University of Pittsburgh

Annual Progress Report: 2010 Nonformula Grant

Reporting Period

July 1, 2013 – June 30, 2014

Nonformula Grant Overview

The University of Pittsburgh received $4,999,916 in nonformula funds for the grant award period June 1, 2011, through May 31, 2015. Accomplishments for the reporting period are described below.

Research Project: Project Title and Purpose

Reducing the Cognitive Consequences of Cannabis Use by Adolescents - Use of marijuana and other forms of cannabis is increasing in young adolescents. Convergent lines of evidence suggest that cannabis use impairs cognitive abilities, but proof of cause-and-effect and the brain mechanisms that explain this association are lacking. In this project we propose a set of integrated aims, employing innovative and complementary research strategies, to test a single hypothesis that onset of cannabis use before age 16 alters the developmental trajectory of neural circuits that are critical for the normal maturation of core cognitive abilities. We will also examine the feasibility of preventive interventions to reduce cannabis use in a high-risk population of 12-year-olds and conduct a research training program to engage underrepresented minorities and other undergraduates in basic, clinical, and health services research on substance abuse.

Anticipated Duration of Project

6/1/2011 - 5/31/2015

Project Overview

Cannabis is the most widely used illicit drug in the United States. In 2009, the number of 12–17-year-olds who reported using marijuana *increased* by ~10 percent, and the proportion of this age group who thought smoking marijuana carried a great risk of harm *declined*. Prolonged cannabis use has been associated with cognitive impairments. However, whether cognitive impairments persist after cessation of cannabis use and whether these impairments are more pronounced and persistent in individuals who start using cannabis during adolescence, when cognitive abilities are still maturing, remain unknown. To address these questions, it is critical to understand the impact of cannabis use on working memory (WM), a core cognitive process that matures during adolescence and is dependent on the development of the dorsolateral prefrontal cortex (DLPFC).

Based on existing literature, we propose in this project that an early age (before age 16) of onset of cannabis use (EAOC) disrupts the normal developmental trajectories of DLPFC circuits mediating WM, resulting in persistent WM impairments. However, the existing data that support this idea have several limitations. First, evidence of the long-term cognitive consequences of cannabis use in humans is limited by insufficient information about cognitive capacity prior to the onset of cannabis use. Second, no prospective studies have used assessments of WM and brain function with the sensitivity required to clearly demonstrate adverse effects of EAOC. Third, demonstrating causality requires experimental evidence of a biological mechanism that links age-related cannabis exposure with WM dysfunction.

We propose a set of integrated aims to test the central hypothesis that EAOC alters the developmental trajectory of neural circuits in the DLPFC, resulting in persistent impairments in WM function. Aim 1 examines the direction of the association between EOAC and cognitive function in epidemiological samples. Aim 2 assesses the neural substrates for the short- and long-term effects of EAOC on WM in a subset of subjects studied by Aim 1. Aim 3 directly tests tetrahydrocannabinol (THC) as a causal agent of WM impairment and DLPFC circuitry dysfunction in a non-human primate model system using the same measures employed in Aim 2. Aim 4 uses health services research methods in a cohort of 12-year-olds recruited by Aim 1 to explore novel strategies for preventing EAOC. Aim 5 seeks to train the next generation of substance abuse researchers, focusing on underrepresented minorities, by engaging talented undergraduates in the research conducted in Aims 1-4.

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Expected Research Outcomes and Benefits

The proposed studies will provide answers to the following questions that are essential for protecting the health and long-term well-being of young Pennsylvanians and for guiding legislation and public policy in substance abuse. Aim 1 will determine the risk and protective factors for EAOC. In addition, by controlling for cognitive capacity and psychiatric symptoms prior to the initiation of cannabis use, this aim will robustly reveal the strength and directionality of the association between EAOC, cognitive functioning, and symptoms of psychosis. Aim 2 will reveal the short- and long-term effects of EAOC on WM, a core cognitive process, and document the neural mechanisms that mediate these effects. By using both a cross-sectional design in young adults and a longitudinal design in 12-year-olds, the results will reveal the magnitude, dose-response relationship, and persistence of the effects of EAOC on WM and brain function. Aim 3 will provide a critical test of the cause-effect relationship between cannabis use during adolescence and WM dysfunction and reveal how cannabis alters brain development to impair WM. Aim 4 will demonstrate the feasibility of a method for implementing a computer-assisted decision support system that provides pediatricians with the resources to conduct assessments of risk for EAOC with adolescents and their parents, provide families with brief interventions to facilitate adherence to recommendations, and identify local treatment referral options tailored to individual family members. Aim 5 will deliver an effective program for engaging talented underrepresented minority and other undergraduate students in research activities designed to recruit and prepare them for a career in substance abuse research. In concert, the findings from these studies will provide critical and robust knowledge about the risk and cognitive consequences of EAOC that is currently lacking and, if our hypotheses are supported by the new data, will provide a compelling rationale for a new strategy to prevent or delay the onset of, and thus reduce the cognitive consequences of, cannabis use by adolescents.

Summary of Research Completed

Aim 1: Determine the predictors and long-term cognitive consequences of EAOC

Goal 1. Conduct secondary data analyses to: (1) Characterize risk and protective factors for EAOC using the unique and extensive measures and developmental time points that are available in unique existing data sets. (2) Explore the association between EAOC, cognitive functioning, and symptoms of psychosis, controlling for the risk factors for EAOC and measures of cognitive and psychiatric development prior to and subsequent to the initiation of marijuana use. These same analyses will be done using measures of the age of onset and the quantity and frequency of cannabis use.

Status of progress on Aim 1, Goal 1.
Analyses have now been completed to examine the first subgoal of Aim 1, Goal 1: 'Examination of the proximal and long-term impact that adolescent cannabis use has on changes in various aspects of cognitive functioning (i.e., inattention, academic performance) across adolescence in males and females. These analyses involved the youngest cohort of the Pittsburgh Youth Study (PYS) (N=503) and all youth in the Pittsburgh Girls Study (PGS) (N=2,451) using data collected

annually from ages 11 to 16. A draft manuscript documenting the findings has been prepared and is currently being finalized for submission to a peer-reviewed journal. The findings are briefly outlined below.

Data Analytic Strategy. The data were analyzed using fixed effects regression models designed to examine the association between changes in marijuana use and changes in attention and academic achievement within individuals. One strength of using fixed effect models is that all possible time-stable and pre-existing characteristics (e.g., intelligence, prenatal substance use) that vary between individuals are eliminated as potential confounds, even without including them in the model (Allison, 2009; Osgood, 2010). In essence, these models estimate the association between within-individual change in a given predictor (e.g., marijuana use) and within-individual change in an outcome of interest (e.g., attention and academic problems) over the course of time series, which is then pooled across all subjects in a sample. Because the focus is on within-individual change, pre-existing and time stable individual differences between participants cannot account for the observed associations, and this strengthens the causal inference (Allison, 2009; Osgood, 2010).

All models were run separately for the PYS and PGS samples. Due to the relatively low frequency of early marijuana use, two dummy-coded variables (0=no, 1=yes) representing whether or not youth used marijuana less than monthly or at least monthly were used as predictors in both studies (non-users were coded 0 on both variables). Contemporaneous associations between changes in marijuana use and changes in the outcomes of attention and academic problems were examined, as well as lagged associations focused on predicting change in the outcomes one year later. Contemporaneous models examined whether youth experienced greater increases in attention and academic problems during years when they used marijuana either less than monthly or at least monthly relative to years in which they did not use marijuana. Lagged models examined whether youth tended to experience greater attention and academic problems in the years immediately after using marijuana either less than monthly or at least monthly relative to the years following no marijuana use. Several potential time-varying confounds were included in the models, including linear and quadratic age trends, family socioeconomic status (SES), change in residence, single parenthood, neighborhood disadvantage, parental stress, peer delinquency, peer substance use, and conduct problems, as well as the use of tobacco, alcohol, and hard drugs.

