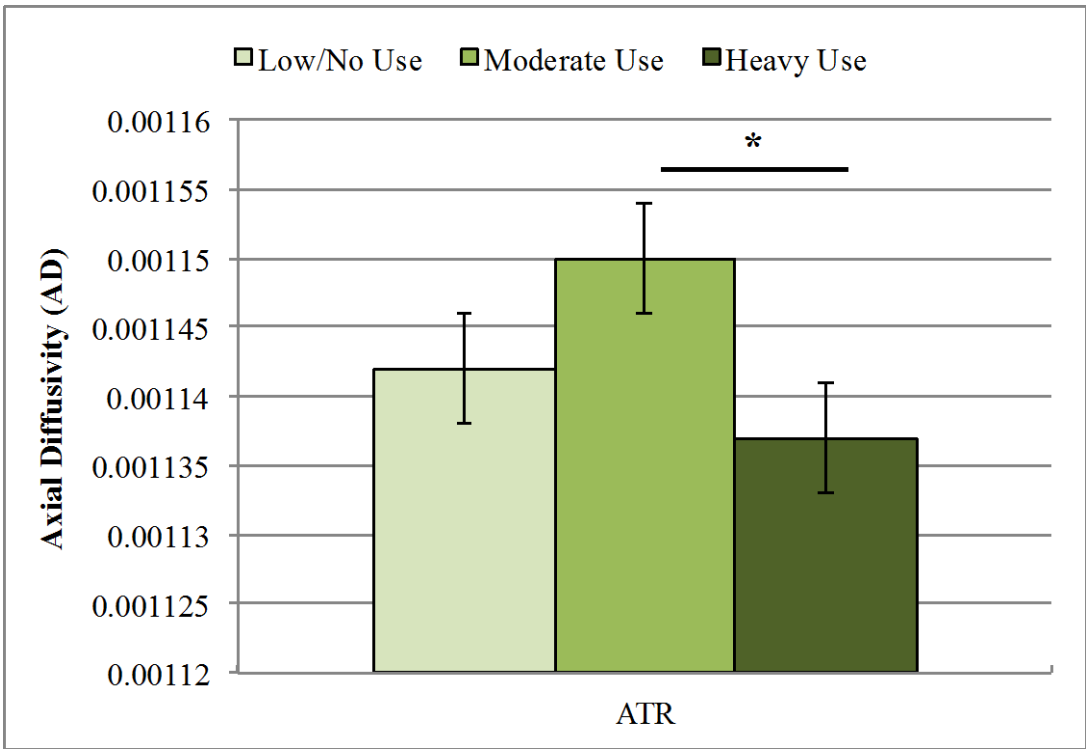

Figure 9. Main effect of adolescent cannabis exposure on ATR AD at age 20. A significant effect of adolescent cannabis exposure on ATR AD was observed (F=4.31, p=.014). Consistent with the pattern observed for ATR FA, the moderate exposure group had higher AD than both other groups. Post-hoc tests demonstrated that the pairwise difference between the moderate and heavy exposure groups was statistically significant ($p_{corrected}$=.012).

3.2.2.3 MD.

Significant effects on ATR MD were observed for adolescent cannabis exposure (F=3.48, p=.032; see Figure 8) and hemisphere (F=8.19, p=.004). The moderate exposure group had higher MD than both other groups. Bonferroni-corrected post-hoc test revealed that the difference between the moderate and the heavy exposure groups was statistically significant ($p_{corrected}$=.031). This effect was no longer significant when alcohol was included as a covariate (F=2.07, p=.13), although the effect of alcohol exposure on ATR MD was also non-significant (F=2.32, p=.13). The effect of hemisphere reflects higher MD in the left ATR. No significant interaction between cannabis exposure and hemisphere was found.

3.2.2.4 Whole-brain results.

Our whole-brain analysis did not reveal any clusters throughout the white matter skeleton in which white matter microstructure differed significantly between adolescent cannabis use groups. Therefore, subsequent analyses of longitudinal cannabis effects no not include any additional ROIs.

3.3 AIM 2. LONGITUDINAL CANNABIS EFFECTS ON DEVELOPING ACC CONNECTIVITY

Model selection procedures demonstrated that none of the potential covariates improved the model fit when predicting the change in FA or MD from age 20 to 22 for either the cingulum or ATR (see Table 5). Therefore, reported models include hemisphere and movement during the age 20 and age 22 scans as covariates.

3.3.1 Cingulum

3.3.1.1 FA change from age 20 to 22.

All cannabis use groups displayed increased FA from age 20 to 22. Significant effects of cumulative cannabis exposure (F=4.43, p=.013) and hemisphere (F=15.52, p=.000) were observed for change in cingulum FA across these 2 years (see Figure 10). Bonferroni-corrected post-hoc tests revealed that the 2-year increase in FA was significantly larger for the no/low use group relative to the moderate exposure group ($p_{corrected}$=.01). Cannabis effects on change in cingulum FA remained significant (F=4.52, p=.012) when controlling for alcohol exposure (F=.37, p=.54). The effect of hemisphere reflects a greater increase in FA for the right cingulum. No cannabis group by hemisphere interaction was found.

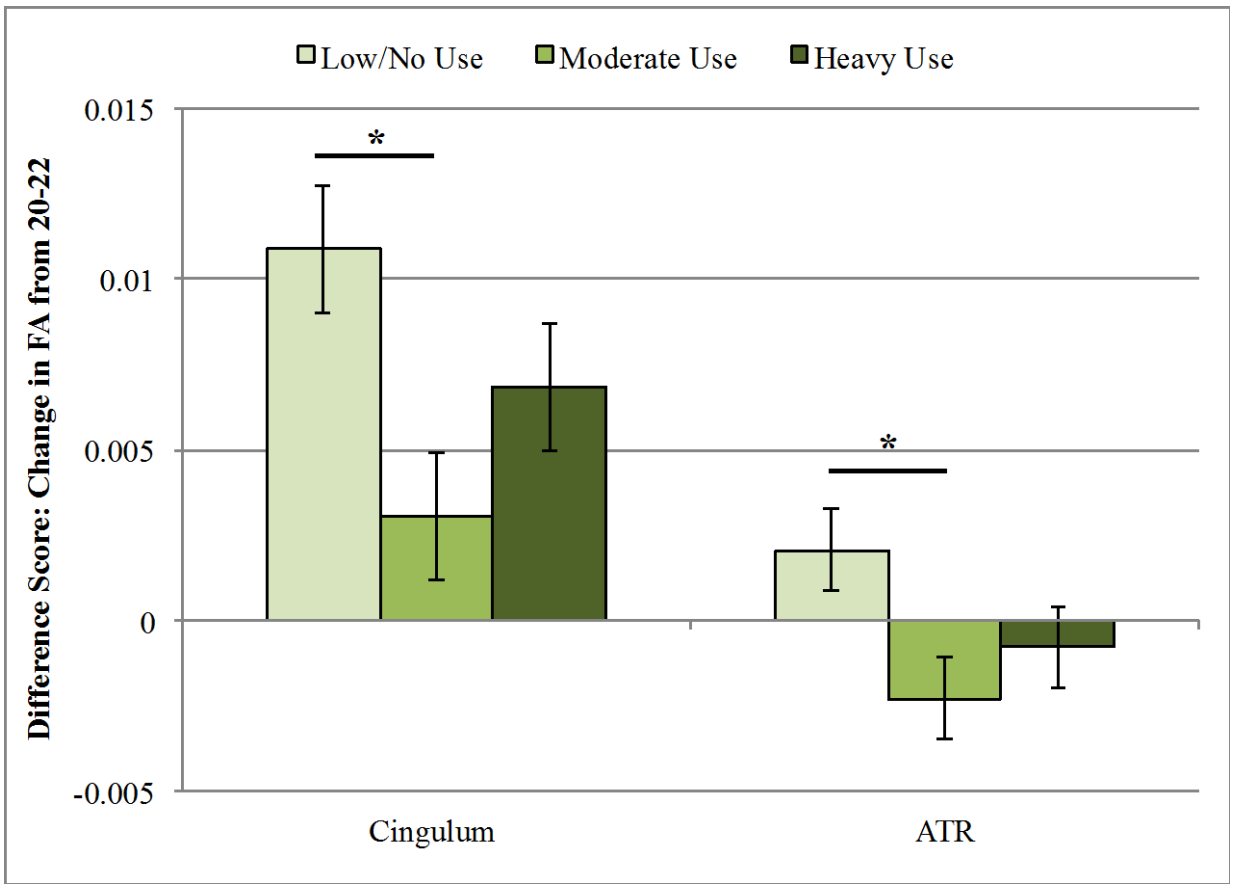

Figure 10. Longitudinal effects of cannabis exposure on FA development from 20 to 22.

