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Morphometric Effects of Marijuana on the Amygdala, Hippocampus, and Nucleus Accumbens

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Introduction

The legalization of marijuana is a growing social, political, and economic issue. In the United States, twenty-four states have decriminalization and medical marijuana laws and four states have legalized recreational use of the drug, including Colorado (1,2). A 2011 report from the Center on Juvenile and Criminal Justice revealed that states which have decriminalized marijuana possession have significantly reduced violent deaths, drop out rates, property crime arrests, marijuana-rated DWIs, and non-marijuana drug arrests in youths ages 15-19 compared to states without such decriminalization laws (3). The report also noted the billions of dollars “wasted” in states without decriminalization due to the arrest and incarceration of marijuana users and the severe impact of such arrests on marijuana possessors, mostly young adults, including fines, jail time, a criminal record, and loss of student loans (4,5). Moreover, recent economic figures from Colorado, the first state to fully legalize marijuana, demonstrate an economic boost from legalization (2,6).

Alongside this burgeoning social and political acceptance of the drug, though, grows a potentially misguided belief that marijuana is also neurologically and physiologically harmless (2). According to a 2012 study by Meier et al. (7), fewer and fewer adolescents believe that regular cannabis use is harmful to health, when in fact, the science is still very much split on the topic. Much of the early medical and neurological studies of marijuana use revealed strong negative impacts, showing marijuana to be addictive and associated with respiratory illnesses and cognitive impairment (2,9,10,11). Many of these earlier studies also concluded that adolescent use of marijuana precipitated the most significant morphological changes compared to an older age of first use (2,11,12). A surge of recent studies, however, have refuted these worrying results, citing instead no significant effect of marijuana use on subcortical morphology and only an uncertain effect on cognition (2,12,13,14,15). As legalization and social acceptance spreads, it is becoming increasingly important to understand the actual effects of long-term marijuana use and the significance of use during adolescence or early age of first use.

A Morphological Approach

Studies of brain morphometry are used to reveal the neuroanatomical changes associated with differences in cognition, psychiatric/neurological status, personality, or health factors (like drug abuse) and provide insights into the underlying neural mechanisms of cognition (16,17,18). General approaches to morphometry include analysis of magnetic resonance imagery (MRI) to yield metrics of grey matter (GM) density, GM volume, and the surface area or shape of various brain structures (16,19), as well as analysis of diffusion tensor imaging (DTI) data to yield metrics of tractography or white matter (WM) integrity. The current study assayed the volume
and shape of the subcortical structures most cited to be affected by marijuana consumption: the hippocampus, amygdala, and nucleus accumbens.

**Regions of Interest**

Previous findings have suggested that cannabis use affects brain morphology most severely in regions associated both with addiction and high endogenous cannabinoid receptor-1, or CB-1, concentration: regions such as the nucleus accumbens, amygdala, and hippocampus (20). Δ9-tetrahydrocannabinol, or THC, is the main psychoactive ingredient in marijuana and is known to bind to the CB-1 receptor (20), which, though abundant throughout the brain, is particularly concentrated in the subcortical structures selected for this study. The nucleus accumbens, amygdala, and hippocampus have also been shown to exhibit neurotoxicity after chronic exposure to THC in animal studies (20,21).

The hippocampus, a component of the limbic system, is considered a main component of semantic and episodic memory acquisition and consolidation (22) as well as emotion and motivation regulation (23). The hippocampus has thus traditionally been studied in drug research due to the memory and emotional defects caused by continual use of addictive drug. In marijuana studies, the hippocampus is also implicated due to a large concentration of CB1 receptors (20). Cannabis use, for example, has been strongly associated with acute and long-term impairment of working memory (15) and multiple studies have substantiated the association between deficient cognitive processing and altered brain structure size or shape (24,25,26,27). Furthermore, as stated, early animal model research found evidence for the morphologic effects of marijuana on the hippocampus (17,20,21).