Overview of Study Results. Models examining linkages between concurrent within-individual changes in marijuana use and changes in attention problems are presented in Table 1. In the PGS, analysis indicated that girls experienced increases in their own attention problems in years that they used marijuana less than monthly and at least monthly. In contrast, no significant contemporaneous association was found between changes in marijuana use and attention problems in the PYS.

Results examining whether youth tended to experience increased attention problems in the years following marijuana use are presented in Table 2. Findings from the PYS and PGS indicated that youth experienced a significant increase in their attention problems in the years immediately

after using marijuana less than monthly relative to years they did not use. However, youth did not exhibit a significant increase in attention problems in the years immediately after they reported using marijuana at least monthly relative to years they did not use.

Table 3 includes results examining concurrent associations between changes in marijuana use and academic problems. In the PGS, results indicated that girls experienced significant increases in their academic problems in years when they used marijuana at least monthly (but not less than monthly) relative to years in which they did not use. Analyses with the PYS indicated there was no significant association between changes in marijuana use and concurrent changes in academic achievement.

The final set of analyses examined whether youth tended to experience an increase in their level of academic problems in the years immediately following marijuana use (see Table 4). In the PYS, analysis indicated that boys experienced increases in their own academic problems in the years that followed the use of marijuana either less than monthly or at least monthly. Analyses with the PGS indicated there was no significant association between within-individual changes in marijuana use and subsequent changes in academic achievement.

Conclusions. The findings provided inconsistent support for the notion that when youth use marijuana in early and middle adolescence, they tend to experience increases in their attention and academic problems. In most instances, significant findings did not replicate across the male and female samples. For example, findings from the PGS indicated that girls experienced increases in attention problems during years when they used marijuana either less than monthly or monthly, but this association was not found in the PYS. The only finding that replicated across both studies indicated that when adolescents used marijuana less than monthly, they tended to experience an increase in their attention problems a year later. However, neither study found that using marijuana at least monthly led to a subsequent increase in attention problems, which runs counter to the notion that there is a cumulative dose-response relation between marijuana use and decreased cognitive functioning (i.e., more frequent marijuana use, more cognitive problems). It is possible that informant biases associated with using parent-reports of attention and academic problems may have led to these inconsistencies. For example, evidence from the current study (see Tables 1 and 3) and prior findings (Youngstrom, Loeber, & Stouthamer-Loeber, 2000) suggest that high levels of stress may lead parents to over-report their children's behavioral problems. Another possibility is that the longer term impact that marijuana use has on cognitive functioning is too subtle to be noticed by parents. To examine these issues further, future studies should measure attention and academic problems using behavioral performance tests or rating scales completed by alternative informants (e.g., teachers).

Future Analysis involving Thought Disorder/Psychotic Symptoms. As part of Aim 1, Goal 1, analyses are currently underway to examine the association between early-onset marijuana use and the development of thought disorder/psychotic symptoms. The investigators have now agreed on a set of items across the PYS and PGS that will be used to assess thought disorder/psychotic symptoms in late adolescence. In addition, a common data analytic strategy has been specified to examine whether growth-curve trajectories of marijuana use across early to

middle adolescence predict thought disorder/psychotic symptoms in late adolescence. Preliminary analyses examining this issue have been run using the PGS data set and will be initiated using the PYS data set within the next month.

Allison, P. D. (2009). *Fixed Effects Regression Models (Quantitative Applications in the Social Sciences).* SAGE Publications Inc., Thousand Oaks, CA .

Osgood, D. W. (2010). Statistical models of life events and criminal behavior. In A. R. Piquero & D. Weisburd (Eds.), (pp. 375-396). New York, NY: Springer.

Youngstrom, E., Loeber, R., & Stouthamer-Loeber, M. (2000). Patterns and correlates of agreement between parent, teacher, and male adolescent ratings of externalizing and internalizing problems. *Journal of Consulting and Clinical Psychology, 68*, 1038-1050.

Status of progress on Aim 1, Goal 2

Interviewing for the third year of the project began November 25, 2013, and is on schedule. The start date reflects the timing of the first assessments in Year 1. Through 6/01/14, we have assessed 78 pairs of mother/child participants in the third year of the protocol and anticipate a retention rate of 98 percent (two participants have not been followed up since Phase 1).

[Relevant Institutional Review Board (IRB) protocol titles and approvals: "Longitudinal Study of Behavior, Mental Health, and Brain Development among Adolescents Aged 12 through 15: Interview, EEG, and fMRI Studies,"1/26/2015; "Early Age of Cannabis Onset and Psychotic Symptoms: Adult Imaging," 3/6/2015.]

<u>Aim 2: Assess the short- and long-term impact of EAOC on WM and DLPFC function</u>

The purpose of this aim is to detect possible short- and long-term effects of cannabis use by assessing brain function during a spatial working memory (WM) task using functional magnetic resonance imaging (fMRI), blood oxygen level dependent (BOLD) measures, and electroencephalography (EEG) measures of gamma-band (30-80 Hz) oscillations. These measures are being made in (1) adult participants from Aim 1 to test the hypothesis that WM performance and DLPFC circuitry differ among young adults as a function of prior cannabis use, and (2) longitudinally in a cohort of 12-year-olds (identified as being at high risk of adolescent cannabis use) and later at 15 years of age to test the hypothesis that the normal developmental improvement in WM performance, and the circuitry that mediates this improvement, is altered by early adolescent cannabis use. We completed the baseline assessments of the 12-year-old participants in Year 1, reported on these data in previous reports, and are prepared for the longitudinal follow-up in year 4, testing the hypothesis that WM performance and DLPFC circuitry differ based on early adolescent cannabis use. The longitudinal studies are set to begin in September. We have also now completed the adult study.

<u>Participant Flow</u>

Longitudinal study. As reported in the initial progress report, after exclusions we successfully tested 94 12-year-old participants during Year 1, and no activities were planned for this Aim during Years 2 and 3. We will begin bringing back these teens in summer 2014.

Adult study. 74 participants ages 27-30 have successfully completed fMRI and EEG studies, comprising the final sample. Seven additional participants who could not participate in the fMRI completed the EEG session. All had a negative urine drug screen.

As a brief reminder, both fMRI and EEG studies are using a spatial working memory task, which is described in detail in the original grant application. Briefly, stimuli of varying set size that are presented serially must be retained in WM over a delay period. Participants are presented with a yellow circle stimulus appearing in one of eight possible locations in either the same location (low load) or three different locations (high load). Following a delay period, four probes located among the eight possible locations indicate a required response (button press) as to whether one of the probes had occurred in any of the previous cue locations (50 percent trials) versus all novel locations (50 percent trials). Behavioral analyses examine reaction time and accuracy across the two load conditions. EEG and fMRI studies are completed for each participant within a two-week period.

Goal 1: DLPFC Activity: Human fMRI Studies
Longitudinal Study. We reported our initial results of the Time 1 longitudinal study in previous reports. In summary, we found that participants had good performance in the scanner (accuracy=88.2+/-8.8 percent), which varied by design with task dimensions (primarily worse performance at high load). fMRI results demonstrated that a widely distributed circuitry was engaged throughout the working memory task as reflected in the extant literature including: posterior parietal cortex (PPC), thalamus, caudate, insula, pre-supplementary motor are (preSMA), superior frontal sulcus (SFS), and DLPFC.