Significant effects of cumulative cannabis exposure (F=4.43, p=.013) and hemisphere (F=15.52, p=.000) were observed for change in cingulum FA across these 2 years. The main effect of cumulative cannabis exposure on change in ATR FA was also statistically significant (F=3.48, p=.032), Bonferroni-corrected post-hoc tests revealed that the difference between the no/low use and moderate exposure groups was statistically significant for change in both cingulum and ATR FA ($p_{corrected}$<.05).

3.3.1.2 AD/RD change from age 20 to 22.

No significant effect of cumulative cannabis exposure or hemisphere was observed for change in cingulum AD or RD from age 20 to 22.

3.3.1.3 MD change from age 20 to 22.

No significant effect of cumulative cannabis exposure or hemisphere was observed for change in cingulum MD across the 2-year period.

3.3.2 ATR

3.3.2.1 FA change from age 20 to 22.

The no/low exposure group displayed increased ATR FA from age 20 to 22, whereas both the moderate and heavy exposure groups exhibited a decrease in ATR FA across the 2-year follow-up. The overall ANOVA revealed a significant effect of cumulative cannabis exposure on change in ATR FA (F=3.48, p=.032; see Figure 10). Bonferroni-corrected pairwise post-hoc tests demonstrated that the no/low exposure and moderate exposure groups differed ($p_{corrected}$=.03). This effect only met trend-level significance (F=2.58, p=.08) after controlling for alcohol exposure, although the effect of alcohol exposure on change in ATR FA was not significant (F=.01, p=.92). Change in ATR FA did not differ significantly based on hemisphere.

3.3.2.2 AD/RD change from age 20 to 22.

The pattern of change in ATR RD was consistent with the results for ATR FA: whereas the moderate exposure group displayed the largest *decrease* in ATR FA, they also displayed the largest *increase* in ATR RD from age 20 to 22. ANOVA results demonstrated a significant effect of cumulative cannabis exposure on change in ATR RD (F=3.28, p=.039; see Figure 11). Bonferroni-corrected post-hoc pairwise tests showed that the difference between the no/low exposure and moderate exposure groups was statistically significant ($p_{corrected}$=.037). Change in ATR RD did

not differ significantly based on hemisphere. No significant effect of cumulative cannabis exposure or hemisphere was observed for change in ATR AD from age 20 to 22.

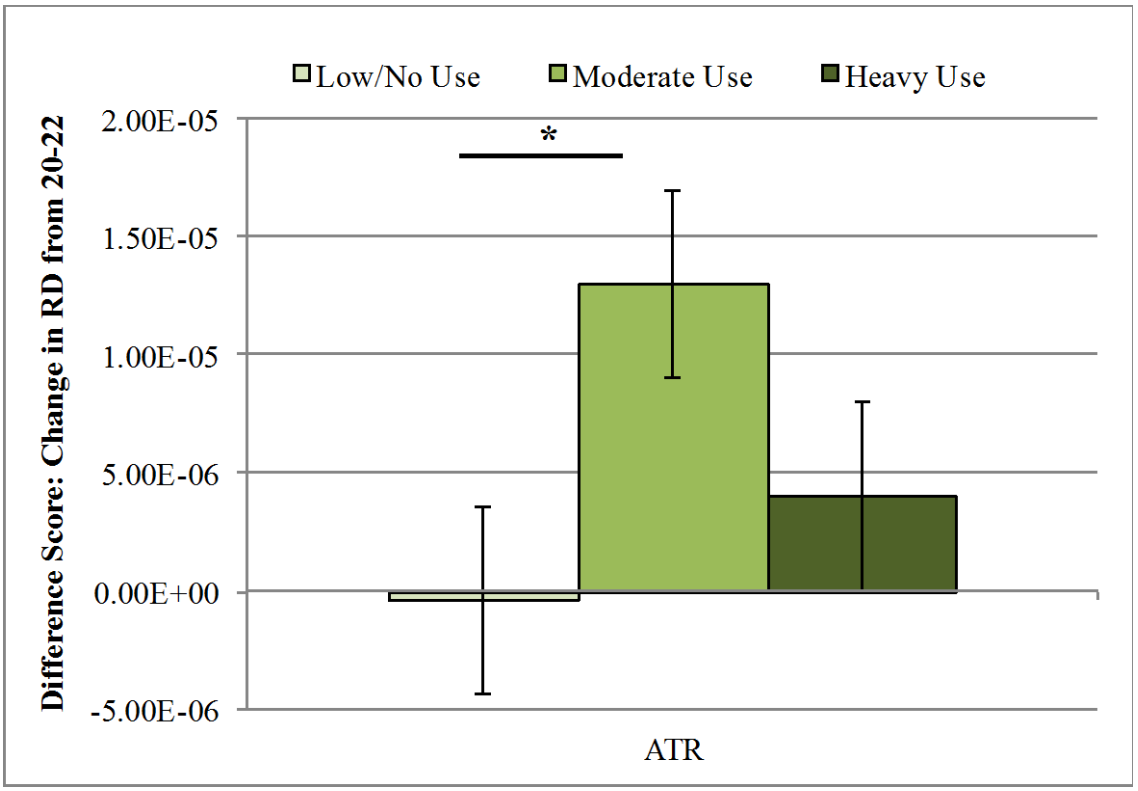

Figure 11. Longitudinal effects of cannabis exposure on ATR RD development from 20 to 22. The effect of cumulative cannabis exposure on ATR RD was statistically significant (F=3.28, p=.039), and Bonferroni-corrected post-hoc pairwise tests showed that the difference between the no/low exposure and moderate exposure groups was statistically significant ($p_{corrected}$=.037).

3.3.2.3 MD change from age 20 to 22.

No significant effect of cumulative cannabis exposure or hemisphere was observed for change in ATR MD across the 2-year period.

3.4 AIM 3. MEDIATION OF CANNABIS EFFECTS ON PSYCHOSOCIAL ATTAINMENT BY ACC CONNECTIVITY

3.4.1 Cannabis effects on psychosocial adjustment

3.4.1.1 Educational attainment.

Cumulative cannabis exposure was a significant predictor of educational attainment at age 22 (F=8.58, p<.001; see Figure 12). Bonferroni-corrected post-hoc pairwise tests demonstrated that heavy cannabis users reported significantly lower educational attainment relative to both the low ($p_{corrected=}$.011) and moderate ($p_{corrected}$<.001) use groups. On average, heavy users reported their highest level of education to be high school/GED, whereas low and moderate cannabis users reported that they had completed some college at age 22. Early childhood SES (F=5.37, p=.006), alcohol (F=21.19, p<.001) and tobacco use (χ^2=28.76, p<.001) also varied significantly as a function of cumulative cannabis exposure. Therefore, the relationship between cumulative cannabis exposure and educational attainment was also assessed controlling for these factors. The relationship between cumulative cannabis exposure and educational attainment remained significant when accounting for SES, alcohol and tobacco use (F=6.79, p=.002), and the pattern of results remained consistent (heavy users reported significantly less educational attainment than both other groups, $ps_{corrected}$<.05).

