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I, Timothy McQueeny, hereby submit this original work as part of the requirements for the degree of:

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Amygdala Morphometry in Adolescent Marijuana Users

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Amygdala Morphometry in Adolescent Marijuana Users

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by

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Abstract

Adolescent developments in limbic structures and the endogenous cannabinoid system suggest that teenagers may be more vulnerable to the negative consequences of marijuana (MJ) use. This study examined the relationships between amygdala volume and mood symptoms in teenaged chronic MJ users. Participants were 35 MJ users and 47 controls aged 16-19 years. Extensive exclusions included psychiatric or neurologic disorders, including mood and anxiety disorders. Substance use, mood (anxiety/depression) symptoms and brain scans were collected after 28 days of abstinence. Reliable raters (ICCs>.85) manually traced amygdalar and intracranial (ICV) volumes on high-resolution MRIs. Female MJ users had larger right amygdala volumes and more mood symptoms than all males and female controls, after covarying ICV, alcohol, nicotine and other substance use. For female controls and males, worse mood was linked to smaller right amygdala volume, whereas increasing mood problems was associated with bigger right amygdalas in female MJ users. Gender interactions may reflect MJ-related interruptions to sex-specific neuromaturation. Subtle neurodevelopmental amygdala abnormalities may underlie unique vulnerabilities to sub-diagnostic depression and anxiety in teenage female MJ users. Longitudinal studies are needed to determine the extent to which MJ impacts regional brain changes and the emergence of internalizing psychopathology.
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Chapter I
Introduction

Statement of the Problem

The latest report from the national Monitoring the Future study found that more than 20% percent of 12th graders report marijuana (MJ) use in the past month (Johnston, O’Malley, Bachman, & Schulenberg, 2009). According to these recent findings, the prevalence in MJ use has remained steady over the last few years with a trend for a slight increase in rate of use over time. Negative outcomes associated with adolescent MJ use include psychosocial problems, psychopathology, increased polysubstance use, antisocial behaviors and, most consistently, lowered educational attainment (Kloos, Weller, Chan, & Weller, 2009; Macleod, et al., 2004). While not all of these findings have been replicated (Macleod, et al., 2004), MJ use reliably predicts poorer academic performance among teenage users. Although teenage MJ use could have major implications for ongoing educational, occupational and social attainment, the impact of MJ on brain functioning and neuromaturation remain unclear.

Adolescent Brain Development

Important neuromaturational processes occur during adolescent years and continue into young adulthood. Gray matter volumes peak in early adolescence and then decrease throughout teen years (Giedd, et al., 1999; Sowell, Trauner, Gamst, & Jernigan, 2002). During this period, declining cerebral gray matter volumes coincide with cortical thinning (Gogtay, et al., 2004; Sowell, et al., 2004), which represents an ongoing process of synaptic pruning during adolescence. White matter volume and integrity increases throughout and beyond teen years (Eluvathingal, Hasan, Kramer, Fletcher, & Ewing-Cobbs, 2007; Giedd, et al., 1999; Giorgio, et al., 2008; Schmithorst, Holland, & Dardzinski, 2008; Sowell, et al., 2002). These white matter developments also coincide with gray matter reduction (e.g., Giorgio et al., 2008; Sowell et al.,
suggesting that adolescent brain changes involve the pruning of excessive connections and enhancement of remaining networks.

Demonstrating changes in cortical thickness throughout adolescence and in emerging adulthood, the prefrontal cortex and other higher-order association areas appear to mature later than somatosensory cortices and phylogenetically older regions, like the limbic system (Giedd, 2004; Gogtay, et al., 2004). Whereas cortical gray matter volumes decline, limbic areas (e.g., hippocampus, amygdala) increase in size through adolescence (Giedd, Vaituzis, et al., 1996), which is likely explained by relatively high plasticity observed in these regions across the lifespan (Gould, 2007; Leuner & Gould, 2010; Shapiro, Ng, Zhou, & Ribak, 2009).

Given the dynamic developmental patterns of the limbic forebrain, limbic structures may be particularly vulnerable to the neurotoxic impact of substances like MJ. Indeed, animal models provide evidence supporting this hypothesis. Administering cannabinoids to adolescent rat pups is associated with altered neuron structure and function compared to those exposed in neonatal or adult periods (Scallet, 1991). Heavy MJ use is also associated with abnormal DNA synthesis, reduced chromosomal integrity and alterations in cannabinoid signaling (Ellgren, et al., 2008; Li & Lin, 1998; Zimmerman & Zimmerman, 1990). These adolescence-specific changes may result from greater cannabinoid receptor densities that are unique to adolescents (Burston, Wiley, Craig, Selley, & Sim-Selley, 2010). Taken together, these findings suggest that the introduction of MJ during teen years may induce long-term cellular changes and affect neurodevelopment.

**Gender Differences in Brain Development**

Boys and girls may differ in their vulnerability to drug-induced brain changes because of differences in neuromaturational trajectories and gross brain differences. For example, gray matter in the frontal lobe peaks in girls around 11.0 years old and in boys at around 12.1 years, with similar differences observed in the parietal lobe gray matter (girls exhibit peak volumes at 10.2 and boys at 11.8 years, respectively; Giedd, 2004). Conversely, white matter volumes
increase across adolescence, during which boys show a faster rate of growth compared to girls (Giedd, et al., 1999). Further, specific regions demonstrate different courses of white matter development. For example, Nagel and colleagues (2005) found that female adolescents exhibited *declining* white matter of the prefrontal cortex with age when volumes were expressed as a proportion of overall intracranial space, and the magnitude of this decrease was more accentuated in girls compared to boys. White matter organization also differs between males and females. Adolescent boys show enhanced white matter integrity with age lateralized in the left hemisphere whereas girls showed increased axonal quality in the right hemisphere (Schmithorst, et al., 2008).

Compared to their male peers, adolescent females typically exhibit smaller global brain volumes (Durston, et al., 2001; Giedd, et al., 1999; Giedd, Vaituzis, et al., 1996), but after controlling for head size, girls show relatively larger volumes in the mesial temporal cortex (including the hippocampus/parahippocampal gyrus), caudate, pallidum and thalamus whereas larger relative amygdalar sizes are observed in boys (Caviness, Kennedy, Richelme, Rademacher, & Filipek, 1996; Durston & Casey, 2006; Giedd, Snell, et al., 1996; Sowell, et al., 2002). Adult females also demonstrate larger relative hippocampal volumes and smaller amygdalar and frontal lobe volumes in nonhuman primates (Knickmeyer, et al., 2009) and humans (Good, et al., 2001). The introduction of MJ during the development of these areas may differentially impact sexually dimorphic brain regions.

Adolescent girls and boys differ in the timing of neuromaturational sequences, hormone exposure and neuronal organization (Lenroot & Giedd, 2010; Lenroot, et al., 2007; Schulz, Molenda-Figueira, & Sisk, 2009; Spear, 2000); thus, male and female teens may be differentially susceptible to gender-specific brain changes resulting from adolescent MJ exposure. In addition, females teens exhibit elevated cannabinoid receptor densities relative to same-aged males as well as adult men and women (Burston, et al., 2010), suggesting that girls may be furthermore disproportionately sensitive to MJ.
Neurobehavioral Consequences of MJ Use

Neuropsychological studies report decrements across various cognitive domains in adult chronic MJ users. For instance, acute administration of delta(9)-tetrahydrocannabinol (THC), a psychoactive compound in MJ, is linked to poorer processing speed, reaction time, attention, inhibition, and learning in adults (Ilan, Gevins, Coleman, ElSohly, & de Wit, 2005; Kelleher, Stough, Sergejew, & Rolfe, 2004; Ramaekers, Kauert, Theunissen, Toennes, & Moeller, 2008; Wadsworth, Moss, Simpson, & Smith, 2006). Adult chronic MJ users also display deficits in executive functioning, psychomotor speed, verbal learning, and delayed memory (Bolla, Brown, Eldreth, Tate, & Cadet, 2002; Cadet, Bolla, & Herning, 2006; Fried, Watkinson, & Gray, 2005; Harrison, Gruber, Hudson, Huestis, & Yurgelun-Todd, 2002; Pope & Yurgelun-Todd, 1996; Ramaekers, et al., 2008). However, with abstinence, there is evidence of recovery from persistent cognitive deficits (Gonzalez, Carey, & Grant, 2002; Grant, Gonzalez, Carey, Natarajan, & Wolfson, 2003) with gradual normalization of neuropsychological performance among chronic MJ users throughout 28 days of verified abstinence (Pope, Gruber, Hudson, Huestis, & Yurgelun-Todd, 2001).