WM development: adult versus teens
No adults were excluded due to head motion or performance. Two teen participants were excluded for >20 percent volumes discarded for head motion (21 percent and 23 percent). As expected, due to known developmental improvements in working memory, the adult group had significantly higher accuracy than the 12-year-old group (chisq=13.5, p=0.00032; see Aim 2, Fig. 1). An age-by-sex interaction was also seen, such that females performed better in the teen group, but males caught up by adulthood (chisq=4.9, p=0.029). Additionally, all participants performed worse in high-load trials (chisq=142.7, p=7e-33), and there was a trend for a greater performance decrement in teens (chisq=3.1, p=0.08). Establishing developmental improvements in performance is critical to assess possible consequences of EOAC use in normative WM development.

WM Adults: age of onset and quantity/frequency of cannabis use:
In the adult group, 34 (21 female) began to use cannabis before age 16 (early adolescent onset cannabis use, EAOC), and 40 did not use before age 16. Of the non-EAOC group, 21 (14 female) began to use cannabis before age 22 (late adolescent onset cannabis use, LAOC) and 19 (13 female) did not use by age 22 (no cannabis use, NC).

Overall, participants showed high performance (accuracy=92.6 percent+/-5.4 percent) that did not differ by cannabis onset (two groups: chisq=0.0, p=0.85; three groups: chisq=2.1, p=0.12);

although not significant, the NC group performed the worst relative to both EAOC and LAOC (see Aim 2, Fig. 2). WM load effects also did not differ by cannabis group (all p>0.2). Equivalent performance in the cannabis groups is important for probing differences in DLPFC function.

In addition to the timing of the onset of marijuana use, we also examined quantity/frequency measures of past cannabis use (average daily joints; ADJ) at ages 14, 16, and 22 (ages at which use was assessed for all adult participants). This measure was further log transformed to account for skewed cannabis use data (see Aim 2, Fig. 3). We found an effect at age 16 (chisq=4.1, p=0.047), such that more use was associated with poorer WM performance (see Aim 2, Fig. 3). This main effect and interaction were not seen with cannabis use at ages 14 or 22 (both p>0.1).

We have processed all of the fMRI data for 85 teens and the 74 adults whose behavior is detailed above. We examined only correct trials to ensure that any brain differences were not driven by trial performance. Findings show widespread activation across all participants in regions known to be part of the working memory network, including DLPFC, PPC, thalamus, basal ganglia, preSMA, and SFS. Further, many of these regions were active to a greater degree during high load relative to low load trials (see Aim 2, Fig. 4).

Groups comparisons are investigated both at the level of *a priori* defined regions of interest (ROIs) that include DLPFC and PPC and at the whole brain voxelwise level. Here we report the ROI analyses focusing on age and cannabis group comparisons on the right DLPFC and right PPC. These were defined using anatomical landmarks for the rostral middle frontal gyrus and the inferior parietal lobule, respectively, and individualized ROIs for each participant and condition were generated as the top 20 percent most active voxels for each participant in that region and condition. We examined the mean BOLD signal change in these voxels.

FMRI Development: Adult versus Teen: Results show that there were significant differences between adults and teens in right PPC during the cue (chisq=6.0, p=0.015) and target (chisq=4.5, p=0.036) periods, but not delay (chisq=2.3, p=0.13), all reflecting greater activation in adults than teens (See Aim 2, Fig. 5). There were no significant age differences in right DLPFC (all p>0.1). Connectivity analyses among ROIs showed consistent and strong positive correlations across the task (see Aim 2, Fig. 6). We found a trend towards lower connectivity during the delay period in adults versus teens (chisq=3.3, p=.073), but not during cue or target (both p>0.2).

fMRI in Adults: Age of Onset and Use of Cannabis: Effects of age of onset of use were seen in right DLPFC during the cue period (3 group: chisq=3.6, p=0.033; 2 group: chisq=3.1, p=0.085) and the target period (3 group: chisq=3.7, p=0.03), such that the NC had greater activation than both the LAOC and EAOC groups (see Aim 2, Fig. 7). No significant effects of cannabis use onset were seen in right PPC activation nor its connectivity with right DLPFC, and there were no correlations with quantity/frequency of use at any age. Next we will perform voxelwise group difference analyses across the entire brain that may reveal differences absent from the ROI approach.

In conclusion, behavioral findings confirm developmental improvements in WM performance and suggest no effect of cannabis on WM performance in adult participants. There was, however, a significant effect of quantity/frequency of cannabis use. Those who reported the greatest use at age 16, but not 14 or 22 years of age, showed decreased WM performance.

fMRI results confirmed engagement of prefrontal and parietal regions during WM performance in both groups, with adults showing greater frontoparietal activation than teens. ROI analyses in DLPFC and PPC showed significantly decreased DLPFC activation in both cannabis groups compared to the no cannabis group during the cue and target periods. We are currently performing whole brain voxelwise analyses to probe differences in the adult group regarding age of onset and frequency/amount of use. These may reveal effects on specific regions of prefrontal cortex as well as other regions in the circuitry supporting WM performance. We have begun work on a manuscript based on these findings.

Goal 2: Prefrontal Gamma Oscillations: Human EEG Studies

A total of 81 adults completed the EEG sessions (74 of whom also completed fMRI). Our analyses focused on the cross-sectional behavioral performance and EEG findings in this cohort. Participants were divided into three groups as defined above: EAOC (N=34), LAOC (N=28) and NC (N=19). Poor quality data from three participants were excluded.

WM behavioral performance

ANOVA testing of the adult participants' error rates (ER) showed main effects of working memory load ($F=51.7$, $p<.001$;) and group ($F=2.9$, $p=.063$), with NC having the highest ER and LAOC the lowest ER. There was also a group by load interaction ($F=3.78$, $p=.027$), with the high ER for high load in the NC group primarily driving this effect. Preliminary correlation analyses with neuropsychological measures examined the association between IQ and performance. High minus low load differences in the ER and reaction time (RT) were negatively correlated with IQ assessments (Stanford-Binet) at age 6 ($r=-.389$, $p<.01$; $r=.286$, $p<.05$, respectively). Accordingly, to rule out potential confounds of IQ, which was lower in the NC group, IQ scores were then entered as a covariate in the ANOVA, which decreased the interaction effect ($F=2.61$, $p=.080$). RT also showed a main effect of load ($F=394$, $p<.001$) with no group effect ($F=1$, $p=.35$) and no group by load interaction ($F=1.1$, $p=.343$).

As a more focused comparison of EAOC versus LAOC, an ANOVA was performed with just these two groups, excluding NC. Load was highly significant (p's< .001 for RT and ER) but no other main effects or interactions were significant (for ER, main effect for Group was $F= 3.0$, $p=.087$)

EEG data

For the EEG data, we have conducted preliminary analyses on 78 participants. Shown in Figure 9 are t-statistic maps comparing the high versus low load conditions across the frequency (y-axis) and time (x-axis) in the frontal (left and right, labeled Front L and Front R) and parietal (left and right, labeled Post L and Post R) regions. There was modest delay-related gamma

activity in right frontal region for the EAOC and the left frontal region for LAOC, particularly over the late-delay period. More prominent activity was observed over the parietal area over this same period. Also striking was an absence of modulation and even reversal of gamma differences for the NC group over this same period. These differences between the groups are highlighted in Figure 10. The comparisons between EAOC versus NC and LAOC versus NC both show relatively strong differences in favor of the cannabis use groups. Comparisons of the EAOC versus LAOC show minimal differences between groups frontally, while EAOC appeared to show greater right parietal gamma activity over the delay. Closer scrutiny of the gamma frequency band (30-50 Hz; Figure 11) shows that, in the late delay period of the left frontal region, there is suggestion of an ordinal progression consistent with behavioral performance, with the LAOC versus NC being the highest, followed by EAOC versus NC, with EOAC versus LAOC being slightly negative.