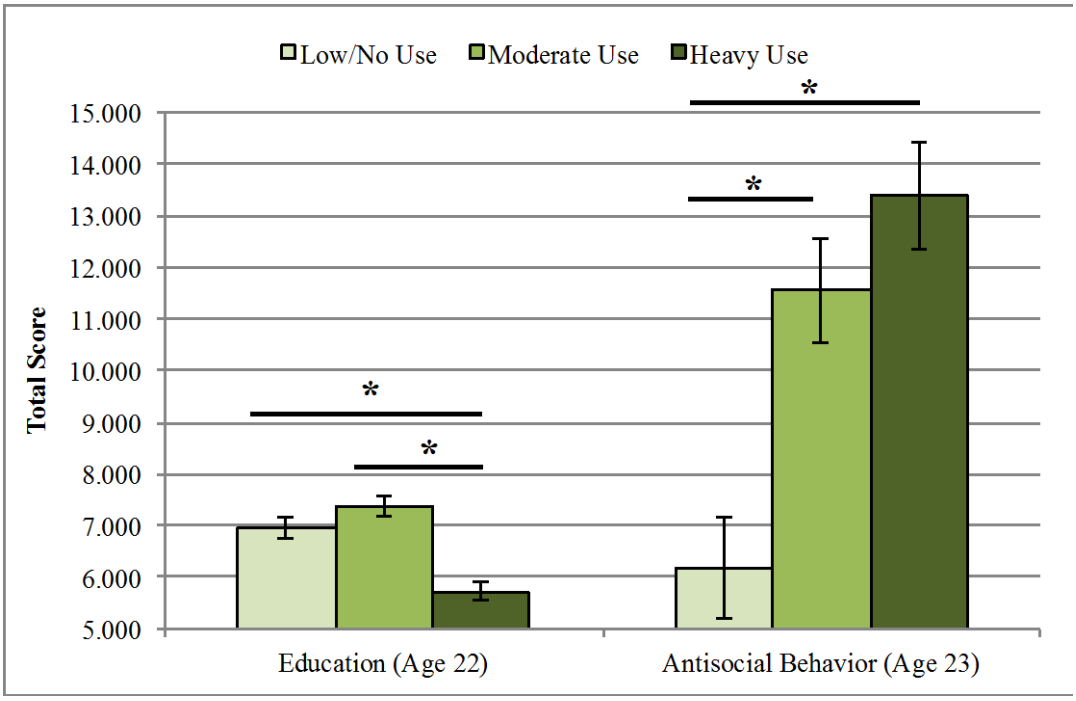

Figure 12. Associations between cannabis use and psychosocial adjustment. Greater cumulative cannabis exposure was associated with lesser educational attainment at age 22 (F=8.58, p=.000) and higher self-reported antisocial behavior at age 23 (F=13.86, p=.000003). On average, heavy users reported their highest level of education to be high school/GED, whereas low and moderate cannabis users reported that they had completed some college at age 22.

3.4.1.2 Occupational attainment.

Cumulative cannabis exposure did not predict whether participants were employed at age 23 (χ^2=3.19, p=.20), nor did it predict their self-reported job satisfaction (F=1.29, p=.28).

3.4.1.3 Interpersonal functioning.

Cumulative cannabis exposure did not predict relationship distress at age 23 (F=1.16, p=.32).

3.4.1.4 Depressive symptoms.

Cumulative cannabis exposure did not predict depressive symptoms at age 22 (F=2.65, p=.07).

3.4.1.5 Externalizing behavior.

Cumulative cannabis exposure predicted participants' self-reported antisocial behavior (F=13.86, p=.000003), such that individuals with higher lifetime cannabis exposure also reported more antisocial behavior at age 23 (see Figure 12). Bonferroni-corrected post-hoc pairwise tests demonstrated that the minimal use group differed significantly from both the moderate ($p_{corrected}$=.001) and heavy using groups ($p_{corrected}$<.001). This effect remained significant when controlling for SES, alcohol, and tobacco use (F=3.91, p=.023). This effect also remained significant after controlling for self-reported antisocial behaviors prior to cannabis use onset (mean total score on Self Report of Delinquency from age 10, 11, and 12; F=18.34, p= 0.0000001), suggesting that cannabis effects on antisocial behaviors in young adulthood are not entirely attributable to premorbid differences or stability in antisocial behavior.

3.4.2 ACC connectivity and psychosocial adjustment

3.4.2.1 Educational attainment.

White matter microstructure of the cingulum and ATR did not predict educational attainment at age 22.

3.4.2.2 Occupational attainment.

White matter microstructure of the cingulum and ATR did not predict employment status or job satisfaction at age 23.

3.4.2.3 Interpersonal functioning.

There was a trend-level association between change in right ATR MD from age 20 to 22 and relationship distress (model F=2.64, p=.056, right ATR MD change β=.-.208, p=.066) at age 23, such that higher MD predicted lower relationship distress at age 23.

3.4.2.4 Depressive symptoms.

White matter microstructure of the cingulum and ATR did not predict depressive symptoms at age 22.

3.4.2.5 Externalizing behavior.

There was a trend-level association between change in right ATR MD from age 20 to 22 and self-reported antisocial behavior (model F=5.26, p=.002, right ATR MD change β=.153, p=.067) at age 23, with higher MD associated with more antisocial behavior.

3.4.3 Mediation of cannabis-outcome associations by white matter microstructure

3.4.3.1 Educational attainment.

No indirect effect of cumulative cannabis exposure through white matter microstructure of the cingulum or ATR was observed for educational attainment at age 22.

3.4.3.2 Occupational attainment.

No indirect effect of cumulative cannabis exposure through white matter microstructure of the cingulum or ATR was observed for employment status or job satisfaction at age 23.

3.4.3.3 Interpersonal functioning.

No indirect effect of cumulative cannabis exposure through white matter microstructure of the cingulum or ATR was observed for relationship distress at age 23.

3.4.3.4 Depressive symptoms.

No indirect effect of cumulative cannabis exposure through white matter microstructure of the cingulum or ATR was observed for depressive symptoms age 22.

3.4.3.5 Externalizing behavior.

A significant indirect effect of cumulative cannabis exposure on antisocial behavior through change in left cingulum MD was observed. Greater cumulative cannabis exposure was linked to an increase in left cingulum MD from age 20 to 22, which in turn predicted higher antisocial behavior (indirect effect = -256.63, $p<.05$; see Figure 13). Based on the temporal sequencing of variables in the mediation model, change in left cingulum MD can be interpreted as a mediator of the relationship between cannabis exposure and antisocial behavior (Kraemer et al., 2008).

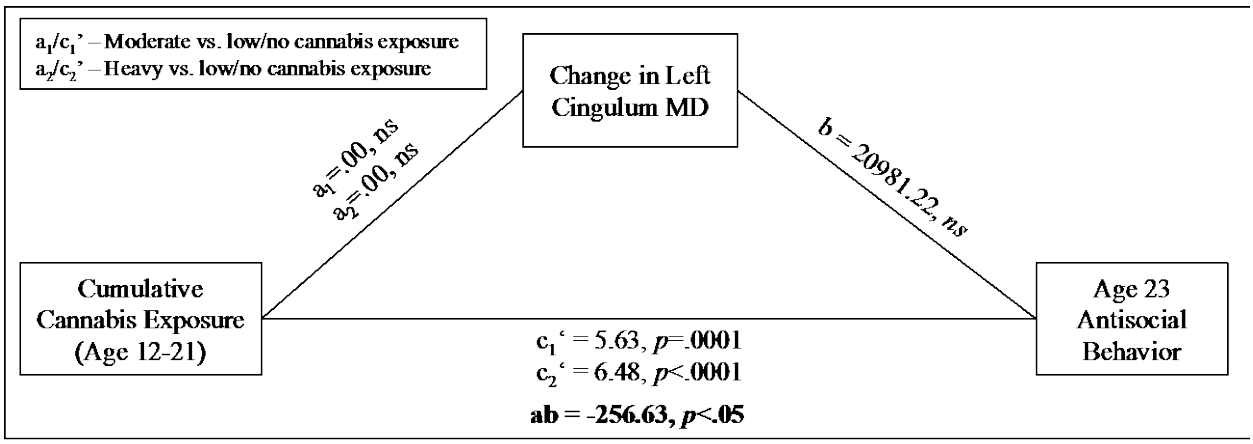

Figure 13. Indirect effect of cumulative cannabis exposure on antisocial behavior in young adulthood through change in left cingulum MD from age 20 to 22. According to Hayes and Rockwood (2016), "by contemporary thinking, tests of significance for the individual paths *a* and *b* are not required to determine whether *M* mediates the effect of *X* on *Y*, contrary to the causal steps logic which requires that both a and b are statistically significant" (p. 5). Therefore, this approach allows for the examination indirect effects, even when individual paths in a mediation model are not significant (Hayes, 2013). The direct effect of cumulative cannabis exposure on antisocial behavior at age 23 was significant, such that individuals in the moderate (coefficient c_1') and heavy (coefficient c_2') exposure groups reported more antisocial behaviors relative to individuals in the no/low exposure group. The indirect effect of cumulative cannabis exposure on age 23 antisocial behavior through change in left cingulum MD from age 20 to 22 (coefficient ab) was also significant.