Given the aforementioned neuromaturation during adolescence, these adult findings may not necessarily generalize to teens. Thus far, teenage MJ exposure has been associated with poorer attention, working memory, processing speed, and learning which persist even beyond a month of abstinence (Medina, Hanson, et al., 2007; Tapert, Granholm, Leedy, & Brown, 2002). Similar neuropsychological deficits have been observed in late adolescents and young adults following a week of abstinence (Medina & Price, under review). Early MJ use is also related to psychological consequences. Teenage MJ use is linked to developing psychopathology, especially in syndromes of emotion dysregulation like mood and anxiety disorders (Georgiades & Boyle, 2007; Hayatbakhsh, et al., 2007; Patton, et al., 2002; Windle & Wiesner, 2004). Earlier MJ use is also linked to an accelerated transition into a cannabis use
disorder (Behrendt, Wittchen, Hofler, Lieb, & Beesdo, 2009; Copeland & Swift, 2009; Palmer, et al., 2009; Winters & Lee, 2008). It is probable that these pharmaco-behavioral relationships may be explained by alterations in emotional neurocircuitry (e.g., frontolimbic network); however, the underlying neural mechanism for MJ disruption is not yet known.

Adolescent studies document aberrant structural morphometry among brain areas that are associated with these cognitive functions. Irregularities are observed in the volumes of the prefrontal cortex, hippocampus and cerebellar vermis as well as white matter organization in teenage MJ users compared to non-using peers, and these abnormalities were associated with poorer neurobehavioral outcomes (Bava, et al., 2009; Jacobus, et al., 2009; Medina, et al., 2009; Medina, Nagel, Park, McQueeny, & Tapert, 2007; Medina, Nagel, & Tapert, 2010). There is also evidence for gender-dependent alterations in brain morphometry as a result of MJ use. Our lab has previously found subtle gender differences in prefrontal cortex volumes in MJ users in that female users had larger volumes compared to same gendered controls while males had the opposite, and less robust, pattern (Medina, et al., 2009). Gender may represent a differential risk factor for acquiring psychopathology. Specifically, there is evidence suggesting that female adolescent substance users experience negative consequences of drug use earlier than male peers and are more likely to suffer from a mood disorder, whereas male substance abusers have more externalizing behaviors (Kloos, et al., 2009). The neuroanatomical bases for gender-related risk factors for MJ use and drug use consequences remain relatively unclear, especially with respect limbic structures like the amygdala.

Why the Amygdala

One possible mechanism underlying MJ-related changes in cognition, mood and behaviors is alterations to the amygdala. Extant literature has characterized the role of the amygdala in emotion processing and cognition (see review by Phelps & LeDoux, 2005). Of note, animal and human models demonstrate the activation of the amygdala in response to salient
emotional cues with associated influences on learning, memory, attention, working memory and 
self-regulation (LeDoux, 2000; Phelps, 2006; Phelps & LeDoux, 2005). Joined directly to the 
frontal lobe via the uncinate fasciculus, the amygdala forms connections with the prefrontal 
cortex and other limbic structures such as the hippocampus (Aggleton, Burton, & Passingham, 
1980; Hasan, et al., 2009). Through the frontolimbic system, the amygdala modulates higher 
order cognitive processes like working memory, behavioral control, emotion regulation and 
memory by reallocating attentional resources to negative and appetitive affective stimuli (Hazy, 
Frank, & O'Reilly R, 2007; Ishikawa, Ambroggi, Nicola, & Fields, 2008; Miserendino, Sananes, 
Melia, & Davis, 1990; O'Reilly & Frank, 2006; Talmi, Anderson, Riggs, Caplan, & Moscovitch, 
2008).

In addition to connections with the frontal lobe and the hippocampus, the amygdala 
receives inputs from and projects to sensory cortices and the thalamus (Aggleton, et al., 1980). 
With direct sensory input, the amygdala has a privileged ability to process emotional events 
prior to slightly slower, higher-order brain centers, thereby providing the opportunity to 
commandeer various cognitive systems. As part of the mesolimbic dopamine system (Krettek & 
Price, 1978), which broadly functions in regulating goal-directed behavior (Ernst & Fudge, 
2009), the amygdala is implicated in reward-seeking and withdrawal-avoidance behaviors 
(Koob, 2003) that typify substance use disorders (SUD; APA, 2000). In short, the amygdala 
interacts with cognitive networks to appraise the emotional content of a stimulus then facilitate 
an appropriate behavioral response (e.g., approaching a reward or avoiding an aversive event).

**MJ and the Amygdala**

Using functional magnetic resonance imaging (fMRI), researchers have documented 
abnormal amygdalar functioning in relation to the cannabinoid system. During an emotional 
processing paradigm, enhanced endocannabinoid signaling is associated with decreased 
threat-related amygdalar reactivity (Hariri, et al., 2009). Preliminary data from a longitudinal
study found reduced amygdalar reactivity in association with increased MJ use (Cornelius, Aizenstein, & Hariri, 2010). Even sober MJ users show less amygdalar reactivity during affective processing tasks (Gruber, Rogowska, & Yurgelun-Todd, 2009). However, the authors of these studies reported on experimental conditions (e.g., angry faces, threat stimuli) contrasted to neutral control trials, so it is possible that the reported lower task-related response actually reflected greater baseline amygdalar activity. Indeed, cannabinoid administration to non-using adults is linked to attenuated amygdalar reactivity during intoxication, but exaggerated amygdalar activation during acute withdrawal (Fusar-Poli, et al., 2009). In sum, the cannabinoid system appears to be involved in attenuating amygdalar functioning. MJ use may exert chronic changes to cannabinoid signaling resulting in long-term alterations to amygdalar function.

Evidence from structural neuroimaging studies suggests that chronic MJ use may result in abnormal brain morphology, especially in regions rich in binding sites for the psychoactive components of MJ, namely cannabinoid (CB1) receptors (Medina, et al., 2009; Yucel, et al., 2008). The amygdala is one such area possessing a high density of CB1 receptors (Ashton, Darlington, & Smith, 2006; Glass, Dragunow, & Faull, 1997; Iversen, 2003). Chronic MJ use may alter the cellular properties of neurons comprising the amygdala and potentially impact its structure. Animal studies show reduced neuronal volume and density in hippocampal cells following long-term cannabinoid administration (Chan, 1998; Lawston, 2000; Scallet, 1987), and the cellular mechanism may be mediated by the glucocorticoid system (Landfield, 1988).

Provided that the amygdalar and hippocampus express comparable levels of CB1 and glucocorticoid receptors (Glass, et al., 1997; Spear, 2000), the amygdala may also be exhibit similar patterns of neurotoxicity. To date, there is one volumetric study of adult MJ users, and the authors of this report described smaller amygdalar volumes in MJ users (Yucel, et al., 2008). However, in consideration of ongoing brain developments, results from adult samples may not extend to adolescents. For example, larger regional volumes have been observed among
adolescent MJ users including in the prefrontal cortex and cerebellar vermis (Medina, et al., 2009; Medina, et al., 2010).

Summary and Proposed Study

In summary, MJ use in the midst of adolescent neuromaturational processes may adversely affect the amygdala and its associated functions. Gender differences in amygdalar morphometry are apparent during adolescence, with greater amygdalar volume increases observed in boys compared to their female peers (Giedd, Vaituzis, et al., 1996). Thus, amygdalar morphometry is likely influenced by early MJ use, gender and their interaction. However, it remains unknown whether MJ use is related to altered amygdalar structure in adolescents or whether such relationships differ between boys and girls. Because adolescent MJ use is linked to altered cognition (Medina, Hanson, et al., 2007), and to developing further mood and anxiety disorders as adults (Georgiades & Boyle, 2007; Hayatbakhsh, et al., 2007; Patton, et al., 2002; Windle & Wiesner, 2004), it is critical to understand the impact of MJ exposure on specific brain regions like the amygdala. To date, there is no known study that has examined the impact of MJ use on amygdalar morphometry in healthy teens.