Conclusions. Behavioral findings suggest that early cannabis use (EAOC) was associated with worse performance compared to late use (LAOC), though there were no specific interactions of this effect with working memory load. Somewhat surprisingly, the group with no cannabis use history (NC) showed the worst performance. Before making strong inferences from this observation, the reliability of this finding will be further explored with examination of the influence of other demographic factors such as IQ. EEG analyses appear congruent with regard to the NC group showing poor delay-related gamma activity. Comparisons for EAOC versus LAOC showed largely comparable activity in frontal regions, with suggestions of greater gamma activity in the left frontal region for LAOC compared to EAOC.

In conclusion, our initial results do not show strong effects of age of onset on working memory and brain systems. This may be due in great part to the modest cannibis use at earlier ages and variability across this cohort (Fig. 3). Hence, we are currently developing models to integrate age of onset and amount of use to more precisely identify possible associations with effects of cannabis use in adolescence.

<u>Aim 3: Impact of THC exposure on cognition and brain function in adolescent monkeys</u>

Goals. Reducing cognitive disabilities caused by EAOC requires understanding how cannabis exposure affects the neural cognition mechanisms. This investigation will require controlled experiments in animals. Consequently, achieving the three goals of this aim involves training and carrying out behavioral and electrophysiological tests on adolescent monkeys subjected to chronic THC exposure and matched vehicle-exposed control animals. By simultaneously measuring spatial WM performance and recording gamma oscillations over the DLPFC, we will meet Goal 1 (to assess the influence of chronic THC exposure on WM performance) and Goal 2 (to assess the influence of chronic THC exposure on DLPFC gamma oscillations accompanying WM performance). By carrying out anatomical studies on brains from THC-exposed monkeys and controls, we will meet Goal 3 (to assess the influence of chronic THC exposure on DLPFC circuitry).

Early in the 2013-2014 reporting period, the surgical implantation of intravenous catheters and

vascular access ports was completed in the monkeys. These catheters allowed intravenous administration of the primary psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC). An intravenous route of administration was chosen to model the pharmacokinetics of Δ^9-THC derived from smoked cannabis. In total, 14 adolescent rhesus macaques (eight male, six female) were implanted with intravenous catheters and vascular access ports. After several months, one of the female monkeys in the drug-treated group developed a persistent catheter blockage. A second catheter was implanted in the contralateral internal jugular vein to continue treatment. The monkey's recovery was unremarkable, and she returned to drug treatment and task training after one week.

Also early in the 2013-2014 reporting period, a study to determine the minimum dose of Δ^9-THC required to impair performance in the 2-sample Sternberg task was completed. Data collected during this dose-determination study were used to ensure that each monkey would receive an efficacious dose of Δ^9-THC during the chronic dosing phase of this experiment while still allowing for individual differences in drug sensitivity. Drug doses (VEH, 0.12 mg/kg, 0.18 mg/kg, 0.24 mg/kg and 0.30 mg/kg) were administered in an ascending dose regimen, and each monkey received every dose. The dose range used in these studies was based on earlier evidence of effective dose ranges for peri-adolescent rhesus macaques. Drugs were administered 30 minutes prior to each session via intravenous catheter and subcutaneous vascular access ports. The 30-minute pretreatment period was based on published data regarding the pharmacokinetics of Δ^9-THC in nonhuman primates and previous work in this lab with chronic Δ^9-THC administration in rhesus macaques. In these studies, the threshold dose was defined as the dose beyond which response rates were less than half of that observed after vehicle-treatment. Only response rates observed during the first 60 minutes of each session were used to determine the threshold dose to minimize the influence of drug metabolism on this measure. The resulting individualized threshold doses were used during the subsequent chronic drug treatment period.

Chronic treatment with Δ^9-THC began in October 2013 and has continued throughout the remainder of the reporting period. Monkeys in the drug groups (three male, three female) are treated with an individualized threshold dose of Δ^9-THC immediately after their daily Sternberg training session. Mean Δ^9-THC doses are the same for both sexes (i.e., 0.24 mg/kg). Monkeys in the vehicle control groups (three male, three female) follow the same treatment schedule. All monkeys have been treated Monday through Friday of each week since treatment began. To date, treatments have been well tolerated, and no alterations in food/water intake or body weight gains have been noted.

Within four months of the onset of chronic treatment, the cognitive impact of Δ^9-THC on spatial working memory was evident in male monkeys, but female monkeys were generally unaffected. After analyzing those preliminary data, there was some concern that the detection of group differences might be impaired by a floor effect in one part of the task. The parameters were temporarily modified to boost performance in that portion of the task so that potential drug effects could be characterized accurately. Male monkeys chronically treated with Δ^9-THC continue to show impaired response accuracy and alterations in response-time distribution while

completing the 2-sample Sternberg task. In contrast, female monkeys in the drug-treated group display task performance that is indistinguishable from controls. These data continue to indicate that, in adolescent rhesus monkeys, there are sex differences in the cognitive impairment produced by chronic treatment with the primary psychoactive constituent of cannabis.

Aim 4: Assess intervention options to reduce the risk and cognitive consequences of EAOC

The goal of this project aim is to develop and pilot test a computer-assisted decision support system that provides pediatricians with resources for screening, brief intervention, and referral to treatment (SBIRT) to reduce early age onset cannabis use (EAOC). The activities for this project period July 1, 2013, through June 30, 2014, involve initiating testing in our second pediatric practice, the CCP Armstrong (Kittanning) office, as well as both practices completing recruitment for both phases of the study.

Pediatric Practice Collaborations: In addition to the Bass Wolfson (Cranberry) office, the CCP Armstrong (Kittanning) office was identified to be the second pediatric practice that would be best for initiating our study. On July 2, 2013, Dr. Clark; Ms. Damara Walters, research coordinator; and Dr. Scott Wood, database administrator, met with Carrie C. Fascetti, LSW, Pediatric PittNet administrator, in addition to CCP Armstrong research nurse Ms. Dawn Meta, to begin the training process for the assessment only condition.

This study has two sequential conditions: (1) assessment only; and (2) full implementation. Since CCP Armstrong (Kittanning) is a large practice (nine providers) with a substantial patient base in the target ages (12 through 15 years), we elected to increase the sample size for this practice from 40 to 80 (i.e., 40 participants per condition).

The next step was for our research team (Clark, Wood, Walters, Meta) to meet with the CCP Armstrong (Kittanning) practitioners and staff to describe study procedures and answer questions. The August 8, 2013, visit also provided an opportunity to install and test the tablet computers, docking station, WiFi connection, and printer. Our research team continued to be available to address any problems.

Data collection. The study design included a practitioner assessment prior to collecting parent and teen data in the assessment only condition. Data collection for the study in this practice was initiated in August 2013 with the collection of practitioner assessments on their current practices with adolescent patients. This practitioner assessment was completed by all nine providers at the CCP Armstrong (Kittanning) office (nine MDs, two CRNPs, and one PA) and entered into the database.

On August 21, 2013, parent and teen assessments for the assessment only condition were initiated in the CCP Armstrong (Kittanning) office.

Computer Assisted Decision Support System. Previously, the computer system had been developed and refined in collaboration with the University's Center for Research on Health

Care's Data Center. To assure that the computer system and related databases are functioning correctly, the data being collected are frequently reviewed by Dr. Wood. To determine the influence of this decision support system, two implementation stages will be conducted (1) an assessment only control condition and (2) a full implementation, including practitioner feedback, interpretation of assessments, intervention suggestions, and a tailored print-out provided to the patient and parents.