4.0 DISCUSSION

The current study aimed to examine the effects of cannabis exposure on developing ACC connectivity during the transition to adulthood and to explore the implications of these effects for psychosocial adjustment in young adulthood. Contrary to our expectations, moderate adolescent cannabis use was associated with higher FA and MD of the cingulum and ATR cross-sectionally at age 20. However, the results supported our hypothesis that cannabis exposure alters white matter maturation of the cingulum and ATR longitudinally from age 20 to 22. Our findings also demonstrated that cannabis use in adolescence and the transition to adulthood is associated with worse psychosocial adjustment in young adulthood, including lower educational attainment and more self-reported antisocial behavior. Furthermore, we found preliminary evidence to suggest that white matter microstructure may mediate cannabis effects on later psychosocial functioning.

4.1 CROSS-SECTIONAL CANNABIS EFFECTS ON ACC CONNECTIVITY AT AGE 20

Looking cross-sectionally at the association between adolescent cannabis use and white matter microstructure at age 20, we found an unexpected pattern of results. Contrary to our hypothesis, we found that adolescent cannabis users displayed higher FA in the cingulum and ATR relative to minimal/non-users, even after controlling for alcohol exposure. In both pathways, moderate adolescent cannabis use was associated with the highest FA values, and moderate users displayed significantly higher FA relative to both minimal and heavy adolescent users within the ATR (see

Figure 6). A complementary pattern of ATR AD was also observed, such that moderate adolescent cannabis users were also characterized by the highest ATR AD (see Figure 9).

4.1.1 Concordance with extant cross-sectional literature

Prior cross-sectional investigations of cannabis effects on cingulum and ATR microstructure have yielded mixed results. The current pattern of findings aligns with a small number of previous studies that have reported higher FA of the cingulum (Delisi et al., 2006) and ATR (Bava et al., 2009) among cannabis users relative to controls. However, these findings contradict several studies reporting lower FA of the cingulum or ATR among cannabis users relative to non-users (Gruber et al., 2014; Gruber et al., 2011; Shollenbarger et al., 2015), and lower ATR FA as a predictor of greater late adolescent substance use (Jacobus, Thayer, et al., 2013). Furthermore, several prior studies have failed to detect significant group differences in FA of either pathway based on cannabis use, using both whole brain (Ashtari et al., 2009; Jacobus et al., 2009; Jacobus et al., 2009; Jakabek et al., 2016; Thatcher et al., 2010; Yucel et al., 2010) and region-of-interest (Clark et al., 2012; Gruber & Yurgelun-Todd, 2005; Jakabek et al., 2016) approaches.

4.1.2 Concordance with literature on neural risk factors for substance use

In order to reconcile these discrepant cross-sectional results, it is important to distinguish premorbid neural characteristics associated with risk for substance use from neurobiological consequences of cannabis exposure. Several previous studies have examined differences in white matter microstructure among individuals at high familial risk for substance use, prior to cannabis use onset (Acheson, Wijtenburg, Rowland, Bray, et al., 2014; Herting, Schwartz, Mitchell, &

Nagel, 2010; Squeglia, Jacobus, Brumback, Meloy, & Tapert, 2014). Among these reports, both increased (Squeglia et al., 2014) and decreased (Acheson, Wijtenburg, Rowland, Bray, et al., 2014; Acheson, Wijtenburg, Rowland, Winkler, et al., 2014) FA of the cingulum and ATR has been reported among high-risk relative to low-risk individuals (although others have found no risk group differences (Herting et al., 2010; Hill, Terwilliger, & McDermott, 2013)). These findings suggest that both early-developing and late-developing white matter microstructure may contribute to an increased propensity for substance use disorders, and highlight the need for longitudinal studies to dissociate neural markers of risk from the effects of cannabis exposure on developing ACC connectivity (see **4.4 Risk for Substance Use vs. Effects of Cannabis Exposure** below).

4.1.3 Adolescent cannabis exposure by race interaction

It is interesting to note that there was a race by adolescent cannabis exposure interaction, such that among African American participants there was no significant effect of adolescent cannabis exposure, whereas among Caucasian participants, adolescent cannabis exposure had a significant main effect on cingulum FA at age 20 (see Figure 7). A paucity of research has examined whether white matter microstructure differs by race, although smaller white matter volume has been noted among Asian and African American participants relative to Caucasian individuals (Pfefferbaum et al., 2016). Differences in white matter structure between individuals of different races could be attributable to genetic variation (Vuoksimaa et al., 2017), differential prenatal exposure to environmental pollutants (Peterson et al., 2015), and/or disparate exposure to stress across development (Eluvathingal et al., 2006; Huang, Gundapuneedi, & Rao, 2012).

In particular, racial discrimination - disrespect and poor treatment based on race – is one source of chronic stress that African Americans experience that has been linked to elevated

inflammatory markers, above and beyond the influence of socioeconomic status and other life stressors (Brody, Yu, Miller, & Chen, 2015). Chronic stress has pervasive effects on the endocannabinoid system, including widespread downregulation of CB1 receptor expression (Morena et al., 2015). Therefore, it is possible to speculate that African American participants may be relatively less vulnerable to cannabis effects due to stress-induced alterations in endocannabinoid system functioning. Future research is necessary to examine whether white matter microstructure differs by race, the extent to which this effect is mediated by exposure to chronic stressors including racial discrimination, and to elucidate the extent to which racial disparities may mediate cannabis effects on the developing brain.

4.1.4 Association between adolescent cannabis use and mean diffusivity

Significant effects of adolescent cannabis exposure were also observed for MD of both the cingulum and ATR at age 20. Heavy adolescent cannabis use was associated with significantly lower MD in both pathways relative to moderate use, whereas moderate users did not differ significantly from participants who engaged in minimal/no cannabis use in adolescence (see Figure 8). This pattern could reflect a dose effect on MD, such that it is necessary to exceed a certain amount of exposure to observe significant effects on MD. Alternatively, these results may also reflect premorbid risk for substance use. During typical development, MD decreases with age in both the cingulum and ATR across adolescence and the transition to adulthood (Lichenstein et al., 2016). As mentioned above, there is evidence to suggest that early developing white matter architecture may be a risk factor for later substance use (Squeglia et al., 2014). Therefore, it is also possible that the lower MD observed among heavy adolescent cannabis users could reflect a precocious pattern of white matter development that may contribute to a higher propensity to

engage in substance use (see **4.4 Risk for Substance Use vs. Effects of Cannabis Exposure** below). Previous cross-sectional reports have yielded inconsistent results, with increased (Shollenbarger et al., 2015) and decreased (Delisi et al., 2006) MD reported among cannabis users relative to controls, as well as several studies finding no effect on MD within these tracts (Ashtari et al., 2009; Bava et al., 2009; Gruber et al., 2014; Gruber et al., 2011; Gruber & Yurgelun-Todd; Jacobus et al., 2009). Again, it is difficult to reconcile these disparate results with a cross-sectional design, and longitudinal data is essential to disentangle the overlapping influences of risk and exposure.

4.2 LONGITUDINAL CANNABIS EFFECTS ON DEVELOPING ACC CONNECTIVITY

Consistent with our hypothesis, we observed an increase in FA of the cingulum and ATR from age 20 to 22 among minimal cannabis users, which was reduced or even reversed among cannabis users (see Figure 10). For the cingulum pathway, we found that cannabis users showed a diminished increase in FA relative to minimal users. In the ATR, we found that cannabis users displayed a pattern of decreased FA and increased RD across the 2-year follow-up, whereas increased FA and decreased RD was observed among minimal/non-users (see Figure 11).

4.2.1 Concordance with extant longitudinal literature on the cingulum

To our knowledge, 5 previous longitudinal studies have examined cannabis effects on white matter integrity (Bava et al., 2013; Becker et al., 2015; Epstein & Kumra, 2015; Jacobus, Squeglia, Bava,

et al., 2013; Jacobus, Squeglia, Infante, et al., 2013). Our findings are consistent with Becker et al.'s (2015) recent study, in which they reported less positive change in cingulum FA from age 20 to 22 among cannabis users relative to controls (albeit their results were in the posterior, not anterior, cingulum). However, the majority of previous longitudinal studies have not reported differences in cingulum FA among cannabis users relative to controls (Bava et al., 2013; Epstein & Kumra, 2015; Jacobus, Squeglia, Bava, et al., 2013).