Therefore, the primary aim of the current study was to examine cross-sectional morphometric differences in amygdalar structure between abstinent adolescent MJ users and non-drug using controls. We hypothesized that MJ users would demonstrate larger amygdalar volumes than will non-drug using controls. As a secondary aim, we examined whether gender moderated the effects of MJ on amygdalar volume. Given our previous finding of bigger prefrontal cortex volumes in MJ using girls in a different sample of teens (Medina, et al., 2009), we hypothesize that MJ using girls would show larger amygdalar volumes compared to drug-free peers than will boys. To aid interpretation of the primary findings, follow-up analyses examined whether brain-behavior relationships between amygdalar structures and mood symptoms (Sheline, Gado, & Price, 1998) or performance on measures of attention (Phelps, 2006) differ
between the groups. It was hypothesized that MJ users would demonstrate abnormal brain-behavior relationships compared to controls.
Chapter II

Method

Participants

Participants were two groups of healthy, demographically similar adolescents that included 35 chronic MJ users (8 females) and 47 non-using peers (11 females) who successfully completed a month of monitored abstinence. The current study represents an archival analysis of a sub-sample of youths recruited as part of a larger study of adolescent substance users at the University of California, San Diego’s Adolescent Brain Imaging Project (PI: Tapert, NIDA R01 DA21182). Inclusion criteria included having a minimum of 180 total episodes (i.e., days) of MJ use (for users), being between the ages of 16-18, right-handed, proficiency in English and having written informed consent to participate (parent/guardian consent for minors). The age criterion (16-18 years) reflects our focus on an age group that is still experiencing neuromaturation, but is old enough to be most likely to use MJ (Johnston, et al., 2009). To maximize our ability to detect effects and minimize the influence of potential confounds, extensive exclusionary criteria were utilized, assessed by trained research assistants on separate, confidential teen and parent interviews. Exclusionary criteria included a history of psychotropic medication use, presence of psychiatric illness, neurologic problems (e.g., sensory impairments), previous head injury or loss of consciousness greater than two minutes, learning disability, prenatal drug exposure and significant birth complications (e.g., premature birth, anoxia). In order to optimize MJ-related effects, participants were excluded if criteria were met for abuse or dependence of substances (other than cannabis and nicotine) or if teens reported other illicit drug use exceeding 25 lifetime episodes. MRI contraindications like irremovable metal, pregnancy, and claustrophobia were also exclusionary.

The sample for the current study was selected from the pool of eligible youth who were successfully screened and completed the protocol ($n = 108$). Twenty-six cases were excluded
due to excessive (greater than 25 episodes) polysubstance use \( n = 13 \), poor quality of MRI data \( n = 12 \) and neuroanatomical abnormality as determined by a neuroradiologist \( n = 1 \). It is worth noting that six participants turned 19-years old between enrollment and completion of the study, which resulted in an extended age range of 16.0 to 19.2. Because recent MJ intoxication may not result in observable macrostructural changes (Jager, et al., 2007), four individuals were included in the analyses after discovering they used MJ between their neuropsychological assessment and neuroimaging exam (based on positive lab results with confirmatory self-report). Therefore, the final sample \( N = 82 \) represents two groups of healthy, typically developing youth differing primarily on substance use.

**Measures**

*Detailed Screening Measures and Demography.* A Structured Clinical Interview was used to collect basic demographic information including age, gender, ethnicity, socioeconomic status (Hollingshead Index; Hollingshead, 1965), household income, academic standing (e.g., grade point average), and psychosocial functioning. The Computerized Diagnostic Interview Schedule for Children (C-DISC; Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000) self-report and parent modules were administered by trained research assistants to screen for Axis-I psychiatric disorders of the Diagnostic and Statistical Manual-IV (DSM-IV-TR; APA, 2000). The Family History Assessment Module (Rice, et al., 1995) was used to assess familial SUD among biological parents and second degree relatives (Schweinsburg, et al., 2004; Spadoni, Norman, Schweinsburg, & Tapert, 2008; Tapert & Brown, 2000).

*Premorbid Intellectual Functioning.* Intellectual abilities were estimated using the Wide Range Achievement Test-3rd Edition (WRAT) Reading (Wilkinson, 1993) and the Wechsler Abbreviated Scale of Intelligence (WASI) Vocabulary (Wechsler, 1999) subtests.

*Youth Drug Use.* Lifetime MJ, alcohol, nicotine, and other drug use, frequency, withdrawal symptoms, DSM-IV-TR abuse and dependence criteria (APA, 2000), and substance-
related life problems were assessed using the well-standardized Customary Drinking and Drug Use Record (CDDR; S. A. Brown, et al., 1998). On the final day of the study a revised version of the CDDR was administered to gather substance involvement and related experiences within the previous three-months (i.e., recent drug use).

**Mood Measures.** Mood measures were collected just prior to the MRI scan and included the Beck Depression Inventory (BDI; Beck, 1978), Hamilton Depression and Anxiety Rating Scales (HAM-D; HAM-A; M. Hamilton, 1959, 1960; M. Hamilton, 1986) and Spielberger State-Trait Anxiety Inventory (STAI; Spielberger, Gorusch, & Lushene, 1970). The BDI is a 21-item self-report questionnaire that measures depressive symptoms over the previous two weeks. Previous studies have employed the BDI with adolescent samples (Bennett, et al., 1997; Medina, Nagel, et al., 2007). The HAM-D and HAM-A, which have also been used with adolescents (Nixon, Milin, Simeon, Cloutier, & Spenst, 2001; Rynn, Siqueland, & Rickels, 2001), are semi-structured scales that assess reported and observed depressive and anxiety symptoms during the previous 7 days. The STAI measures transient or situational ‘state’ anxiety symptoms as well as more chronic dispositional ‘trait’ anxiety. Standardized z-scores based on the overall sample were computed from total raw scores of the BDI, HAM-D and HAM-A. Age corrected T-scores from the STAI were transformed to z-scores. In the interest of condensing the list of depression and anxiety variables, a composite scale was calculated based on the average of each z-score from the BDI, STAI, HAM-D and HAM-A. The resulting Mood Composite demonstrated good internal consistency (Cronbach’s $\alpha = 0.81$).

**Complex Attention.** As part of a larger comprehensive neuropsychological battery, measures of simple and sustained attention, working memory, psychomotor processing speed and verbal fluency were collected. Specific attention measures included: the California Verbal Learning Test-II (CVLT-II; Delis, Kramer, Kaplan, & Ober, 2001) List A Trial 1 recall; Delis-Kaplan Executive Function System (D-KEFS) Letter Fluency total score (Delis & Kaplan, 2000); Wechsler Adult Intelligence Scale-Third Edition (WAIS-III; Wechsler, 1997) Digit Symbol scaled
score, Arithmetic scaled score, and Digit Span backwards total score; and the Paced Auditory Serial Addition Test (PASAT) 2-second trial total score (Gronwall, 1977). Each task has been shown to be related to complex attention (Zimmermann & Fimm, 2002; Zomeren & Brouwer, 1994) and has been used previously as cognitive construct with adequate internal consistency (Medina, Hanson, et al., 2007). For the current study, age-normalized performance scores were transformed to z-scores, and then a complex Attention Composite score was computed as the average z-score (Cronbach's $\alpha = 0.75$).

Procedures

The stepwise protocol for teens completing the study involved: (1) recruitment; (2) initial teen and parent screens; (3) informed consent/assent; (4) detailed teen and parent interviews; (5) 28 days of monitored abstinence; (6) neurocognitive assessment; and (7) MRI exam. Participants were recruited by posting flyers and distributing handouts at college and high school campuses in the San Diego vicinity. Interested teens contacted the project and were administered a confidential initial questionnaire, screening for basic inclusionary and exclusionary criteria (e.g., age, handedness, MRI suitability, previous diagnosis of a psychiatric or neurologic problem, substance use cutoffs). If eligible, a similar screener was administered to a parent of the teen for collateral information as well as further basic exclusions (e.g., prenatal drug exposure, developmental delays/learning disabilities). In accordance with the UCSD Human Research Protections Program, volunteers remaining eligible after the preliminary screens provided assent or consent (depending on adult age status) and a parent or legal guardian also provided consent to participate in a detailed phone interview as well as permission for the participation of a minor as appropriate.