Assessment only condition. In the past year, we have completed recruitment for the assessment only condition in both of our pediatric practices (n=80). On August 30, 2013, Dr. Clark and Ms. Walters met with the Bass Wolfson (Cranberry) practitioners and presented the results from the assessment only condition, in addition to providing a brief training on physician reports and resources that will be used during the next study phase (full implementation). Prior to transitioning to the full implementation condition, practitioners were asked to complete a second practitioner survey. On December 11, 2013, results were presented for the assessment only condition to CCP Armstrong (Kittanning) practitioners, and the same training was provided for the next study phase. CCP Armstrong practitioners also completed a survey.

Full implementation condition. In the past year, we have completed recruitment for the full implementation study condition in both pediatric practices (n=80). On April 17, 2014, Dr. Clark and Ms. Walters presented the overall study results to Bass Wolfson (Cranberry) practitioners, and to CCP Armstrong (Kittanning) practitioners on May 21, 2014. All were asked to complete a third and final practitioner survey at that time.

Follow-up. Beginning in early August 2013, participants were emailed a link to the two-month follow up questionnaire. Upon questionnaire completion, Ms. Walters receives e-mail notification, contacts them via phone, and pays them through the UPMC WePay system. Thus far, 149 (93 percent) two-month follow ups have been completed. Twelve-month follow up assessments were initiated in early June for the Bass Wolfson (Cranberry) office and, thus far, eight have been completed.

<u>Aim 5: Promote training in substance abuse research for minority undergraduate students</u>

The goal of the PA State Undergraduate Training Program is to recruit two under-represented minority undergraduate students per year and facilitate their training in substance abuse research. The undergraduate research program in substance abuse has been highly successful in identifying and recruiting promising student candidates. Since the program's initiation, two freshman or sophomore minority students have been recruited each year, bringing the total of program participants to eight.

All applications for the 2011, 2012, 2013, and 2014 training programs were evaluated by a Selection and Advising Committee using a scoring sheet that ranked applicants according to the strength of their letters of recommendation, grade point average, and personal statements. The most promising candidates had two interviews for the fellowship and for laboratory placement.

For the third summer program (2013), 51 applications were received. Applicants who were not under-represented minorities were considered and/or referred to other programs; and applications were evaluated according to the strength of their letters of recommendation, GPA, and personal statements. Two students were recruited:

(1) Raissa Berry, mentored by John Enwright, PhD, and David Lewis, MD
(2) Alana Carr, mentored by Duncan Clark, MD, PhD

For our current summer program (2014), 54 applications were received. Successful candidates (n=2) had three interviews for the fellowship and for laboratory placement:

(1) Jennifer Cortes, a female freshman student mentored by Charles Bradberry, PhD
(2) Sennay Ghenbot, a male sophomore student mentored by Ken Fish, PhD, and David Lewis, MD

All are minority students (seven African Americans and one Hispanic/Latina) at the University of Pittsburgh in their freshman or sophomore year at the time of application.

During each summer program, the students spend 40 hours a week working on a research project under direct mentorship by a faculty researcher from the Department of Psychiatry. Each week, students attend journal club discussions (training sessions designed exclusively for the undergraduate student researchers). During the weekly training series, students meet with a variety of faculty members from the Department of Psychiatry, who discuss their research approaches and translational strategies for the study of substance abuse and related mental disorders.

Since the May 19 start of the summer portion of the 2014 program, students have also attended

two didactic meetings per week: (1) a training series designed exclusively for the undergraduate student researchers (attendance required), and (2) the Translational Neuroscience Program Seminar Series (attendance voluntary). Training sessions thus far have included lab data maintenance and ethics, animal models and substance abuse research, an introduction to postmortem human brain tissue research, and a seminar led by a psychiatry resident and neuroscience graduate student, who discussed their research training experiences.

Students were also required to attend the University of Pittsburgh's Department of Psychiatry 14th Annual Research Day on June 5, 2014. This event exposed the students to the diverse basic, translational, clinical, and health services research in our large department. The students attended the morning poster session, which featured 120 presentations by faculty, postdoctoral trainees, residents, students, and staff. They attended afternoon oral presentations, which included 12 speed "dat(a)ing" presentations given by independent investigators in the Department of Psychiatry.

Furthermore, students have attended a weekly journal club with other undergraduate neuroscience fellowship students. For this journal club, the fellows have led a discussion on papers relevant to the research conducted by their mentors (and therefore relevant to their own

projects). The papers have been presented to other students in the program and the discussions have been facilitated by Dr. John Enwright, instructor in psychiatry, who works in close collaboration with Dr. David Lewis.

Since the start of the summer, students have also participated in a clinical-exposure program that provides seven different opportunities for the students to shadow psychiatric clinicians. This program creates an opportunity for the students to become familiar with the human experience of mental illness, including substance abuse disorders. The aim of the clinical exposures is to assist the participants in developing a broader perspective from which to approach the complex problem of mental illness through research.
Upon completion of each summer program, students are given a questionnaire and asked to rate on a five-point scale the extent to which they agree or disagree with a set of statements. The students are also interviewed individually. In 2011, 2012 and 2013, the program was very well received. Areas identified as needing improvement were implemented for summers 2012, 2013, and 2014 respectively.

Since the first summer program in 2011, three of the eight students recruited into the program have gone to scientific conferences, six have presented posters locally and/or nationally, and two have co-authored papers submitted for publication. All students who completed the summer programs in 2011, 2012, and 2013 continued to participate in the training program throughout the 2011-2012, 2012-2013, and 2013-2014 academic years, spending 10-15 hours a week in their research laboratories.

Aim 1 - Study 1 - Table 1

Concurrent association between within-individual change in marijuana use and attention problems across ages 11 to 16

	Attention Problems							
	Pittsburgh Boys Study				Pittsburgh Girls Study			
Predictors	*B*	*SE*	*T*	*p*	*B*	*SE*	*t*	*P*
Age	0.283	0.301	0.940	0.348	2.014	0.280	7.20	0.000
Age^2	-0.016	0.011	-1.380	0.166	-0.079	0.010	-7.38	0.000
Family SES	0.002	0.005	0.400	0.691	0.272	0.094	2.90	0.004
Moved residence	0.051	0.080	0.630	0.527	0.015	0.080	0.20	0.845
Single Parent	-0.133	0.103	-1.300	0.195	0.169	0.098	1.73	0.084
Neighborhood disadvantage	0.004	0.005	0.680	0.494	0.018	0.006	3.05	0.002
Parental stress	0.041	0.009	4.580	0.000	0.099	0.008	11.72	0.000
Peer delinquency	0.007	0.008	0.940	0.350	0.037	0.015	2.43	0.015
Peer substance use	0.024	0.022	1.080	0.279	0.086	0.077	1.11	0.265
Conduct problems	0.050	0.040	1.260	0.209	0.270	0.042	6.46	0.000
Tobacco use[a]								
Less than monthly	0.298	0.117	2.550	0.011	0.346	0.158	2.20	0.028
Monthly or more	0.298	0.181	1.650	0.099	0.513	0.179	2.86	0.004
Alcohol use[a]								
Less than monthly	0.090	0.144	0.620	0.533	0.043	0.090	0.48	0.632
Monthly or more	-0.049	0.104	-0.470	0.638	-0.036	0.197	-0.18	0.857
Any hard drug use	-0.351	0.240	-1.460	0.145	0.237	0.388	0.61	0.541
Marijuana use[a]								
Less than monthly	0.132	0.125	1.060	0.291	0.421	0.142	2.97	0.003
Monthly or more	0.153	0.155	0.990	0.322	0.472	0.186	2.55	0.011

[a]Estimates represent the average within-individual change in attention problems that occurred during years when youth engaged in the frequency of use indicated relative to years when they did not use.

Aim 1 - Study 1 - Table 2

Within-individual change in marijuana use predicting change in attention problems in the following year across ages 11 to 16.