The nucleus accumbens, part of the ventral striatum within the basal ganglia, also possesses a significant history within drug research, as the proposed ‘limbic-motor’ interface at which motivation- and emotion-related processing bridge into physical action (22). A major component in the reward system, the nucleus accumbens plays a significant role in the processing of motivation, pleasure, and reward and reinforcement learning (28). It receives dopaminergic inputs directly from the ventral tegmental area (VTA) and is strongly activated in the presence of addictive drugs and natural reinforcers, such as water or food (29). Given the nucleus accumbens’ role in reinforcing pleasurable or rewarding actions, it is often implicated in the formation of addiction. In particular, addictive drugs are found to have a strong morphological and functional effect on the outer shell of the accumbens (30). Other inputs to the accumbens include the amygdala and hippocampus, and outputs include projections to the basal ganglia and the ventral pallidum (28).

Finally, the amygdala was selected as a region of interest for this study because of its similarly robust presence in drug and addiction research and its high CB1 receptor density
Widely studied and implicated in a number of psychological conditions, the amygdala is known to regulate fear learning, emotional response, reward-seeking behaviors, and long-term memory consolidation. Drug and dependency literature has strongly implicated the amygdala in drug-seeking behavior and addiction, demonstrating with multiple drugs that amygdala volume reductions correlate with increased drug craving and addiction (4,32,33). Although the amygdala is now considered more a set of independent nuclei and subnuclei than a single, coherent structure (20), most of the gross morphometry literature still reports volume and shape changes as if occurring in a single anatomical structure.

Our Data and Analytic Approach

The demographic and morphometric data of our study were collected by the WU-Minn HCP consortium of Human Connectome Project (HCP), led by the Washington University, University of Minnesota, and Oxford University. Split between two consortia of research institutions, the Human Connectome Project is a five-year project sponsored by the National Institutes of Health that has already made their extremely high-resolution structural MRI, functional MRI, DTI, and functional MEG data for 500 of the target 1200 adults freely available, online, to the research community (35). In fact, the HCP offers the highest quality data yet to be made publicly available, and it contains a diverse, representative community sample.

In the current study, the structural MRI images collected by the HCP were analyzed for subcortical volumetric changes using FreeSurfer, a surface-based brain imaging software package developed at Massachusetts General Hospital. Compared to voxel-based morphometry (VBM), an older volume extraction approach (16), FreeSurfer’s surface-based morphology (SBM) approach has been shown to have greater resolution (sub-voxel) and be more consistent in its estimation of subcortical and cortical volumes (36).

Initially created to solve the EEG/MEG inverse problem, FreeSurfer provides various pipelines to construct cortical surface models, automatically segment subcortical brain structures, and perform inter-subject alignment based on cortical folding patterns, among other functionalities (37-40). To segment subcortical structures, FreeSurfer uses a nonspatial, anisotropically-modified Markov Random Field (MRF) machine-learning model trained on manually-labeled images to produce a surface mesh that delineates a particular structure (37). The FreeSurfer model is as accurate as extensively trained manual raters and has been proven by direct comparison against histological thickness measures in the same tissue, published volumes, manually segregated MRI images in both disease and control populations, and post-mortem dissection (37-39,41,42).

The HCP data was also analyzed using FSL’s FIRST tool which automatically segments subcortical structures and performs vertex analysis for detecting group shape differences. This
software suite performs shape-analysis of subcortical structures, offering a separate means of assessing GM changes in the brain (43,44). Shape analysis offers complementary but distinct information from that of volumetric analysis, as shape analysis can determine region-specific surface deflections that may or may not effect a significant change in volume (45,46). It is expected that significant volume changes in a structure should correlate to shape changes, but significant shape deflections need not yield a significant volume change. Thus, by performing shape and volumetric analysis, our study is able to more precisely detail the morphometric effects of marijuana usage. Shape alterations of subcortical structures have been correlated with psychological illnesses such as addiction, schizophrenia, OCD, Parkinson’s disease, and Tourette’s syndrome, and shape deflections have been noted to increase with the progression of the latter four pathologies (45).