After enrolling in the study, confidential comprehensive phone interviews were administered to teens and parents separately. The phone interview verified eligibility by assessing detailed symptoms of psychopathology according to self and parent collateral reports.
Parents and teens provided basic demographic information during their respective phone interviews. In addition, adolescents were administered a detailed substance use inventory to collect lifetime use patterns of nicotine, alcohol, MJ and other drugs as well as withdrawal, abuse and dependence symptoms (CDDR).

Following the detailed screening interviews, teens began a 28 day abstinence period verified by semi-weekly urine toxicology screens. To ensure against recent intoxication, breath samples were collected to verify 0.000 breath alcohol concentration (AlcoSensor IV Intoximeter) and urine samples were obtained to assess for recent drug use (measuring metabolites indicating recent use of cannabis, amphetamines, barbiturates, benzodiazepines, cocaine, codeine, morphine, phencyclidine, and ethanol using cloned enzyme donor immunoassay; CEDIA DAU, Microgenics, Fremont, CA, USA). In the event that biological tests indicated drug use during the abstinence period, participants were offered the opportunity to restart the study one more time or voluntarily discontinue. Teens with negative or near-negative MJ metabolite levels in their third week of abstinence were scheduled for two longer appointments in their final week of participation. Approximately two to three days prior to their neuroimaging appointment, teens attended a behavioral session during which cognitive abilities and intellectual functioning were assessed. At their final appointment trained research assistants administered measures of mood symptoms (BDI, STAI, HAM-D, HAM-A) and assessed recent substance use involvement in the previous three-months (CDDR) then each participant underwent a structural MRI exam. A urine sample for pregnancy testing was also collected and any pregnant female was excluded (n = 0). Participants were scanned on a 3-Tesla GE Scanner at the Keck Center for fMRI on UCSD’s campus.

MRI Acquisition and Processing.

MRI Acquisition. High-resolution anatomical images of the entire brain used 3D spoiled gradient recalled images that were T1-weighted for gray-white contrast (TR = 8 ms, TE = 3 ms,
flip angle = 12°, field of view = 240 mm, 176 continuous slices, 1 mm³, acquisition time 7:19).

These images have high tissue contrast, permitting volumetric analyses.

**MRI Processing & Amygdalar Region of Interest (ROI).** Imaging data were processed using Analysis of Functional Neuroimages (AFNI; Cox, 1996). Manual tracing of the amygdalar regions of interest occurred on high-resolution grayscale images in standard AC-PC alignment. Left and right amygdalar ROIs (modified protocol created by TM based on Richardson, et al., 2007; Sheline, et al., 1998) were manually traced on contiguous slices in the coronal plane by reliable raters (ICCs > 0.86), who were blind to group status and gender. Boundaries were as follows: anterior boundary: first coronal slice in which the temporal stalk merges with the white matter of the insula; dorsal boundary: entorhinal sulcus separating the basal forebrain and temporal lobe; posterior boundary: head of the hippocampus as evident in the sagittal view; ventral boundary: horizontal boundary extending from the anterior and ventral hippocampal edge; medial boundary: presence of the subarachnoid space; and the lateral boundary: surrounding white matter (see Figure 1). Amygdalar volumes were calculated by extracting the number of voxels within the hand-drawn ROI, which were multiplied by the voxel dimensions. In order to account for individual variability in head size, intracranial volumes (ICV) were treated as a covariate in multiple regression models. ICVs were created by an automated watershed program that employs a deformable surface-based algorithm to produce a brain-only mask (Segonne, et al., 2004), which were then inspected and hand-edited for accuracy by reliable raters (ICCs > 0.99).
Figure 1. Manually traced amygdalar ROI

**Power Analysis**

Previous studies examining amygdalar morphometry in adult samples demonstrate large effect sizes (Cohen’s $d = 0.80-0.99$; (Sheline, et al., 1998; Yucel, et al., 2008). Our previous work examining prefrontal cortex morphometry in a similar adolescent sample yielded a more modest effect size ($f^2 = 0.11$); these results were produced from a multivariate model that included similar covariates to those in the current study. The large effect sizes from adult studies may be explained by greater years of exposure to MJ; thus, the medium effect size resulting from adolescent studies may be a more accurate approximation for the current analysis. Given that the sample was derived from an archival dataset, the sample size of current study ($N = 82$) represents the maximum number of eligible participants. An estimation of power to detect relationships between amygdalar volumes and MJ user group status, gender and group X gender interactions controlling for nicotine, alcohol and other drug use was conducted. With an expected medium effect size ($f^2 = 0.15$), an alpha of 0.05, and a sample of 82 teenagers, the power to detect these relationships is estimated to be 0.83.
Data Analysis

Potentially Confounding Variables. Healthy control participants were recruited from the same sites as MJ users to achieve group similarity in ethnicity, age, and socioeconomic status. However, in the event that demographic variables are disproportionate or significantly different between groups, those variables were treated as possible confounds. Family histories of SUD are associated with amygdalar size abnormalities (Hill, et al., 2001). Past-month cigarette use in the recruitment schools used in this study is approximately 21% and nicotine use is associated with structural changes in the brain (Gallinat, et al., 2006); therefore, nicotine smoking will be accounted for statistically. Similarly, alcohol use is related to changes in brain structure in adolescents (Medina, et al., 2008; Medina, Schweinsburg, Cohen-Zion, Nagel, & Tapert, 2007); thus, alcohol use will be controlled for statistically. For covariate selection, bivariate relationships between amygdalar volume and each index of alcohol and nicotine involvement will be analyzed for the strongest association then subsequently used as a covariate in the main analyses.

In order to determine whether groups differ on potentially confounding variables, continuous measures will be compared using independent samples t-tests or Mann-Whitney U-tests, and categorical variables will be examined with a chi-square test of equal proportion. If group disparities emerge, these discrepant variables will be entered into the multivariate analyses as covariates. Similarly, self-reported frequency, duration and recency of alcohol, nicotine and other drug use will be compared.

Primary Analyses. To ensure assumptions for the analyses are met, dependent variables will be tested for normality using a Kolmogorov-Smirnov test with confirmatory visual inspection of distributions. Positively skewed dependent variables were transformed to optimize symmetry of distribution. A series of multiple regressions were run in SPSS 17.0 (SPSS for Mac, Rel. 17.0.1. 2008. Chicago: SPSS Inc.) to model right or left amygdalar sizes after controlling for ICV and potentially confounding factors (alcohol, nicotine and other drug uses) in
a first block (Block 1). Main effects for MJ user status and gender were entered in Block 2. The last block (Block 3) included the centered group by gender interaction term to examine whether gender moderates the effects of MJ use on amygdalar morphometry. Statistical decisions were made based on an $\alpha$ of 0.05.

Secondary Analyses. In order to help interpret the results of the main analyses, behavioral analyses were performed. Multiple regressions were used to determine whether gender, group, amygdalar volumes, or their centered interactions are related to mood or attention composites, after controlling for ICV.
Chapter III

Results

Sample Characteristics

Descriptive statistics were computed for demographic and potentially confounding variables (Table 1). MJ users \((n = 35)\) and controls \((n = 47)\) were similar in age \((t = 1.43, p = 0.16)\), gender ratio \((\chi^2<0.01, p = 0.95)\), ethnic proportion \((\chi^2 = 0.11, p = 0.74)\), socio-economic status \((\text{Household Income}, t = 0.50, p = 0.46; \text{Hollingshead Code}, t = 1.50, p = 0.14)\), family history of SUD \((\chi^2 = 3.82, p = 0.15)\) and intellectual functioning \((\text{WASI Vocabulary}, t = 1.43, p = 0.16; \text{WASI Block Design}, t = 0.88, p = 0.39; \text{WRAT-3 Reading}, t = 1.43, p = 0.16)\). MJ users had significantly lower academic grade point averages \((t = 2.19, p = 0.03)\), which our group has previously observed (Medina, Hanson, et al., 2007) and has been similarly reported in other adolescent samples (Miller & Miller, 1997). Sample characteristics were also compared according to group and gender using a one-way ANOVA with post-hoc Tukey tests, and the pattern of results were similar to the group comparisons (the ANOVA results not reported due to redundancy; see Appendix A for descriptive statistics by group and gender).