There are several possible reasons that prior studies may not have found longitudinal cannabis effects in the cingulum pathway. First, previous studies had more limited power to detect this effect. The majority of prior longitudinal studies used a whole-brain analysis approach (Bava et al., 2013; Jacobus, Squeglia, Infante, et al., 2013), and all included smaller sample sizes than the current study. Specifically, the largest previous report included 48 participants (Epstein & Kumra, 2015), less than one-third of the current sample. Furthermore, previous reports may have failed to detect this effect because they did not distinguish between different levels of cannabis use. In the current study, the pattern of altered cingulum development was more robust among the moderate cannabis use group relative to the heavy use group (see **4.5 Cannabis Dose Effects on White Matter Microstructure** below). Therefore, this effect may have been attenuated in previous reports by including all cannabis users in a single group regardless of their level of use.

Alternatively, the inconsistency in cingulum findings may be attributable to the developmental time point at which different studies were conducted. According to Simmonds et al. (2014), the cingulum pathway undergoes periods of significant developmental change during early adolescence (age 8-14) and late adolescence (19-21), with no statistically significant growth occurring during mid-adolescence. The majority of prior studies included participants aged 16-18 (Bava et al., 2013; Jacobus, Squeglia, Infante, et al., 2013; Jacobus, Thayer, et al., 2013), and one

study included participants ranging from 10-23 with a mean age of 16.6 (Epstein & Kumra, 2015). It is possible that the cingulum is relatively less vulnerable to cannabis effects during mid-adolescence when growth in this pathway plateaus, and studies targeting late adolescence/emerging adulthood may be better designed to detect cannabis-related changes in this pathway (Becker et al., 2015; current study).

4.2.2 Concordance with extant longitudinal literature on the ATR

The current findings align with previous longitudinal investigations of cannabis effects on white matter development. Of the four previous longitudinal studies that examined cannabis-related changes in white matter microstructure across the whole brain, all reported a decrease or lesser increase in FA within regions of the ATR (Bava et al., 2013; Becker et al., 2015; Jacobus, Squeglia, Bava, et al., 2013; Jacobus, Squeglia, Infante, et al., 2013). The fifth longitudinal study in the literature used a ROI approach, and did not examine cannabis effects on this pathway (Epstein & Kumra, 2015). Collectively, this body of research provides strong evidence that cannabis exposure during adolescence and the transition to adulthood is associated with altered white matter maturation of the ATR.

4.3 PSYCHOSOCIAL IMPLICATIONS OF CANNABIS USE

4.3.1 Cannabis use predicts lower educational attainment

Higher cannabis use across adolescence and the transition to adulthood predicted lower educational attainment at age 22. In fact, individuals in the heavy use group reported their highest level of education to be high school/GED, whereas minimal/non-users and moderate cannabis users advanced to complete some post-secondary education by age 22. This effect may have may have a significant influence on participants' long-term trajectories of achievement into adulthood. In fact, it has been reported that individuals with a college degree earn twice as much as high school graduates (Haveman & Smeeding, 2006), suggesting that the observed cannabis effect on educational attainment could have a meaningful impact on participants' earning potential and future socioeconomic status.

However, the mechanisms underlying this association remain to be fully understood. It is possible that certain characteristics may predispose individuals to both cannabis use and poor educational achievement. For example, lower cognitive or attentional capabilities may increase the likelihood of both outcomes. Indeed, childhood attention-deficit/hyperactivity disorder (ADHD) has been associated with a higher likelihood of using and abusing cannabis (Lee, Humphreys, Flory, Liu, & Glass, 2011), as well as lower academic achievement (Fleming et al., 2017). Additionally, cannabis use may lead to academic difficulties due to cannabis effects on cognitive functioning. Indeed, persistent adolescent cannabis use has been found to prospectively predict decreased neuropsychological performance across a range of domains (Meier et al., 2012). Therefore, there may also be a causal relationship between cannabis use and academic underachievement via impaired cognitive functioning.

4.3.2 White matter microstructure mediates cannabis effects on later antisocial behavior

Cannabis use was also associated with greater self-reported antisocial behavior at age 23, which is linked to criminality and substance use disorders in adulthood (Shaw, 2013). Interestingly, this association remained significant when accounting for antisocial behavior prior to the onset of cannabis use. This pattern of results supports the possibility that cannabis exposure may directly contribute to increased antisocial behavior over time, although the specific mechanisms whereby cannabis exposure may lead to increased antisocial behavior remain to be fully elucidated. Furthermore, we also observed a significant indirect effect of cannabis exposure on antisocial behavior through change in cingulum MD from age 20 to 22, suggesting that changes in cingulum microstructure may partially account for cannabis effects on antisocial behavior.

These results are consistent with previous studies reporting altered white matter microstructure of the cingulum in association with antisocial behavior (Waller, Dotterer, Murray, Maxwell, & Hyde, 2017). Furthermore, the cingulum pathway has been strongly implicated in connections among regions of the default mode network (DMN), a canonical resting-state network including the medial prefrontal cortex, posterior cingulate cortex, lateral parietal cortex and ACC (Greicius, Krasnow, Reiss, & Menon, 2003), which has been implicated in an array of functions related to self-referential, autobiographical mental activity (Fair et al., 2008). Aberrant DMN connectivity has been is hypothesized to contribute to violent behavior and emotional detachment among antisocial and psychopathic individuals by impairing introspective social, moral, and affective processing (Waller et al., 2017). Therefore, cannabis exposure effects on white matter microstructure of the cingulum my contribute to later antisocial behavior via alterations in DMN functioning. However, additional research is needed to replicate the indirect effect of cannabis

exposure on antisocial behavior through change in cingulum MD, and to elucidate the mechanisms of this effect.

4.4 RISK FOR SUBSTANCE USE VS. EFFECTS OF CANNABIS EXPOSURE

Taken together, the results of our cross-sectional and longitudinal analyses highlight the need to distinguish premorbid neural characteristics associated with risk for substance use from the neurobiological effects of cannabis exposure. A variety of different patterns of aberrant white matter development may contribute to risk for psychiatric problems (Di Martino et al., 2014). As described above, both delayed (Acheson, Wijtenburg, Rowland, Bray, et al., 2014; Acheson, Wijtenburg, Rowland, Winkler, et al., 2014) and accelerated (Squeglia et al., 2014) patterns of white matter development have been identified among individuals at high risk for substance use. Of particular relevance to the current sample, Belsky's evolutionary theory of socialization proposes that familial psychosocial stress leads individuals to mature more rapidly and reproduce earlier to improve their reproductive fitness in the context of an insecure environment (Hochberg & Belsky, 2013). Congruently, an emerging body of animal and human literature has suggested that early adversity may accelerate the development of cortical-subcortical connectivity (McPherson et al., 2013). Given that participants in the current study were recruited based on low socioeconomic status, this sample is characterized by high rates of neighborhood impoverishment, low income, and maternal depression, among other sources of childhood adversity (Shaw, Sitnick, Reuben, Dishion, & Wilson, 2016), which could potentially lead to a compensatory acceleration in white matter development. In turn, precocious white matter development may contribute to earlier autonomy, exploration, and socialization, which may be alternatively advantageous or risky

depending upon individuals' environments (Squeglia et al., 2014). Indeed, prior research has reported that higher FA in the ATR is linked to more self-reported risky behaviors (Berns, Moore, & Capra, 2009) as well as greater behavioral measures of risky behavior (Kwon, Vorobyev, Moe, Parkkola, & Hamalainen, 2014) among adolescents.

Collectively, our findings of higher FA among cannabis users at age 20 coupled with decreased FA across 2 years of cannabis exposure suggest a pattern of white matter development in which a subset of participants are characterized by higher FA prior the onset of cannabis use (potentially due in part to early adversity) that may increase their liability to experiment with drugs, followed by a deceleration of white matter maturation with extended cannabis use. This pattern of results led us to modify our hypothesized model of cannabis effects on developing ACC connectivity (Figure 4) to reflect potential premorbid characteristics of risk for cannabis use in addition to exposure effects on white matter microstructure (see Figure 14 for revised model).