	Attention Problems (T+1)							
	Pittsburgh Boys Study				Pittsburgh Girls Study			
Predictors (T)	*B*	*SE*	*T*	*p*	*B*	*SE*	*t*	*P*
Age	0.113	0.407	0.280	0.782	0.869	0.448	1.940	0.052
Age^2	-0.009	0.016	-0.570	0.570	-0.037	0.017	-2.170	0.030
Family SES	0.005	0.006	0.960	0.338	0.111	0.109	1.020	0.306
Moved residence	-0.032	0.092	-0.350	0.726	0.023	0.087	0.260	0.791
Single Parent	0.001	0.121	0.010	0.991	-0.051	0.114	-0.450	0.653
Neighborhood disadvantage	0.004	0.006	0.700	0.486	0.009	0.007	1.300	0.195
Parental stress	-0.003	0.010	-0.320	0.753	0.020	0.010	2.070	0.039
Peer delinquency	0.017	0.009	1.930	0.054	0.014	0.018	0.770	0.439
Peer substance use	-0.026	0.027	-0.980	0.325	0.156	0.089	1.760	0.078
Conduct problems	-0.047	0.047	-0.980	0.325	0.030	0.050	0.610	0.542
Tobacco use[a]								
Less than monthly	0.161	0.139	1.160	0.247	-0.158	0.191	-0.83	0.407
Monthly or more	0.183	0.207	0.880	0.377	0.060	0.238	0.25	0.801
Alcohol use[a]								
Less than monthly	0.096	0.179	0.540	0.592	-0.025	0.107	-0.240	0.814
Monthly or more	-0.224	0.120	-1.870	0.062	-0.224	0.271	-0.830	0.409
Any hard drug use	0.150	0.324	0.460	0.643	0.392	0.487	0.80	0.421
Marijuana use[a]								
Less than monthly	0.307	0.150	2.050	0.041	0.371	0.181	2.040	0.041
Monthly or more	-0.005	0.201	-0.030	0.979	-0.172	0.251	-0.690	0.493

[a]Estimates represent the average within-individual change in attention problems that occurred in the year after youth engaged in the frequency of use indicated relative to years when they did not use.

Aim 1 - Study 1 - Table 3

Concurrent association between within-individual changes in marijuana use and academic problems across ages 11 to 16

	Academic Problems							
	Pittsburgh Boys Study				Pittsburgh Girls Study			
Predictors	*B*	*SE*	*T*	*p*	*B*	*SE*	*t*	*P*
Age	1.049	0.353	2.970	0.003	-0.412	0.151	-2.720	0.006
Age^2	-0.034	0.013	-2.590	0.010	0.015	0.006	2.660	0.008
Family SES	-0.008	0.005	-1.420	0.157	0.035	0.051	0.680	0.493
Moved residence	0.183	0.094	1.950	0.051	0.052	0.042	1.260	0.208
Single Parent	0.113	0.121	0.940	0.349	0.061	0.053	1.160	0.247
Neighborhood disadvantage	0.002	0.006	0.300	0.765	0.000	0.003	0.010	0.989
Parental stress	0.046	0.011	4.360	0.000	0.018	0.005	4.030	0.000
Peer delinquency	0.002	0.009	0.210	0.837	0.011	0.008	1.360	0.174
Peer substance use	0.051	0.026	1.930	0.054	0.028	0.042	0.670	0.505
Conduct problems	0.078	0.047	1.650	0.099	0.109	0.023	4.790	0.000
Tobacco use[a]								
Less than monthly	0.104	0.138	0.760	0.450	-0.005	0.085	-0.060	0.949
Monthly or more	-0.150	0.210	-0.710	0.476	0.081	0.098	0.830	0.408
Alcohol use[a]								
Less than monthly	0.251	0.170	1.480	0.139	0.030	0.049	0.620	0.538
Monthly or more	-0.034	0.121	-0.280	0.779	0.154	0.108	1.420	0.154
Any hard drug use	-0.045	0.286	-0.160	0.875	0.080	0.211	0.380	0.705
Marijuana use[a]								
Less than monthly	-0.051	0.146	-0.350	0.728	0.112	0.077	1.450	0.146
Monthly or more	0.302	0.184	1.640	0.101	0.213	0.101	2.100	0.035

[a]Estimates are the average within-individual change in academic problems during years when youth engaged in the frequency of use indicated relative to years when they did not use.

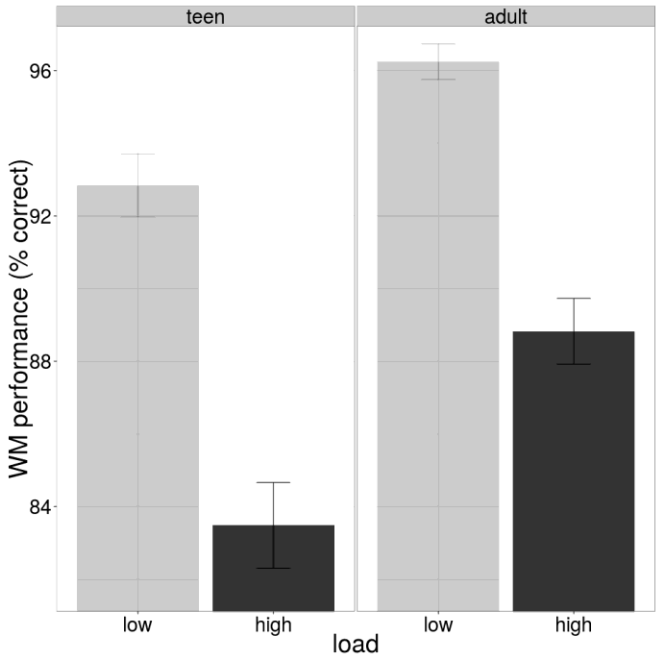
Aim 1 - Study 1 - Table 4

Within-individual change in marijuana use predicting change in academic problems in the following year across ages 11 to 16.

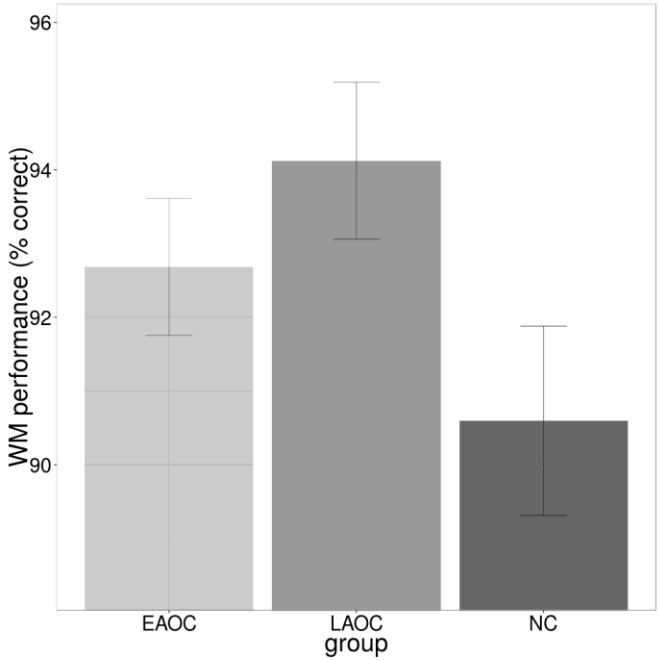
	Academic Problems (T+1)							
	Pittsburgh Boys Study				Pittsburgh Girls Study			
Predictors (T)	*B*	*SE*	*t*	*p*	*B*	*SE*	*t*	*P*
Age	1.057	0.493	2.150	0.032	-0.515	0.242	-2.120	0.034
Age^2	-0.035	0.019	-1.870	0.062	0.020	0.009	2.160	0.031
Family SES	-0.003	0.007	-0.430	0.667	0.058	0.059	0.980	0.329
Moved residence	-0.069	0.111	-0.620	0.536	0.005	0.047	0.100	0.918
Single Parent	0.161	0.148	1.090	0.277	0.091	0.062	1.470	0.143
Neighborhood disadvantage	-0.002	0.007	-0.270	0.788	0.008	0.004	2.200	0.028
Parental stress	0.018	0.012	1.430	0.154	0.007	0.005	1.320	0.186
Peer delinquency	0.027	0.011	2.430	0.015	-0.016	0.010	-1.690	0.091
Peer substance use	-0.031	0.032	-0.960	0.338	0.043	0.048	0.900	0.369
Conduct problems	-0.100	0.058	-1.720	0.085	0.020	0.027	0.76	0.448
Tobacco use[a]								
Less than monthly	-0.150	0.170	-0.880	0.378	-0.077	0.104	-0.740	0.459
Monthly or more	-0.407	0.249	-1.640	0.102	0.141	0.132	1.070	0.284
Alcohol use[a]								
Less than monthly	0.048	0.220	0.220	0.828	-0.004	0.058	-0.06	0.950
Monthly or more	-0.247	0.145	-1.700	0.090	-0.184	0.148	-1.25	0.459
Any hard drug use	-0.207	0.388	-0.530	0.594	-0.055	0.271	-0.20	0.839
Marijuana use[a]								
Less than monthly	0.360	0.181	1.980	0.048	0.115	0.099	1.170	0.241
Monthly or more	0.574	0.248	2.310	0.021	0.068	0.138	0.490	0.622