Independent samples \(t\)-tests assessed whether groups differed on drug use, mood and neuropsychological variables. As expected MJ using and control teens differed in substance use and behavioral measures (see Table 1 for descriptive statistics). On average, users reported 445.66 lifetime MJ episodes, ranging from 180 to 1,800 occasions. For duration and frequency of MJ use, teens had been smoking MJ for about 2.61 years prior to their participation and they reported approximately 11.54 occasions in the past month with around 9.97 hits per episode. MJ users reported greater lifetime \((U = 85.00, p<0.01)\) and recent alcohol experiences \((U = 187.00, p = <0.01)\). Although minimal, nicotine use was also higher in user teens, with about half \((48.57\%)\) reporting having smoked a cigarette in the month before participation compared to 4.30\% among controls \((\chi^2 = 22.13, p<0.01)\). On average, MJ users smoked cigarettes 5.57 days
per month, with just two users reporting daily use (10 and 20 cigarettes per day); however, no participant met criteria for nicotine dependence (Fagerström score; Range [0, 3], maximum possible = 10). Lifetime uses of other illicit drugs were greater ($U = 267.00$, $p < 0.01$) in users (Range [0, 27]) compared to controls (Range [0, 13]).

Higher scores on the mood composite were observed in the MJ users ($t = 2.30$, $p = 0.02$) but no difference between groups was detected for the Executive Attention Composite ($t = 0.89$, $p = 0.38$). Non-parametric correlations were calculated between each of the drug use variables (other than MJ) and amygdalar morphometry variables in order to select the covariates to control for alcohol, nicotine and other drug use (see Appendix B for correlations). The strongest bivariate relationships were observed between right amygdalar (ICV ratio) with years of regular drinking ($\tau_B = -0.13$, $p = 0.12$), Fagerström Nicotine Dependence scores ($\tau_B = 0.11$, $p = 0.24$) and lifetime other drug uses ($\tau_B = -0.13$, $p = 0.05$). Therefore, these variables were entered into multiple regression models as covariates.

**Characteristics of Volumetric Variables**

Volumetric variables are summarized in Table 2. ICV differed by gender ($F_{1,80} = 13.02$, $p < 0.01$) as well as by group*gender class ($F_{3,78} = 4.40$, $p = 0.01$), but not group alone ($F_{1,80} = 0.06$, $p = 0.81$). Post-Hoc Tukey tests revealed that females had smaller overall ICVs, with female MJ users showing the smallest values relative to males and female controls.
## Table 1

**Sample Characteristics by MJ User Status and Gender**

<table>
<thead>
<tr>
<th></th>
<th>Female Controls</th>
<th>Male Controls</th>
<th>Female MJ</th>
<th>Male MJ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$M \ (SD)$ [R] or %</td>
<td>$M \ (SD)$ [R] or %</td>
<td>$M \ (SD)$ [R] or %</td>
<td>$M \ (SD)$ [R] or %</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>11</td>
<td>36</td>
<td>8</td>
<td>27</td>
</tr>
<tr>
<td><strong>Caucasian</strong></td>
<td>36%</td>
<td>40%</td>
<td>25%</td>
<td>48%</td>
</tr>
<tr>
<td><strong>Positive family history of SUD</strong></td>
<td>9%</td>
<td>17%</td>
<td>57%</td>
<td>26%</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>17.85 (0.73) [16.41-18.83]</td>
<td>17.65 (0.90) [16.13-19.16]</td>
<td>18.15 (0.86) [16.55-19.07]</td>
<td>17.92 (0.91) [16.41-19.12]</td>
</tr>
<tr>
<td><strong>Household income ($k)</strong></td>
<td>137.00 (86.32) [36-305]</td>
<td>114.39 (65.84) [13-275]</td>
<td>177.86 (180.96) [60-565]</td>
<td>125.57 (106.74) [25-450]</td>
</tr>
<tr>
<td><strong>Vocabulary ($T$)</strong></td>
<td>58.82 (9.91) [46-75]</td>
<td>59.89 (8.76) [43-76]</td>
<td>58.63 (8.35) [49-70]</td>
<td>57.70 (8.98) [36-75]</td>
</tr>
<tr>
<td><strong>Block design ($T$)</strong></td>
<td>59.91 (8.18) [44-67]</td>
<td>55.42 (6.94) [41-66]</td>
<td>54.75 (7.46) [49-65]</td>
<td>56.58 (7.58) [38-68]</td>
</tr>
<tr>
<td><strong>Reading standard score</strong></td>
<td>109.64 (5.63) [103-121]</td>
<td>110.06 (7.49) [93-126]</td>
<td>106.38 (9.09) [93-119]</td>
<td>106.74 (8.55) [89-119]</td>
</tr>
<tr>
<td><strong>Grade point average</strong></td>
<td>3.50 (0.63) [2.3-4]</td>
<td>3.38 (0.59) [1.9-4]</td>
<td>3.40 (0.61) [2.5-4]</td>
<td>2.97 (0.83) [0.5-4]</td>
</tr>
<tr>
<td><strong>Mood ($z$)</strong></td>
<td>-0.43 (0.58) [-0.97-0.51]</td>
<td>-0.47 (0.69) [-1.07-2.57]</td>
<td>0.51 (1.25) [-0.85-2.22]</td>
<td>-0.33 (0.50) [-0.99-0.96]</td>
</tr>
<tr>
<td><strong>Complex attention ($z$)</strong></td>
<td>0.41 (0.34) [-0.11-1.04]</td>
<td>0.31 (0.63) [-0.99-1.5]</td>
<td>0.01 (0.54) [-0.63-0.83]</td>
<td>0.26 (0.80) [-1.1-1.98]</td>
</tr>
<tr>
<td><strong>Lifetime alcohol</strong></td>
<td>26.60 (61.24) [0-196]</td>
<td>21.03 (27.53) [0-106]</td>
<td>239.38 (211.15) [90-736]</td>
<td>185.7 (138.93) [14-450]</td>
</tr>
<tr>
<td><strong>Drinks per month</strong></td>
<td>3.64 (8.72) [0-29]</td>
<td>7.56 (12) [0-53]</td>
<td>39.75 (46.84) [0-152]</td>
<td>49.07 (45.87) [0-179]</td>
</tr>
<tr>
<td><strong>Alcohol withdrawal (symptoms)</strong></td>
<td>0.00 (0.00) [0-0]</td>
<td>0.69 (1.35) [0-5]</td>
<td>1.75 (1.91) [0-6]</td>
<td>1.85 (2.09) [0-7]</td>
</tr>
<tr>
<td><strong>Alcohol use duration (years)</strong></td>
<td>0.14 (0.45) [0-1.5]</td>
<td>0.00 (0.00) [0-0]</td>
<td>1.86 (2.01) [0-5.75]</td>
<td>1.01 (1.26) [0-4.08]</td>
</tr>
<tr>
<td><strong>Lifetime MJ</strong></td>
<td>0.36 (0.81) [0-2]</td>
<td>1.58 (2.56) [0-9]</td>
<td>397 (243) [200-814]</td>
<td>460 (391) [180-1800]</td>
</tr>
<tr>
<td><strong>MJ hits per month</strong></td>
<td>0.00 (0.00) [0-0]</td>
<td>0.42 (1.54) [0-8]</td>
<td>127.25 (265.41) [0-780]</td>
<td>163.44 (185.32) [0-810]</td>
</tr>
<tr>
<td><strong>MJ dependence (symptoms)</strong></td>
<td>0.00 (0.00) [0-0]</td>
<td>0.03 (0.17) [0-1]</td>
<td>2.50 (2.93) [0-7]</td>
<td>3.81 (2.47) [0-9]</td>
</tr>
<tr>
<td><strong>Lifetime other drugs</strong></td>
<td>0.18 (0.60) [0-2]</td>
<td>0.47 (2.20) [0-13]</td>
<td>8.13 (6.10) [1-19]</td>
<td>4.48 (7.11) [0-27]</td>
</tr>
<tr>
<td><strong>FTND (symptoms)</strong></td>
<td>0.00 (0.00) [0-0]</td>
<td>0.00 (0.00) [0-0]</td>
<td>0.00 (0.00) [0-0]</td>
<td>0.30 (0.78) [0-3]</td>
</tr>
<tr>
<td><strong>Cigarettes per month</strong></td>
<td>0.00 (0.00) [0-0]</td>
<td>0.11 (0.67) [0-4]</td>
<td>0.00 (0.00) [0-0]</td>
<td>45.93 (128.87) [0-600]</td>
</tr>
</tbody>
</table>