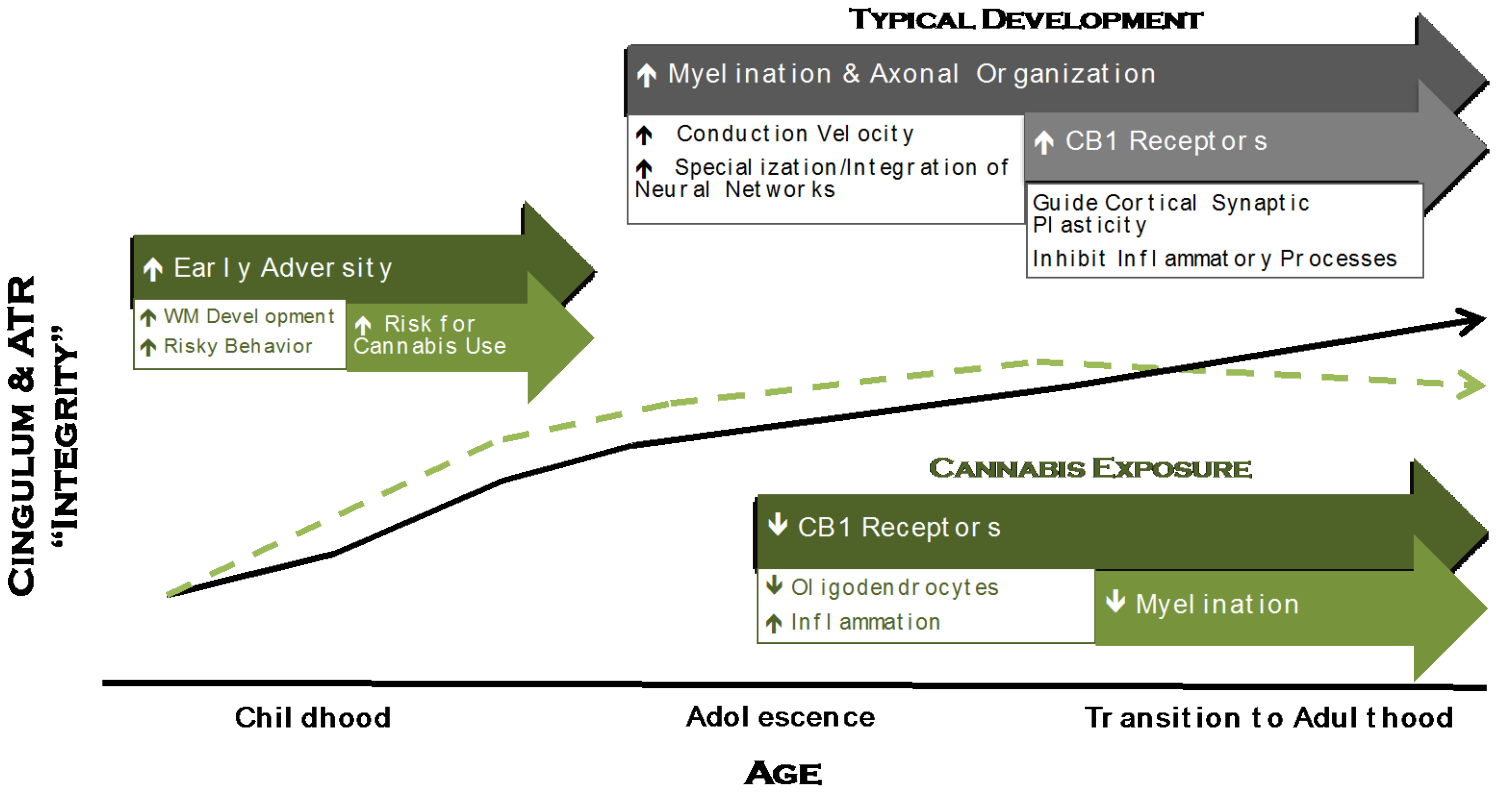

Figure 14. Revised theoretical model of developing ACC connectivity among individuals with and without cannabis exposure. In typical development (black line), increased myelination and axonal organization, as well as increased CB1 receptor expression are thought to give rise to increased white matter integrity across adolescence and into adulthood. Exposure to adversity early in development may lead to a compensatory acceleration of white matter development, which may increase risky behavior and risk for cannabis use among a subset of individuals (green dashed line). Conversely, cannabis exposure is associated with altered white matter maturation of the cingulum and ATR during the transition to adulthood, an effect that may be mediated by a downregulation of CB1 receptor expression and/or direct effects on oligodendrocyte survival and myelination.

Although speculative, the current model provides promising avenues for future research to disentangle neural risk factors from cannabis effects on the developing brain. Specifically, future research is necessary to confirm whether early adversity accelerates white matter development of the cingulum and ATR, and to determine what type(s) of stressors are most likely to have this effect at what developmental time points. Furthermore, studies will need to demonstrate the mechanisms whereby higher FA may lead to increased substance use behavior, as well as to distinguish how precocious and delayed trajectories of white matter development may each contribute to risk for substance use problems by acquiring prospective neuroimaging data at multiple time points before and after cannabis use onset.

4.5 CANNABIS DOSE EFFECTS ON WHITE MATTER MICROSTRUCTURE

It is very interesting to note that cannabis effects on white matter microstructure varied substantially between the moderate and heavy cannabis use groups. At age 20, adolescents reporting moderate adolescent cannabis exposure displayed the highest FA in both the cingulum and ATR and moderate cumulative cannabis exposure was associated with more substantial alterations in white matter development from age 20 to 22, relative to heavy cumulative exposure. To our knowledge, no previous studies have compared white matter development between cannabis users with different levels of use. However, to speculate about what may be driving this pattern, it is possible that the heavy users are further along the cannabis exposure trajectory of white matter development illustrated in Figure 14. In the current sample, individuals in the heavy cannabis use group initiated use at a mean age of 14.68 (*SD*=1.8) whereas the moderate cannabis

use group began using at a mean age of 16.12 (*SD*=2.1). Therefore, participants in the heavy use group have greater exposure to cannabis over a longer time course.

The time course of cannabis effects on the brain remains poorly understood, although data on CB1 receptor changes with cannabis use are informative. Chronic cannabis use has been shown to lead to a downregulation of CB1 receptors (Hirvonen et al., 2012). However, this finding was based on a comparison between control subjects and daily cannabis smokers who had been using for a mean of 12 years (Hirvonen et al., 2012). Therefore, it is not clear whether the downregulation in CB1 receptor expression occurs quickly and is then sustained by continued use, or whether this effect occurs gradually over the course of 12 years of chronic exposure. Nonetheless, follow-up data demonstrated that receptor levels normalized after ~4 weeks of abstinence (Hirvonen et al., 2012). Therefore, it is likely that the observed downregulation in receptor expression takes place on a timescale of weeks to months, rather than gradually over the course of years. Therefore, there may be a plateauing of cannabis effects with protracted use, which could be reflected in the current pattern of results, such that cannabis effects on change in white matter microstructure from 20 to 22 may have been more robust among moderate cannabis users relative to heavy cannabis users because the heavy users are at a later point on the trajectory (see Figure 14), when cannabis effects have begun to plateau. Future studies should examine differential effects of different levels of cannabis use, as well as longitudinal effects of prolonged use, in order to better characterize the nature and timing of cannabis exposure effects on white matter microstructure.

4.6 DEVELOPMENTAL PERSPECTIVE

The pattern of results observed in the current study highlights the need for longitudinal, developmentally-informed data to adequately characterize cannabis effects on white matter microstructure. It is essential to obtain neuroimaging data before and after cannabis use onset, as well as rich information about family history and early adversity to distinguish neural characteristics of risk from consequences of cannabis exposure. Furthermore, it is critical to assess neural structure and function at multiple time points to capture the time course of cannabis effects on the developing brain. Ongoing initiatives, such as the NIH-funded Adolescent Brain Cognitive Development (ABCD) study, which plans to collect neuroimaging data on 10,000 adolescents over the course of a decade beginning at age 9-10, will be instrumental in furthering our understanding of cannabis effects on the adolescent brain from a developmental perspective. The ABCD study will recruit a representative sample of US teens that will be adequately powered to compare the effects of different trajectories of cannabis use on the brain. However, the current results also highlight late adolescence and the transition to adulthood as an important period for cannabis effects on white matter maturation. Therefore, similar initiatives are also necessary to examine cannabis effects on the brain during late adolescence and into adulthood.