Using these two morphology metrics, the current study attempts to clarify the lack of consensus among previous marijuana morphology studies, some of which have found GM and WM changes in the brain that significantly correlate with marijuana consumption (e.g. 5,12,47-49) while others have not (e.g. 2,14,15). The current study is uniquely poised to clarify the actual impact of marijuana consumption in that it utilizes MRI data of exceptionally high resolution, cutting-edge preprocessing techniques, a statistically sophisticated analysis of both volume and shape of our regions of interest, and a large dataset (n=438) over ten times the size of many previous publications. Only a study of this sample size has the power to adequately analyze the impact of marijuana on the hippocampus, amygdala, and nucleus accumbens while controlling for the required number of covariates: tobacco use, alcohol abuse, age, and gender. Such a data-collection task that would have been insurmountable for this thesis without access to the incredible resource of the Human Connectome Project which provided this study with a much larger but also more diverse and representative population sample than is targeted in many other studies.

Review of Literature

Cannabis research has been conducted for millennia, as evidenced by medical texts from as far back as 4000 BC discussing the analgesic uses of the plant (7). In the United States, a modern scientific approach was instigated in the 1970s following the ‘Reefer Madness’ paranoia established in the 1920s, but only recently have technologies made possible the in vivo analysis of the effects of marijuana, both acute and long-term (7). Prior to modern imaging techniques, morphology studies were animal based and often involved neurobehavioral, neurohistological, and post-mortem structural evaluation. These early animal studies linked marijuana consumption to altered structure and function of the rat hippocampus (17), and demonstrated early support for the age-of-first use and duration-of-use effects seen in later human brain imaging studies. However, a 1991 review of such studies noted that while rodents displayed persistent effects
from long-term THC administration, similar exposure to marijuana smoke in peripubertal rhesus monkeys showed no neurotoxic effects, behavioral or morphological (17). Thus, from the beginning of morphological inquiry into the effects of marijuana of subcortical structures, studies have yielded seemingly contradictory results. While results have varied for subcortical structures, the effect of this drug on the cortex has generally been found to be not significant. A 2015 study by Lorenzetti and colleagues, for example, looked at the effect of marijuana on the cortex as well as on mediotemporal subcortical structures, and reported that marijuana had a selective and detrimental impact on the morphology of the mediotemporal lobe (50).

In general, a review of the effects of marijuana on various subcortical structures paints an unclear picture. A 2013 review by Rocchetti and colleagues demonstrated that only two of four region of interest (ROI) studies analyzing the amygdala found volumetric reductions, and then only in the left amygdala (51). This same review reported that only three of five ROI studies of the hippocampus found volumetric differences, here bilateral volume reductions. Another recent review noted that while bilateral volumetric reductions in the hippocampus are the most consistently reported finding, it is not uncommon for studies to concluded that no hippocampal reductions correlate with marijuana consumption (2,12,15,52), and one study even reported volumetric increases in the hippocampus with marijuana use (53). A separate 2013 review by Batalla et al. noted that the lack of uniformity of sample size (with some studies reporting structural results from as few as 15 participants), methodology, and analysis techniques across studies make it difficult to determine the true effect of marijuana on subcortical morphology (46).

These mixed results may also mean that more factors than have previously been included are at play. For example, while it has historically been very common for marijuana studies to control for alcohol use, only recently have the majority of studies controlled for tobacco use. Tobacco has been shown to interact with many of the tracts and subcortical structures implicated in marijuana studies (25,52,53,54), and the lack of consistent inclusion of this as a factor may explain the diversity of findings. In fact, a 2015 study by Wieland and colleagues stated that many morphological cannabis studies suffer from poorly-controlled or discounted comorbid tobacco and alcohol use (14).

This 2015 Weiland study, in particular, presented a strong case that marijuana is not associated with brain morphometric changes (14). In the study, 29 marijuana users and 29 age-, gender-, handedness-, race-, and education level-matched adult non-users, and 50 similarly matched adolescent users and non-users, were analyzed by Weiland and team who found no statistical shape or volume differences in the cerebellum or bilateral nucleus accumbens, amygdala, or hippocampus (14). This study carefully controlled for tobacco and alcohol use, in addition to other factors, and attributed their lack of significant morphologic effects despite
regular marijuana use in their using participants to their proper handling of these covariates. Another recent, well-controlled study similarly found that neither GM density, volume, nor shape of the hippocampus or amygdala varied significantly with marijuana use or age of first use (17), though a sub-trend-level (p > 0.1) concavity in the right amygdala was found to correlate with drug use behavior (45). These recent studies make the strong claim that previous studies citing subcortical volume effects of marijuana use may have failed to sufficiently control for environmental and behavioral factors (e.g. tobacco use).