FTND=Fagerström Nicotine Dependence; SUD = substance use disorder

*a* Wechsler Abbreviated Scale of Intelligence; *b* Wide Range Achievement Tests - 3rd Edition; *c* Total use episodes

*p < .05, MJ users differ from controls*
Table 2

*Morphometric Values by MJ User Status and Gender*

<table>
<thead>
<tr>
<th></th>
<th>Female Controls</th>
<th>Male Controls</th>
<th>Female MJ</th>
<th>Male MJ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Intracranial Volume (cc)</td>
<td>1,512.61</td>
<td>160.00</td>
<td>1,616.23</td>
<td>131.51</td>
</tr>
<tr>
<td>Right Amygdala (cc)</td>
<td>2.03</td>
<td>0.19</td>
<td>2.38</td>
<td>0.33</td>
</tr>
<tr>
<td>Left Amygdala (cc)</td>
<td>1.84</td>
<td>0.23</td>
<td>2.04</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*Primary Analysis: Amygdalar Morphometry with MJ Status and Gender*

Multiple Regression analyses were performed in order to determine whether right and left amygdalar volumes were associated with MJ user status, gender and their group*gender interaction after controlling for ICV, alcohol, nicotine and other drug use. Significant interactions between group and gender were observed in modeling right amygdalar volume (see Table 3; $t = -2.17$, $\beta = -0.22$, $p = 0.03$), whereby female MJ users had significantly larger volumes compared to female controls; males had similar volumes and were larger than female controls but smaller than female users on average (see and Figure 2). The effect size for this finding was in the small range ($f^2 = 0.05$). Other drug use was also associated with right amygdalar morphometry, with greater polysubstance use predicting smaller volumes ($t = -2.03$, $\beta = -0.23$, $p = 0.05$). The model for left amygdalar volume revealed no significant relationships after controlling for ICV, alcohol, nicotine and polysubstance use ($R^2_{\Delta} = 0.02$, $F_{3,74} = 0.60$, $p = 0.62$).

Table 3

*Regression Statistics for Modeling Right Amygdalar Volume*

<table>
<thead>
<tr>
<th></th>
<th>$F$</th>
<th>df</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Model (all blocks)</td>
<td><strong>4.32</strong></td>
<td>7,74</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>$t$</th>
<th>$\beta$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracranial Volume</td>
<td><strong>3.20</strong></td>
<td>0.35</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Years of Alcohol Use</td>
<td>0.93</td>
<td>0.11</td>
<td>0.36</td>
</tr>
<tr>
<td>Fagerström Nicotine Dependence</td>
<td>1.52</td>
<td>0.16</td>
<td>0.13</td>
</tr>
<tr>
<td>Lifetime Other Drug Use</td>
<td><strong>-2.03</strong></td>
<td><strong>-0.23</strong></td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>Group</td>
<td>-0.05</td>
<td>-0.01</td>
<td>0.96</td>
</tr>
<tr>
<td>Gender</td>
<td>1.42</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>Group*Gender</td>
<td><strong>-2.17</strong></td>
<td><strong>-0.22</strong></td>
<td><strong>0.03</strong></td>
</tr>
</tbody>
</table>

**BOLD:** $p < 0.05$
Figure 2. Right amygdalar volumes (residuals) by gender and MJ user status controlling for alcohol, nicotine and other drug use.

Relationships with Mood and Cognition

To elucidate brain-behavior relationships linked to right amygdalar volume, multiple regressions were run to determine how group, gender, right amygdalar volume and all centered second-order interactions were related to behavioral measures of mood or complex attention. Right amygdalar volume ($t = -2.18, \beta = -0.27, p = 0.03$), group ($t = 2.58, \beta = 0.28, p = 0.01$) and the group*gender interaction ($t = -2.04, \beta = -0.24, p = 0.04$) independently accounted for significant variability in mood and anxiety symptoms (see Table 4 and Figure 3). Specifically, smaller amygdalar volume was linked to greater mood and anxiety symptomatology. However, plotting the relationship between right amygdalar volume and mood scores (adjusted for ICV, main effects and interactions) reveals that the inverse relationship among amygdalar and mood is driven by female controls and males (see Figure 3, part A). In contrast, female MJ users show the opposite brain-behavior pattern (i.e., larger amygdala is linked to worse mood in female MJ users), though a three-way (group*gender*amygdala) interaction was not significant ($t = -1.00, \beta$
= 0.14, p = 0.32). In light of this finding, the Mood composite term was added to the model of right amygdalar volume (Table 3) as a covariate without altering the significance of the group*gender interaction (p > 0.05). Independently of amygdalar morphology, *MJ users* had *higher* mood composites and *female MJ users* showed the greatest mood scores (see Figure 3, parts B and C). A main effect for gender was not found (t = -0.55, β = -0.07, p = 0.59), nor any second-order interaction with morphometry (gender*right amygdalar: t = 1.34, β = 0.17, p = 0.18; group*right amygdalar: t = 0.61, β = -0.07, p = 0.55). Modeling the Executive Attention Composite did not yield significant results (F_{7,73} = 0.41, p = 0.89) with group, gender, right amygdalar volume or their interactions (p’s < 0.05).

Table 4

*Regression Statistics for Modeling Mood Composite Scores from Right Amygdalar, Group and Gender*

<table>
<thead>
<tr>
<th>Overall Model (all blocks)</th>
<th>F</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.55</td>
<td>6,75</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>t</th>
<th>β</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracranial Volume</td>
<td>0.68</td>
<td>0.08</td>
<td>0.50</td>
</tr>
<tr>
<td>Right Amygdalar Volume</td>
<td>-2.18</td>
<td>-0.27</td>
<td>0.03</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.55</td>
<td>-0.07</td>
<td>0.59</td>
</tr>
<tr>
<td>Group</td>
<td>2.58</td>
<td>0.28</td>
<td>0.01</td>
</tr>
<tr>
<td>Gender*Right Amygdala</td>
<td>1.34</td>
<td>0.17</td>
<td>0.18</td>
</tr>
<tr>
<td>Group*Right Amygdala</td>
<td>-0.61</td>
<td>-0.07</td>
<td>0.55</td>
</tr>
<tr>
<td>Group*Gender</td>
<td>-2.04</td>
<td>-0.24</td>
<td>0.04</td>
</tr>
</tbody>
</table>

**BOLD:** p < 0.05
Figure 3. Higher mood composite scores are independently related to smaller right amygdalar volume, being a MJ user, especially female MJ users. Mood score residuals plotted (a) against right amygdalar volume separately for gender and group (b) by MJ user status and (c) by group and gender. Each y-axis represents the residuals of the model with the variable of the each respective x-axis omitted.
Chapter IV
Discussion

Various lines of research have suggested that adolescence represents a period of increased vulnerability to neurobehavioral consequences of MJ use. The amygdalar may be a site that is particularly sensitive to marijuana use. The primary purpose of the current study was to compare amygdalar volumes among adolescent MJ users and healthy control teens, as well as determine whether relationships between MJ user status and amygdalar morphology vary by gender. We found that gender moderated the relationship between MJ user status and right amygdalar morphometry, although there were no main effects for group. More specifically, after controlling for intracranial volume, alcohol, nicotine and other drug use, female MJ users exhibited larger right amygdalar volumes relative to all males and female controls. These findings are consistent with our lab’s previous study of a separate sample of adolescents which reported slightly larger prefrontal cortex volumes in female MJ users compared to non-using female peers (Medina, et al., 2009).