[a]Estimates represents average within-individual change in academic problems in the year following the substance use level indicated relative to years of non-use.

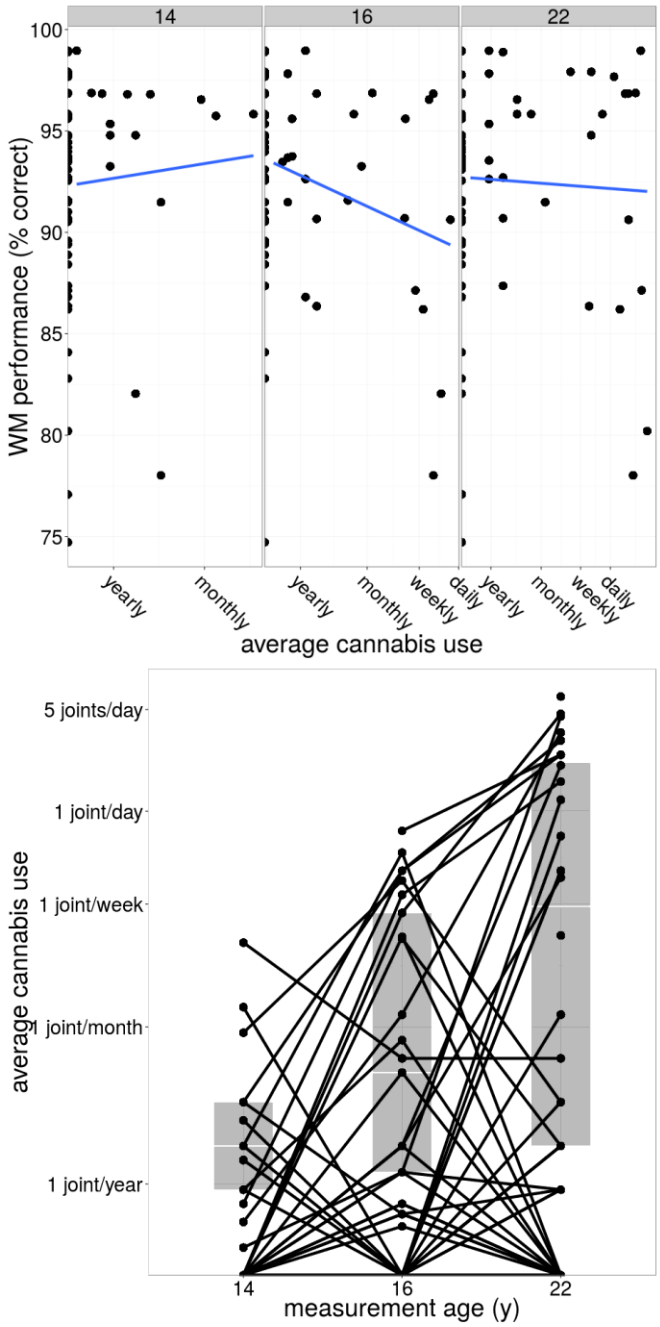
Aim 1 - Study 2 -Table 1	Total Study Year 3	03/11/14-06/01/14
Total eligible	108	65
Number with Year 3 interviews	78	45
Remainder	30	



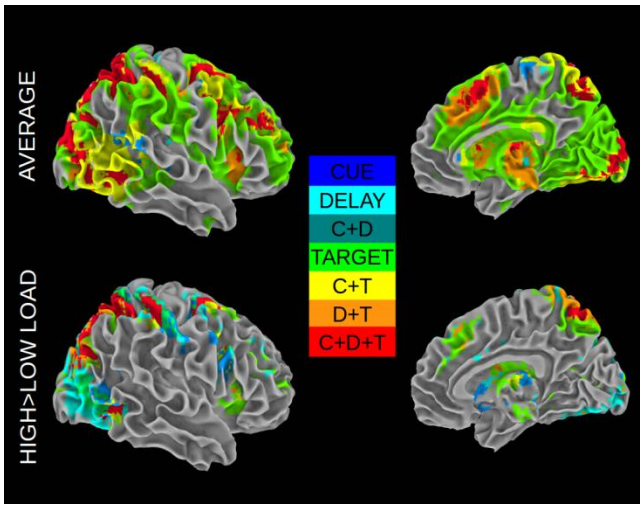
Aim 2 - Figure 1: Developmental improvement in WM performance (percent correct), and effects of load. Standard error bars shown for each group.



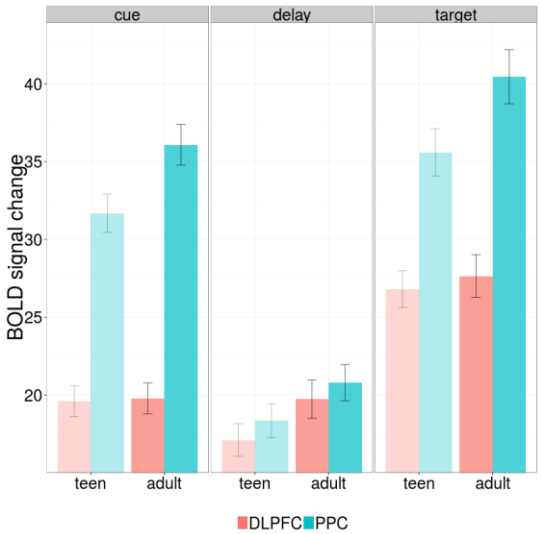
Aim 2 - Figure 2: Timing of the onset of cannabis use on WM performance (percent correct). Standard error bars shown for each group.



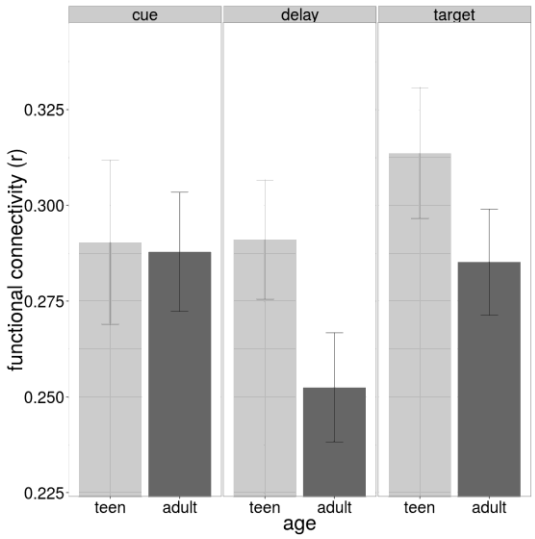
Aim 2 - Figure 3: (Top Panel) Cannabis use at age 16 but not at ages 14 and 22 was significantly associated with poorer WM performance in adulthood. (Bottom panel) Quantity/frequency of cannabis use at three earlier measured time points; data is log transformed, with within-individual data points connected by lines and box plots highlighting quartile ranges.