Confounds of Marijuana Research

Tobacco use has been shown to modulate the orbital and medial prefrontal cortex (OMPFC) network that regulates cravings and drug use behavior, a network that extends into the ventral striatum and nucleus accumbens (54). Previous studies have shown significant morphological effects of marijuana use on this dependency-indicating network, but few controlled for tobacco use (8,46,47,55), thus neglecting the likely impact of tobacco in these regions. Tobacco smoke exposure in young children has also been linked to a reduction the amygdala, thalamus, and pallidum, and these morphological effects are known to persistent into adolescence and adulthood (56). Also, nicotine addiction, as assessed by the Fagerstrom Test of Nicotine Dependence (FTND), has been shown to alter cue-induced activity in the amygdala, insula, hippocampus, cerebellum, the occipital cortex, and middle frontal gyrus (53,57,58); the FTND score is the metric used in this study. While functional activation does not directly correlate with morphometry, a link between the activity and morphology has been characterized in numerous regions in the brain -- for example, between poor spatial memory and the size of the hippocampus (25, 26). Importantly, tobacco has been shown to interact with marijuana in mediating alterations to the OMPFC and may mediate the effects of marijuana in other brain regions, as well (59). Given these results, tobacco use likely impacts the same brain regions purportedly harmed by marijuana use and must therefore be controlled for in marijuana research.

Another common confound in drug studies is alcohol. The metric used in our study tabulated the self-reported frequency of drinking 5 or more alcoholic drinks in the heaviest 12-month drinking period of the participants lifetime. We chose this metric because “binge drinking” has been correlated with significant morphological changes in the hippocampus that are not present with regular, lower-dose alcohol consumption (60). Animal and human studies have also shown that binge drinking affects neurotransmitter levels in forebrain structures such as the hippocampus and nucleus accumbens, correlates with reduced hippocampal volumes, may cause apoptosis by inflammatory processes, and may also cause changes in the fractional anisotropy (FA) of many frontal white matter tracts (60,61). Moreover, for those with a large number of binge drinking episodes, alcohol use disorders have been shown to positively correlate with overall reductions in GM density and cortical volume (53).
Genetic predisposition may also be a confound and only longitudinal studies are able to directly ascertain whether morphological effects associated with drug use are caused by the drug or are indications of predisposition. It was initially assumed that strong correlation between morphology changes and marijuana use would eventually be proven to be causation, though recent genetic studies of marijuana addiction have instead demonstrated that differences between cannabis users and non-users are likely present before first use (62). A 2014 genetics study concluded that genetic variation in the endocannabinoid system expressed as variants of a specific type of cannabinoid receptor, CNRI, may mediate altered brain morphology in the orbitofrontal cortex (OFC) and anterior cingulate cortex (63), supporting the link between genetic predisposition and morphometry in at least some parts of the brain. Another study combined morphology and genetic analysis and found that heavy cannabis users have bilaterally reduced hippocampal volumes and that the lowest volumes were found in heavy users with the rs2023239 G allele (63,64), implicating both genetic and non-genetic factors in the volume reductions. Finally, a 2012 longitudinal study by Churchwell and colleagues concluded that, after controlling for age, gender, and IQ, a smaller OFC volume at age 12 correlates to cannabis initiation prior to age 16 (65). This last study, linking genetic morphology in the OFC to future marijuana use, specifically elucidates the difficulty presented by genetic predisposition.

As the recent trend in recent marijuana morphology studies has been to conclude that no significant volumetric or shape deflections due to early or regular marijuana use (2,12,15,36), it was likewise our hypothesis that the HCP data would demonstrate that neither regular use nor a young age of first use significantly impacts the shape or volume of the amygdala, hippocampus, or nucleus accumbens. While most prior studies have compared heavy users to non-users, we were able, given the quality of our data, to take an individual difference approach in examining a wide-range of levels of use in the presence of tobacco and alcohol use. Additionally, long-term marijuana use (duration) was separately considered in our study. We expected no significant morphological changes due to this metric, as well, believing all exposure-dependent metrics would confirm the lack of morphological effect from marijuana exposure.