The secondary aim of this study was to determine whether MJ use status, gender, and amygdalar volumes were independently or interactively predictive of mood and executive attention in adolescents. Although the amygdalar is implicated in directing neural resources to novel and emotional stimuli (Phelps, 2006), we did not detect any relationships between complex attention with amygdalar morphometry, MJ user status, gender or their interactions. With respect to mood, smaller right amygdalar volume and being a MJ user were linked with increased depression and anxiety symptoms. Gender also modulated the relationship between MJ user status and mood with female MJ users having greater symptoms. Whereas smaller amygdalar volume was associated with greater mood scores, female controls and males primarily drove this effect. Collectively these findings are consistent with previous literature connecting depression and anxiety to smaller amygdalar size (J. P. Hamilton, Siemer, & Gotlib, 2008; Richardson, et al., 2007; Rosso, et al., 2005; Sheline, et al., 1998), teenage MJ use
(Georgiades & Boyle, 2007; Hayatbakhsh, et al., 2007; Patton, et al., 2002; van Laar, van Dorsselaer, Monshouwer, & de Graaf, 2007; Windle & Wiesner, 2004) and higher prevalence of depression and anxiety in females (Zahn-Waxler, Shirtcliff, & Marceau, 2008). However, being female and a MJ user was associated with different, unique patterns.

Among female MJ users, larger amygdalar volumes were linked to higher depressive and anxiety scores. This finding is the opposite of what was found among female controls (worse mood linked to smaller amygdalar), and is furthermore inconsistent with adult MJ literature. One study known to date has documented amygdalar volume decrements among adult MJ users (Yucel, et al., 2008). However, gender-specific findings may signify dual effects of internalizing symptoms and teenage MJ use. Larger amygdalar volumes are documented in adult depression and anxiety studies (Frodl, et al., 2003; J. P. Hamilton, et al., 2008; McEwen, 2005). In children, increased amygdalar volumes are related to pediatric depression and anxiety, prenatal stress, maltreatment as well as general fearfulness (De Bellis, et al., 2000; MacMillan, et al., 2003; Salm, et al., 2004; Tottenham, et al., 2010; van der Plas, Boes, Wemmie, Tranel, & Nopoulos, 2010). Here, female MJ users, who endorsed the most depression and anxiety symptoms, exhibited larger right amygdalar volumes, and this relationship was not mediated by reported mood. Moreover, increasing amygdalar size appeared to be predictive of worse mood among the female MJ sub-group. The opposite brain-behavior pattern was observed for female controls and both male groups, but a three-way interaction was not significant, likely due to insufficient power related to low sampling of female users. Therefore, in addition to their distinct contributions, MJ, amygdalar volume and gender may also be interactive in explaining mood symptoms in adolescents. In sum, female MJ users had larger right amygdalar volumes, worse mood symptoms, and poorer mood was associated with larger amygdalar volume in this sub-group.

Discrepancies between adult and adolescent structural MRI findings could be related to the disruption of neuromaturation by early chronic MJ use. Typical brain development entails
increasing brain size through adolescence (Durston, et al., 2001; Giedd, 2004) but decreasing volumes are observed over the course of adult aging (Allen, Bruss, Brown, & Damasio, 2005; Kubota, et al., 2001). In the present study, healthy control teen males had larger right amygdalar volumes compared to females, relative to overall brain volume. This is consistent with developmental literature showing similar gender discrepancies in amygdalar morphometry during and beyond adolescence (Caviness, et al., 1996; Durston & Casey, 2006; Giedd, et al., 1999; Giedd, Vaituzis, et al., 1996; Sowell, et al., 2002). The active components of MJ may interact with developmental and aging processes. For example, exogenous cannabinoid administration is associated with altered astrocyte functioning, when astrocytes play a critical role in eliminating weaker connections and maintaining neuronal health (Bindukumar, et al., 2008; Stevens, et al., 2007). By interfering with these support processes, MJ introduced during early adolescence may impair typical pruning resulting in larger regional brain volumes. Teenage exposure to MJ may also compromise neurogenesis, as reduced levels of nerve growth factor and brain derived neurotrophic factor have also been reported among MJ users (Angelucci, et al., 2008; D'Souza, Pittman, Perry, & Simen, 2009), this effect may have a greater impact after the pruning stage is primarily complete thus resulting in relatively smaller volumes in young adulthood. Taken together, MJ may impact brain development such that interrupted pruning (i.e., maturational delay) are followed by accelerated aging (reduced neurogenesis). Thus, larger right amygdalar brain sizes would be expected among adolescent MJ users whereas continued exposure through adulthood may be related to smaller volumes in adult. Interestingly, a similar biphasic model has been proposed (i.e., initial increases followed by brain volume reductions) for the course of depression (McEwen, 2005).

The regulation of glucocorticoid hormones may be point of convergence for the effects of amygdalar morphology and gender on mood (Boyer, 2000; McEwen, 2005; Merali, Anisman, James, Kent, & Schulkin, 2008). Indeed, glucocorticoid release and receptor distribution are modulated by estrogen hormones (Perlman, Webster, Kleinman, & Weickert, 2004; Solomon &
Disrupted glucocorticoid signaling is linked to neuronal atrophy (McEwen, 2005; Murphy, et al., 2001; Uno, et al., 1994), particularly in the amygdalar (E. S. Brown, Woolston, & Frol, 2008; Desai, Khanani, Shad, & Brown, 2009; Merke, et al., 2003; Zobel, et al., 2008). Neurotoxicity may result from glucocorticoids inhibiting glial support and expression of brain growth factors (Rajkowska & Miguel-Hidalgo, 2007; Walter, et al., 2003). It is possible that our observation of smaller right amygdalas in relation to mood severity primarily in female controls may reflect the neurotoxic potential of sex hormone-glucocorticoid interactions. However, the converse may be true; smaller amygdalas may represent an endophenotype underlying risk for depression or anxiety (Nifosi, et al., 2010). It is worth noting that the current findings are merely exploratory given that the nature of this study was examining sub-diagnostic mood in non-depressed youth. Therefore, our results may not map onto clinically significant levels of depression and anxiety. Future research examining the nature and impact of cannabinoids and hormones on mood and brain morphology are needed to confirm these findings.

The present study’s primary finding for gender moderating the relationship between amygdalar volume and MJ use may reflect neurodevelopmental differences between boys and girls (Lenroot & Giedd, 2010). Our finding of similar amygdalar volumes among male users and controls could be based on boys initiating MJ use at a different developmental stage than girls. For example, male and female users in our sample began using MJ at similar ages (M = 14.19, 14.25 respectively); however, brains of teen girls mature about one year earlier than boys (Giedd, 2004). Thus female specific gender differences may be related to the interruption of pruning at more advanced stages whereas boys’ brains may reflect greater resiliency to deleterious effects of MJ if exposure occurs when pruning was at an earlier stage. Alternatively, male-specific effects may not be measurable until young adulthood.

Pubertal steroids may contribute to altered amygdalar morphometry among adolescent female MJ users (Ahmed, et al., 2008; Sakuma, 2009; Tsukahara, 2009). Cannabinoid receptor density and affinity change with sex steroid distributions across the estrous cycle (Rodriguez de
Fonseca, Cebeira, Ramos, Martin, & Fernandez-Ruiz, 1994). Further, animal studies have shown neurobehavioral ties between endocannabinoid signaling and sex hormones. Intact female rodents self administer cannabinoid agonists and display greater drug seeking and reinstatement behaviors than male or ovariectomized female rats (Fattore, et al., 2007; Fattore, Spano, Altea, Fadda, & Fratta, 2010), suggesting that females may be particularly sensitive to the reinforcing effects of MJ. With adolescent females expressing more CB1 receptors than adults and male peers (Burston, et al., 2010), the cannabinoid system may have a stronger regulatory role in teenaged girls. Thus, negative MJ effects during adolescence may leave teenage females particularly vulnerable to changes in brain volume, despite experiencing fewer adverse effects (Quinn, et al., 2008; Schramm-Sapyta, et al., 2007).