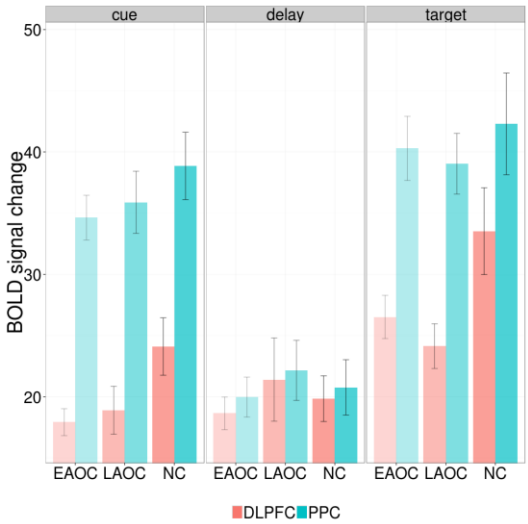
Aim 2 - Figure 4: Lateral (left) and medial (right) brain surface views of activation in right hemisphere during WM task across both loads (top) and activation greater in high load than low load (bottom) during the different WM task components. Colors depict conjunction across task components (see color legend in figure for guide). Widespread activation was seen in many cortical regions; and several of these, including DLPFC, PPC, and visual regions were recruited to a greater degree during high load.



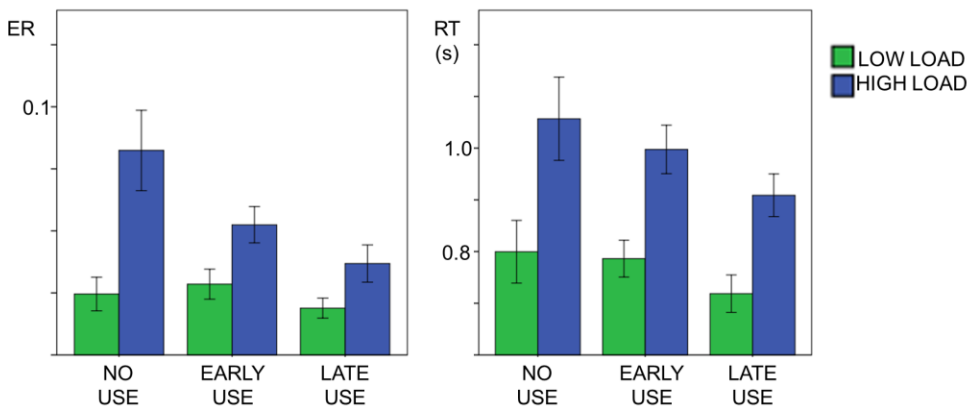
Aim 2 - Figure 5: Activation in right DLPFC (red) and right PPC (blue) during the different WM task components. There was a developmental increase in PPC activation during the cue and delay periods.



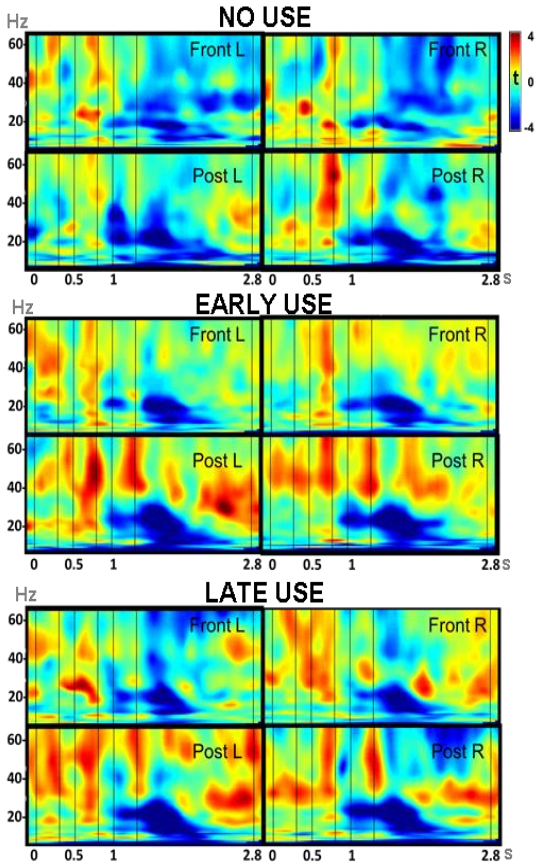
Aim 2 - Figure 6: Right DLPFC and right PPC were positively associated across all periods. There was a trend-level effect of age during the delay, such that connectivity was lower in adulthood.



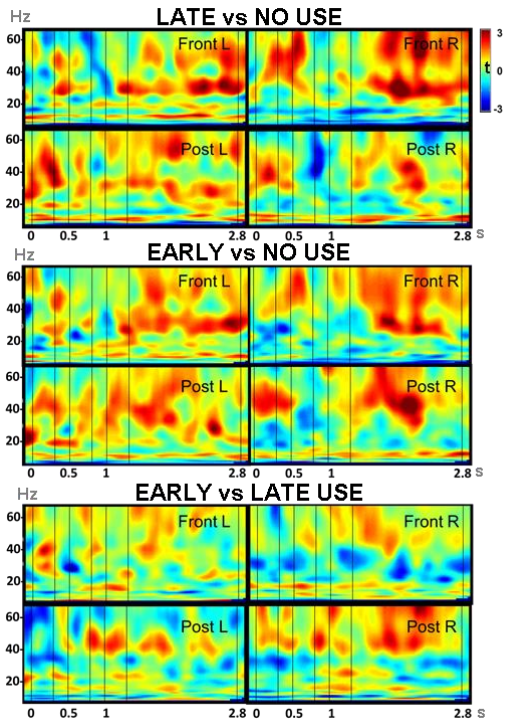
Aim 2 - Figure 7: Activation in right DLPFC (red) and right PPC (blue) during the different WM task components in the adult sample. There was greater activation in DLPFC during the cue and target periods in the NC group relative to both the EAOC and LAOC groups.



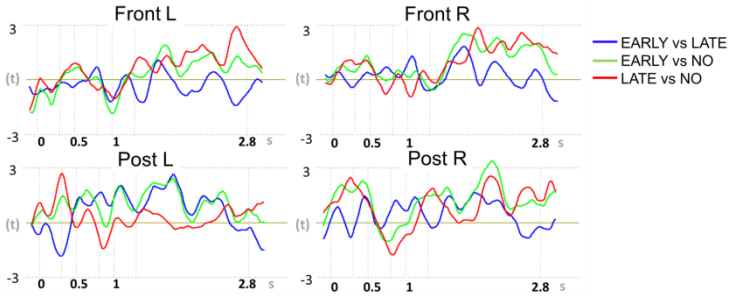
Aim - Figure 8. Behavioral performance comparing no use (NC), early use (EAOC) and late use (LAOC). Left panel shows error rates and right panel.



Aim 2 - Figure 9. Time-frequency plots of t-values for high vs. low load comparisons for no use (NC), early use (EAOC) and late use (LAOC) groups.



Aim 2 - Figure 10. Time-frequency plots of t-values for pairwise group comparisons of high minus low load power differences.



Aim 2 - Figure 11. T-value time series for pairwise group comparisons of high minus low load power differences.