Methods

*Human Connectome Project Participants*

HCP participants are human adult twins (MZ and DZ) and their non-twin siblings, ages 22-35 years. Sibships with individuals having severe neurodevelopmental disorders, documented neuropsychiatric disorders, diabetes, or high blood pressure were excluded, as were twins born before 34 weeks gestation and non-twins born before 37 weeks (66). Participants were recruited to ensure full representation of the ethnic and racial composition of the US population and include those who smoke, are overweight, drink, or use recreational drugs. Demographic, medical, family history, personality, cognitive, and lifestyle information is collected from each
subject over two weeks of phone and in-person interviews as well as through written assessments (e.g. the Semi-Structured Assessment for the Genetics of Alcoholism, SSAGA).

**Human Connectome Project Image Acquisition and PreProcessing**

MRI scans were collected using a Siemens 3T Connectome Skyra magnet with a customized gradient coil and gradient power amplifier that provides a maximum gradient strength of 100mT/m. A 32-channel Siemens receive head coil and a “body” transmission coil was designed for use in the smaller space of the Connectome Skyra; the gradient nonlinearity distortions due to the geometry of these customizations were corrected in the extensive preprocessing, discussed in more detail below. Dynamic head position was tracked using an optical motion tracking camera system, and cardiac and respiratory signal data was collected using a standard Siemens pulse oximeter and a respiratory belt.

Structural MRI scans were acquired at 0.7mm isotropic resolution (FOV = 224mm, matrix = 320, 256 sagittal slices in a single slab) and include a pair of T1-weighted (T1w) and a pair of T2-weighted (T2w) images; ancillary scans (B0 field map, B1- receive field, and B1+ transmit field) were also completed over each ~40-minute MRI session (67, 66). Two T1w image averages were acquired using a 3D magnetization-prepared rapid gradient multiecho (MPRAGE) sequence (TR = 2400ms, TE=2.14ms, TI=1000ms, FA = 8-degrees, band width = 210Hz/pixel, echo spacing (ES) = 7.6ms) and a phase encoding undersampling factor GRAPPA of 2. A non-selective binomial (1:1) water excitation pulse [two 100us pulses with 1.2ms spacing, 10% phase encoding oversampling (anterior-posterior), and asymmetric readout along the superior-inferior direction (z) with a 7.4us dwell time] was used to reduce bone marrow and scalp fat signaling, avoid nose wrap-around, and correct readout distortion with FSL’s FUGUE (67).

HCP imaging data undergoes multiple, cutting-edge pre-processing steps, such as field map distortion correction -- a beneficial but often neglected MRI processing step (67). Broadly, in the preprocessing steps the cortex is spatially smoothed and analyzed using surface-constrained methods that treat the convoluted cortical sheet as a 2D surface. This treatment that keeps functionally-distinct areas that sit near each other, on opposing sulcal banks, for example, as well as nearby CSF and white matter from mixing signals, producing a much cleaner, and more accurate MRI image data set (67). Preprocessing also involves registration of native-space MRI data to a standardized (MNI) template, ICBM152. ICBM152 is an averaged-composite of a population of normal human brains (n=152) that were matched to previous MNI coordinates from an earlier MNI template (68).

For volume analysis, a modified version of the FreeSurfer software suite (version 5.3.1-HCP) and a series of automatic, customized steps combining T1w and T2w scans were
used to develop accurate white and pial surface images, registered volumetrically to MNI152 space using the FMRIB Software Library (FSL) linear FLIRT tool and nonlinear FLIRT algorithm to align subcortical structures. Cortical surface alignments also produced by FreeSurfer used a hemisphere-independent registration based on population-average cortical folding patterns. Lastly, the scans were registered to the Conte69 atlas using interhemispheric landmark-constrained registration.

Shape (i.e., vertex) analyses were carried out using the FMRIB Software Library (FSL) 5.0.7 FIRST tool (69). This tool allows for a model-based segmentation and registration of anatomical images, where volumetric labels are parameterized as surface meshes. Models for each subcortical structure are based on a training set of manually traced images. Vertex locations from each participant were projected onto the surface of the average shape (transformed to MNI space). The scalar projection values were then processed with univariate permutation methods using FSL’s randomise tool, corrected at the cluster-level using Threshold-Free Cluster Enhancement (TFCE) (67,70) to a Family-Wise Error (FWE) rate of \( p \leq .05 \). Permutation testing was completed by FSL’s Randomize function using a determined number of Monte Carlo simulations (5000, in this study) to test for individual differences as a function of marijuana use.