4.7 BRAIN-BEHAVIOR ASSOCIATIONS

It is important to acknowledge that we did not see any significant direct effects of cingulum or ATR microstructure on our psychosocial outcome measures. These results are surprising in light of literature linking FA of these tracts to cognitive performance (Niogi et al., 2010; Peters et al.,

2014; Seghete et al., 2013; Tamnes et al., 2010), inhibitory control (Treit et al., 2014), and social functioning (De Pisapia et al., 2014; Parkinson & Wheatley, 2014), each of which would be expected to impact individuals' academic, occupational, and interpersonal success in young adulthood. Nonetheless, the outcomes examined in the current analyses are more distal than those described in the literature. For example, a myriad of environmental factors can influence the likelihood that an individual will pursue higher education, in addition to that person's individual characteristics and capabilities. An individual's financial resources, the quality of their primary and secondary education, competing familial demands, and the social norms of their family and community can all influence whether they pursue higher education.

Although it is important to assess the real-world implications of aberrant ACC connectivity for psychosocial adjustment in young adulthood, our focus on these broader, more distal outcomes may have obscured our ability to detect significant brain-behavior associations, particularly given the social context of the current sample. Future studies should include both proximal (i.e. measures of cognitive functioning, impulsivity, and/or social competence) and distal outcome measures (i.e. educational/occupational attainment) to identify the behavioral impliations of different patterns of development of ACC connectivity. Additionally, the low socioeconomic status of the current study sample may have made the relationship between cingulum and ATR microstructure and academic and occupational attainment more difficult to detect, as these individuals have many external hindrances to their achievement, potentially making the impact of individual differences less readily apparent.

4.8 IMPLICATIONS

4.8.1 Implications for brain function

Developmental changes in structural connectivity of the ACC are presumed to lay the foundation for maturation of ACC functional connectivity and ultimately impact cognitive, affective and social functions of the ACC. Congruently, functional connectivity of the ACC has been shown to evolve across development, with functional connections associated with higher-level social-cognitive and emotional functions characterized by a more protracted developmental course than connections related to motor and attentional control (Kelly et al., 2009). These data support the notion that age-related changes in functional ACC connectivity may underlie the maturation of key higher-level capabilities across adolescence.

In particular, the structural integrity of the cingulum pathway has been linked to functional connectivity of the DMN, whereas the ATR has been linked to frontothalamic functional connectivity (Fair et al., 2010). Therefore, the current findings of altered maturation of structural ACC connectivity among cannabis users may have downstream effects on functional ACC connectivity. Indeed, reduced resting state connectivity of the DMN has been identified among individuals with cannabis use disorders (Wetherill et al., 2015) as well as between the caudal ACC and the superior frontal gyrus (Camchong, Lim, & Kumra, 2017). Future research is necessary to determine whether altered development of cingulum and/or ATR microstructure is directly linked to altered functional ACC connectivity, as well as to explore the implications of these effects for behavior, mental health, and long term achievement.

4.8.2 Implications for prevention and treatment

The current results demonstrate that cannabis use during late adolescence and the transition to adulthood can significantly impact the course of developing ACC connectivity, which has important implications for mental health and achievement. These findings suggest an urgent need to inform public perceptions about the risks associated with cannabis use, as well as to improve treatment utilization among individuals with cannabis use problems. The perceived risks associated with cannabis use have steeply declined since the mid-2000s (Johnston, O'Malley, Miech, Bachman, & Schulenberg, 2017), and lower risk perception prospectively predicts increased use (Grevenstein, Nagy, & Kroeninger-Jungaberle, 2015). Therefore, the current finding support the need for public health campaigns and prevention programs to provide accurate information on the risks associated with cannabis use. There is initial evidence to suggest that school-based prevention and intervention programs may be effective in reducing rates of cannabis use (Das, Salam, Arshad, Finkelstein, & Bhutta, 2016; Lize et al., 2016). However, effect sizes for these programs are small (Lize et al., 2016; Das et al., 2016), and future research is necessary to improve their efficacy and to better characterize the etiological mechanisms supporting the development and maintenance of cannabis use in order to direct prevention and intervention efforts towards individuals at highest risk.

Additionally, 19% of current cannabis users between the ages of 18-25 meet criteria for CUD, yet only 7.8% of adults with CUD seek cannabis-related treatment (We et al., 2017). Although many individuals with CUD may eventually remit without treatment (Feingold, Fox, Rehnm & Lev-Ran, 2015) the current findings demonstrate that use during important developmental periods, such as the transition to adulthood, can have enduring effects on their brain development and mental health that may influence their long term trajectory of psychosocial

adjustment even in the absence on ongoing cannabis use. Improved awareness about the potential consequences of use may facilitate earlier intervention and higher rates of cannabis use treatment for individuals with CUD to mitigate the potential harms associated with long-term use and foster positive long-term trajectories of psychosocial adjustment for affected individuals.

4.8.3 Implications for cannabis policy

These results are also relevant for ongoing decisions about cannabis policy. Twenty-eight states and the District of Columbia have now legalized the use of cannabis for medicinal purposes, 9 states have legalized recreational cannabis use among individuals above age 21 (McGinty, et al., 2017), and 31 states are considering bills pertaining to cannabis legislation in 2017 (Rough, 2017). Legislation legalizing the medicinal use of cannabis has approved its use for a wide array of conditions, ranging from Alzheimer's Disease to hepatitis C, epilepsy, traumatic brain injury, HIV/AIDS, and glaucoma, among others (Hill, 2015). However, there is only strong evidence to support the therapeutic efficacy of cannabis for a minority of approved conditions, including nausea and vomiting, appetite stimulation, chronic pain, neuropathic pain, and spasticity associated with multiple sclerosis (Hill, 2015). Nonetheless, legalization may increase use among adolescents and emerging adults with and without approved conditions by increasing its availability, encouraging social norms that favor cannabis use, and reinforcing beliefs that cannabis is not harmful (Paschall, Grube, & Biglan, 2017). Indeed, there is evidence to suggest that the perception of risk is lower in states that have legalized cannabis use for medicinal and/or recreational use, although the direction of this association remains unclear (Dirisu, Shickle, & Elsey, 2016; Paschall et al., 2017; Schuermeyer et al., 2014).

Very few previous studies have specifically examined the effects of cannabis use during emerging adulthood, the time point at which new laws permit the use of cannabis for medicinal and/or recreational use. The current results align with recent findings by Becker et al. (2015) to suggest that use during this time can have deleterious effects on developing white matter architecture, and that these effects may have important implications for individuals' long-term trajectories of mental health and achievement. Furthermore, we find that the effects of cannabis exposure are not limited to individuals engaging in frequent, heavy use, but rather impact both moderate and heavy cannabis users, suggesting the need for caution in enacting policy changes to make cannabis readily available to emerging adults.

4.9 STRENGTHS OF THE CURRENT STUDY

This is the largest study to date to examine cannabis effects on developing ACC connectivity, and the PMCP's prospective, longitudinal design allowed us to leverage rich data on participants' childhood environments, their patterns of cannabis use across development, and their later psychosocial adjustment. Additionally, the current report adds to a nascent literature using longitudinal data to track cannabis effect over time, which helps to reconcile a disparate body of cross-sectional findings by beginning to tease apart premorbid risk factors from direct effects of cannabis exposure across time. Furthermore, this is the first study to our knowledge to differentiate between different levels of cannabis use (i.e. moderate vs. heavy use), highlighting important questions about the time course of cannabis effects on the brain.

The developmental time point at which these participants underwent DTI scanning is also particularly well-suited to examine cannabis effects on the cingulum and ATR. Rates of cannabis use peak between 18-25 (SAMHSA, 2014) when development in both the cingulum and ATR is still ongoing (Lichenstein et al., 2016). For the cingulum in particular, Simmonds et al. (Simmonds et al., 2014) reported periods of significant developmental change during early adolescence (age 8-14) and late adolescence (19-21), with no significant growth occurring during mid-adolescence. Therefore, the timing of DTI scanning in the current study is well-aligned with a period in which the cingulum would be most vulnerable to cannabis effects, a factor that may explain why earlier reports with mid-adolescents failed to detect cannabis effects in this pathway.