It is possible that morphologic changes associated with MJ use may be influenced by factors predating drug exposure, such as familial risk for substance use or sub-clinical mood and anxiety symptoms. Children of alcoholics demonstrate reduced right amygdalar volumes irrespective of personal alcohol use (Hill, et al., 2001), which suggests that biological factors, such as genetic loading, may account for variability in amygdalar morphometry. However, we observed larger amygdalar in female users even after statistically controlling for family history of SUD. Abnormal amygdalar volumes in female MJ users may also reflect an early marker for developing clinically significant mood or anxiety symptoms. A recent meta-analysis of amygdalar morphometry and depression reported mixed findings for larger and smaller amygdalar volumes in depressed patients (J. P. Hamilton, et al., 2008). The authors concluded that smaller amygdalar volumes were more consistent in studies of antidepressant naïve patients whereas larger amygdalar volumes may be attributable to medication treatment. Perhaps more relevant to the current study’s developmental interest, larger amygdalar volumes are observed in patients experiencing a first major depressive episode. Therefore, larger right amygdalar sizes among female MJ users may represent premorbid risk for mood disorders.
Limitations and Future Directions

This study represents the first analysis of amygdalar morphometry in adolescent MJ users. The overall sample is relatively large compared to other morphometric studies. There was sufficient power to detect interactions between gender and group, however, the number of females in each group may have been prohibitively small to detect main effects for gender. Analyses may also remain limited by cross-sectional design. Longitudinal investigations are essential to characterize the impact of gender and MJ use on amygdalar morphometry as teens enter adulthood. Prospective studies, currently underway, will be able to examine whether amygdalar dysmorphology is associated with persistent MJ use, or, conversely, amygdalar volumes normalize with sustained abstinence. As noted above, the current morphometry findings could potentially reflect some premorbid mood symptoms, which could also be reassessed with clinical outcome data measuring the development of psychopathology such as mood and anxiety disorder.

Future studies may consider exploring the interrelationships between multiple brain regions with rich cannabinoid receptor distributions, such as the hippocampus, prefrontal cortex, insula, cingulum and cerebellum. These regions are also implicated in mood dysregulation, which may further explain the connection between brain morphology, MJ and gender. Also, combining structural analyses with examining genetic polymorphisms linked to neuronal health, such as brain-derived neurotrophic factor, may shed light on individual variability in MJ-brain morphology relationships. The inhibition of fatty acid amide hydroylase (FAAH), which metabolizes cannabinoids, is associated with enhancing the analgesic, hedonic, anxiolytic and antidepressant effects (Gaetani, et al., 2009). Therefore, exploring genes encoding cannabinoid signaling like FAAH may better elucidate the underlying mechanisms of neurobehavioral risks and consequences associated with teenage MJ use.
Conclusions

In summary, female MJ users exhibit abnormal amygdalar morphometry compared male MJ users and controls. This pattern of amygdalar size deviated from what would be expected in typical brain development in healthy adolescent girls. The results also indicated that increased mood symptoms were predicted by smaller right amygdalar volume (especially in female controls) and being a female MJ user, suggesting that amygdalar morphometry and using MJ during adolescence may additively contribute to mood dysregulation and female MJ users may be particularly vulnerable to the neurobehavioral consequences of MJ use.
References


Appendices

Appendix A

*Covariate Selection: Bivariate Relationships Between Amygdalar Volumes and Substance Involvement Variables Other Than Marijuana*

<table>
<thead>
<tr>
<th></th>
<th>Kendall’s $\tau_B$</th>
<th>Right Amygdala</th>
<th>Left Amygdala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Lifetime Drinks</td>
<td>0.09</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Lifetime Withdrawal Symptoms</td>
<td>-0.10</td>
<td>-0.01</td>
<td></td>
</tr>
<tr>
<td>Recent Withdrawal Symptoms</td>
<td>-0.12</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Drinks Per Month</td>
<td>0.02</td>
<td>-0.02</td>
<td></td>
</tr>
<tr>
<td><strong>Years of Alcohol Use</strong></td>
<td>0.13</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Nicotine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fagerström Nicotine Dependence</strong></td>
<td>0.11</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Cigarettes Per Month</td>
<td>-0.01</td>
<td>-0.04</td>
<td></td>
</tr>
<tr>
<td>Cigarettes Per Day</td>
<td>0.05</td>
<td>-0.02</td>
<td></td>
</tr>
<tr>
<td>Other Drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total Lifetime Other Drug Use</strong></td>
<td>-0.13</td>
<td>-0.05</td>
<td></td>
</tr>
<tr>
<td>Other Drug Use Per Month</td>
<td>-0.13</td>
<td>-0.02</td>
<td></td>
</tr>
</tbody>
</table>

**BOLD**: selected as covariate
### Appendix B

**Regression Statistics for Modeling Right Amygdalar Volume with Inhalant and Opiate Uses**

<table>
<thead>
<tr>
<th>Overall Model</th>
<th>$F$</th>
<th>df</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.00</td>
<td>8,73</td>
<td>$&lt;0.01$</td>
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<table>
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<tr>
<th>Independent Variables</th>
<th>$t$</th>
<th>$\beta$</th>
<th>$p$</th>
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</thead>
<tbody>
<tr>
<td><strong>Intracranial Volume</strong></td>
<td>3.12</td>
<td>0.33</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Years of Alcohol Use</td>
<td>0.73</td>
<td>0.09</td>
<td>0.47</td>
</tr>
<tr>
<td>Fagerström Nicotine Dependence</td>
<td>1.43</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>Lifetime Inhalant Use</td>
<td>-0.77</td>
<td>-0.09</td>
<td>0.44</td>
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<tr>
<td>Lifetime Opiate Use</td>
<td>-1.79</td>
<td>-0.20</td>
<td>0.08</td>
</tr>
<tr>
<td>Group</td>
<td>-0.25</td>
<td>-0.03</td>
<td>0.80</td>
</tr>
<tr>
<td>Gender</td>
<td>1.69</td>
<td>0.18</td>
<td>0.10</td>
</tr>
<tr>
<td>Group*Gender</td>
<td>-2.00</td>
<td>-0.20</td>
<td><strong>0.05</strong></td>
</tr>
</tbody>
</table>

**BOLD:** $p \leq 0.05$
Appendix C

**Bivariate Relationships Between Mood, Attention and Morphometric Variables**

<table>
<thead>
<tr>
<th></th>
<th>ICV</th>
<th>Right Amygdala</th>
<th>Left Amygdala</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mood</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole Sample</td>
<td>-0.07</td>
<td><strong>-0.21</strong></td>
<td>-0.08</td>
</tr>
<tr>
<td>Female Controls</td>
<td>-0.01</td>
<td><strong>-0.57</strong></td>
<td>-0.17</td>
</tr>
<tr>
<td>Male Controls</td>
<td>0.16</td>
<td>-0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>Female MJ</td>
<td>0.41</td>
<td>-0.28</td>
<td>-0.05</td>
</tr>
<tr>
<td>Male MJ</td>
<td><strong>-0.33</strong></td>
<td><strong>-0.34</strong></td>
<td>-0.20</td>
</tr>
<tr>
<td><strong>Executive Attention</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole Sample</td>
<td>0.02</td>
<td>-0.05</td>
<td>-0.09</td>
</tr>
<tr>
<td>Female Controls</td>
<td>-0.36</td>
<td>0.35</td>
<td>0.01</td>
</tr>
<tr>
<td>Male Controls</td>
<td>-0.09</td>
<td>-0.11</td>
<td>-0.14</td>
</tr>
<tr>
<td>Female MJ</td>
<td>0.53</td>
<td>0.19</td>
<td>0.18</td>
</tr>
<tr>
<td>Male MJ</td>
<td>0.11</td>
<td>-0.07</td>
<td>-0.08</td>
</tr>
</tbody>
</table>

**BOLD: p < 0.10**