Our Analysis of the HCP data

Correlational analyses of the FIRST shape data were performed using the following factors: number of self-reported lifetime marijuana use episodes (“times used”), duration of marijuana use, current age, gender, number of self-reported heavy drinking episodes during the heaviest period of drinking, and Fagerstrom (FTND) tobacco dependence score. In one analysis, contrasts were defined to examine positive and negative correlations of both number of marijuana uses and duration of use, controlling for age, gender, alcohol use, and tobacco use. In a separate analysis, contrasts were defined to examine the effects of the confounds age, gender, alcohol use, and tobacco use. As a follow-up analysis, we substituted duration of marijuana use with the age of first marijuana use. This analysis was used to determine if duration of use could be separated from age of first use. As discussed, age of first use has been cited as a strong correlate to morphometric differences in earlier MRI studies, but newer analyses have challenged the significance of early first use.

Current age was converted to a Z-score for each subject and gender was represented numerically (-1 for female, 1 for male) to assist in the analyses. Age of first use and number of times used were self-reported variables listed within ranges. Age of first used was as follows (Figure 1a): 0 (never used), 1 (first use at less than 15 years of age), 2 (15 to 17 years of age), 3 (18 to 20 years of age), and 4 (older than 20 years of age). We approximated these ranges as 14 (if the age of first use score was 1), 16 (if the score was 2), 19 (if the score was 3), and 21 years of age (if the age of first use score was 4) for ease of analysis. This approximated age was then
subtracted from a subject's current age to determine approximate duration (Figure 1c). If the subject had never used, duration was set to zero. Number of times used was also a self-reported range (Figure 1b), with values 0 (never used), 1 (1-5 uses), 2 (6-10 uses), 3 (11-100 uses), 4 (101-999 uses), 5 (above 1000 uses). Duration, approximate age of first use, and number of times used were Z-scored for all subjects prior to analysis. Figure 1 visually demonstrates the distribution of the critical variables of interest: age of first use, duration of marijuana use, and number of times used.

Figure 1. In all graphs, bars are labelled with participant count in each bin. Graph A shows the distribution of age of first use across our sample. The majority of participants who use marijuana began using after 20 years of age. Graph B shows the distribution of Times Used, and graph C the distribution of duration. In both graphs B and C, the obvious majority are non-users (far left column in each graph).

Alcohol abuse and Fagerstrom FTND score were determined using the SSAGA interview protocol and evaluation. Alcohol abuse was collected from the self-reported frequency of drinking 5 or more alcoholic drinks in the heaviest 12-month drinking period of the participant’s lifetime, with scores of 4 (more than 3 days a week), 3 (1-2 days per week), 2 (1-3 days per month), 1 (1-11 days a year), 0 (never). This metric can be considered a “binge drinking” score. As stated, animal and human studies have shown that the effects of binge drinking include changes in neurotransmitter levels in many forebrain structures such as the hippocampus and nucleus accumbens, cell death by inflammatory processes, reduced hippocampal volumes, and changes in fractional anisotropy of many frontal white matter tracts (61,60). Additionally, binge drinking is more common in American society (24.6% of people 18 years or older, in the past month) compared with alcohol use disorders (7.1% of the population), and thus better represents the damaging alcohol experiences of our sample (71).
The Fagerstrom FTND score reflects severity of tobacco dependence, with values of 0 (not dependent), 1-2 (mild dependence), 3-4 (low to moderate dependence), 5-7 (moderate dependence), and 8+ (heavy dependence). In the HCP database, FTND scores greater than 6 were recoded as 6. Both of the tobacco and alcohol scores were converted to a z-score for each subject.

Results

Shape and Duration

A significant positive correlation (p<0.05) was observed between duration of marijuana use and the shape of the left dorsomedial amygdala and the right ventral hippocampus (Figure 2), and a significant negative correlation (p<0.05) was seen between duration of marijuana use and the shape of the left accumbens. Only a trend level negative correlation (p<0.1) with duration was found in the right accumbens (Figure 3).