Additionally, the current study design also allowed us to control for cumulative alcohol exposure across development and to demonstrate that cannabis effects on developing ACC connectivity remained significant after controlling for alcohol use. Many prior studies of cannabis effects on white matter microstructure have not controlled for alcohol exposure, although all studies included participants with both cannabis and alcohol use histories (Bava et al., 2013; Jacobus, Squeglia, Infante, et al., 2013; Jacobus, Squeglia, Bava, et al., 2013; Epstein & Kumra, 2015; Bava et al., 2009; Jacobus et al., 2009; Yucel et al., 2010; Thatcher et al., 2010; DeLisi et al., 2006). However, a recent report found that cannabis effects on brain morphology were attenuated when alcohol exposure was carefully controlled (Weiland et al., 2015), emphasizing the importance of accounting for alcohol use in studies of cannabis effects on the brain.

4.10 LIMITATIONS

Although the current study has many strengths including prospective, longitudinal data on adolescent/emerging adult cannabis use, white matter microstructure and psychosocial outcomes in a large sample of high-risk young men, there are also several limitations to consider.

4.10.1 Sample composition

The Pitt Mother & Child Project was designed to include low-income male participants at high risk for externalizing disorders and other psychopathology in order to collect prospective longitudinal data on factors that impact risk and resilience to these disorders. The characteristics of this sample are advantageous for studying cannabis effects on the brain and psychosocial adjustment, as men with low socioeconomic status are at particularly high risk for both cannabis use and poor educational attainment (Martin et al., 2015). Furthermore, among young men from low-SES backgrounds, educational attainment may be a particularly salient marker of later status, including upward social mobility (Forrest et al., 2011). Therefore, the identification of risk factors for poor academic attainment may be particularly important in this population. Nonetheless, restricting the study sample also places some limitations on the interpretation and application of the present results.

4.10.1.1 Gender.

Because the sample is limited to male participants, our results may not be generalizable to women. Neuromaturation follows sexually dimorphic developmental trajectories, with steeper trajectories and greater overall white matter volume observed for males (De Bellis et al., 2001; Giedd et al.,

1999; Herting, Maxwell, Irvine, & Nagel, 2012; Lenroot et al., 2007; Schmithorst, Holland, & Dardzinski, 2008). Additionally, gender differences in endocannabinoid system functioning have also been reported, including differences in CB1 receptor efficiency, THC metabolism, and the extent of down-regulation and desensitization of CB1 receptors following chronic THC treatment in rodents (Rubino & Parolaro, 2015). Therefore, there is reason to suspect that cannabis affects men and women differently (Ketcherside, Baine, & Filbey, 2016) and future research will be necessary to address the effects of cannabis use during the transition to adulthood on developing ACC connectivity and subsequent academic, occupational, and mental health outcomes in female samples.

4.10.1.2 High-risk status.

It is also possible that our findings may not be generalizable to samples of higher socioeconomic status or participants in suburban or rural communities. There is evidence to suggest that both high- and low-SES increase risk for cannabis use in adolescence (Humensky, 2010; Patrick, Wightman, Schoeni, & Schulenberg, 2012). Cannabis use also predicts poorer academic achievement among upper middle class individuals (Meier, Hill, Small, & Luthar, 2015), but it remains to be determined whether common or distinct mechanisms account for this association in populations with differing socioeconomic status. Future research will be necessary to test these relationships in affluent, suburban and rural samples.

On the other hand, although the current sample is comprised of men from low-SES families, many of the highest risk participants from the total PMCP sample were excluded from the MRI portion of the study due to MRI contraindications. Specifically, 50 PMCP participants were excluded because of prior head injury or concussion, metal fragments or bullets in their body, or current incarceration. Therefore, within the current low-income sample, we may have been

unable to include some participants at the highest risk for both cannabis use and poor educational, occupational, and mental health outcomes.

4.10.2 Limitations of self-report measurements

The current study relies heavily on self-report measurements, including retrospective self-reports of cannabis use. This raises the concern that subjects may not accurately remember their frequency of prior cannabis use, particularly in light of cognitive impairments associated with recent use (Medina et al., 2007). Participants may also underreport their substance use in order to provide more socially desirable responses (Martin et al., 2015). Therefore, future studies should incorporate concurrent estimates of cannabis use from multiple sources.

Additionally, although subjects reported their frequency of cannabis use, it is difficult to determine the quantity of cannabis participants ingested. There are several routes of cannabis administration (Volkow et al., 2014), and the amount consumed on each occasion is highly variable and difficult to measure. Furthermore, cannabis contains more than 60 pharmacologically active cannabinoids (Hill, 2015) and THC content varies widely between different sources (Batalla et al., 2013). Furthermore, cannabidiol, the major nonpsychoactive component of cannabis, has been suggested to counteract some adverse effects of THC (Mandelbaum & de la Monte, 2017), suggesting that the cannabinoid composition of different cannabis strains may meaningfully influence their effects on the brain. Therefore, without measuring the cannabinoid content or dosage of the cannabis ingested by our participants, we are limited in the precision with which we can quantify their actual THC exposure.

4.10.3 DTI limitations

Metrics derived from the tensor model are highly susceptible to distortion from complex fiber geometry (Concha, 2014), and crossing, "kissing", or fanning fibers can all inaccurately diminish measures of anisotropy in affected tensors (Concha, 2014). This poses a significant limitation because as many as 90% of voxels in the human brain contain multiple fiber populations (Tournier, Mori, & Leemans, 2011). Nonetheless, TBSS and region-of-interest approaches that rely on mean diffusivity measures within the core regions of known white matter pathways may be *relatively* less affected by this limitation.

4.11 FUTURE DIRECTIONS

In order to distinguish premorbid neural characteristics that contribute to risk for cannabis use from neurobiological effects of cannabis exposure, future studies will need to acquire neuroimaging data at multiple time points before and after cannabis use onset. This approach could help to identify whether both precocious and delayed trajectories of white matter development may increase risk for cannabis use as well as to characterize what distinguishes individuals on different trajectories. Additionally, future research is needed to follow cannabis users longitudinally after they begin using in order to elucidate the time course of cannabis effects on white matter microstructure. By obtaining neuroimaging data at multiple time points following cannabis use initiation, it will also be possible to examine whether cannabis users' ACC connectivity remains persistently impaired or whether they eventually catch up to non-users later in development. Similarly, this will enable researchers to examine the effects of different patterns of use over time,

and to observe how persistent vs. desisting use may impact the brain, and to determine whether alterations in white matter microstructure may normalize with abstinence.

Additionally, future studies should apply multiple neuroimaging modalities to better characterize the neurobiological mechanisms of cannabis effects on white matter microstructure, as well as the functional implications of these effects. For example, future studies can combine diffusion imaging with position emission tomography (PET) to assess whether changes in CB1 receptor density mediate cannabis effects on white matter microstructure. Furthermore, research combining diffusion imaging and fMRI can elucidate how changes in structural connectivity may impact functional connectivity and patterns of task-evoked neural response.

Furthermore, it is also important to design studies to identify behavioral correlates of changes in white matter microstructure of the cingulum and ATR. Studies that include more proximal characteristics (i.e. emotion regulation, cognitive functioning, decision making), in addition to more distal outcomes (i.e. academic and occupational attainment) can provide insight into the implications of disrupted ACC connectivity, and elucidate mechanisms whereby atypical maturation of ACC connectivity may negatively impact individuals' long term functioning and achievement.

4.12 CONCLUSIONS

Cannabis use is common during adolescence and the transition to adulthood. Although often considered benign among the general public, cannabis use has been associated with a wide array of negative outcomes that can have profound impacts on individuals' long-term trajectory of achievement, health, and well-being. However, the neurobiological mechanisms that underlie the

deleterious effects of cannabis exposure, especially at vulnerable developmental periods and in high-risk populations, remain poorly understood. The current study used longitudinal DTI data to demonstrate that cannabis use is associated with altered white matter maturation of the cingulum and ATR from age 20 to 22. Furthermore, we found preliminary evidence that microstructural changes in the cingulum may mediate the association between cannabis use and antisocial behavior at age 23. These results have important implications for understanding cannabis effects on brain structure and function, informing public perceptions about the risks of cannabis use, directing clinical care for individuals with cannabis use problems, and guiding ongoing cannabis policy decisions. Elucidating the neural basis of cannabis effects can facilitate the development of targeted prevention and intervention strategies to foster positive developmental trajectories among individuals at highest risk for cannabis use and poor psychosocial adjustment.

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