Figure 2. A significant positive correlation (p<0.05) was seen between duration of marijuana use and the shape of the left dorsomedial amygdala (left) and the right ventral hippocampus (right). The convex regions are in orange.

Figure 3. A significant negative correlation (p<0.05) of duration was found in the left anterior nucleus accumbens (left) and a trend level negative correlation (p<0.1) was found in the right accumbens. The concave regions are in blue.
**Shape and the Number of Times Used**

In the left posterior hippocampus and left ventral and dorsal amygdala, a trend-level negative correlation with times used was seen (p<0.1) (Figure 4).

![Figure 4. At the trend level (p<0.1), a negative correlation with times used was seen in the left posterior hippocampus (left) and the left ventral and dorsal amygdala (right). The concave regions are in blue and pointed to by blue arrows.](image)

**Shape and Age of First Use**

The shape of the nucleus accumbens, hippocampus and amygdala were not found to vary with age of first use.

**Volume**

The volumes of the hippocampus, amygdala, and nucleus accumbens did not vary with duration, number of lifetime marijuana use episodes (“times used”), or age of first use.

**Covariates**

Attention was paid to the impact of our covariates (Table 1): age, gender, tobacco dependence (FTND score), and alcohol abuse (“binge drinking” score). In terms of volume, age and gender often correlated with significant volumetric differences among participants. For example, women were seen to have a larger (by volume) left hippocampus than men (Table 1).
Table 1. Results of volume analysis demonstrating trend (T, $p>0.05$) and significant results of age and gender in various subcortical regions of interest (*, $p<0.05$; ***, $p<0.001$).

<table>
<thead>
<tr>
<th>Structure</th>
<th>Factor</th>
<th>$t$</th>
<th>Significance</th>
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</thead>
<tbody>
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<td>0.056 $\tau$</td>
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<tr>
<td></td>
<td>Gender</td>
<td>-4.139</td>
<td>0.000 ***</td>
</tr>
<tr>
<td>Right Hippocampus</td>
<td>Age</td>
<td>2.448</td>
<td>0.015 *</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>-5.146</td>
<td>0.000 ***</td>
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<tr>
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<td>Age</td>
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<td>0.085 $\tau$</td>
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<tr>
<td>Left Accumbens</td>
<td>Gender</td>
<td>-1.661</td>
<td>0.09 $\tau$</td>
</tr>
</tbody>
</table>

In terms of shape, gender presented the greatest significance among the covariates, with males demonstrating a more concave lateral and medial left hippocampus and a more convex dorsal-anterior left hippocampus than females (Figure 5). Additionally, males were seen to have a small convex patch on right dorsal hippocampus ($p<0.05$) and, at a trend level, a more concave right lateral hippocampus ($p<0.1$) (Figure 6).

![Figure 5. Left hippocampus shape differences between men and women. Men demonstrated more concavity around the lateral borders and posterior of the left hippocampus than women ($p<0.05$, left and right), and a small convex protrusion on the left dorsal-anterior hippocampus compared to women ($p<0.05$, left).](image)
Figure 6. Males demonstrated a small, trend-level convex region dorsal on the right hippocampus (p<0.1, left) and a significant concave region on the right lateral hippocampus compared to females (p<0.05, middle and far right). The far right view is of the posterior right hippocampus. Concave regions are in blue, convex in orange.

Lastly, males demonstrated a more convex right nucleus accumbens, and a more concave left nucleus accumbens than females (Figure 7).

A significant positive correlation (p<0.05) was also seen between alcohol use and the shape of the right nucleus accumbens and a significant negative correlation (p<0.05) was seen between age and the shape of the right hippocampus. At the trend level, a negative correlation was seen between tobacco dependence and the shape of the left hippocampus.

To determine factor covariance, Pearson’s r was computed for each factor pair in SPSS (Table 2).
Table 2. Correlation pairs are presented with the r value, two-tailed significance, and N listed for each factor. Times used and duration are highly correlated with nearly every factor. Age = current age; AFU = age of first use; Alc = alcohol score; Tob = tobacco (FTND) score; Dur = duration.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>TimesUsed</th>
<th>AFU</th>
<th>Alc</th>
<th>Tob</th>
<th>Gender</th>
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<td>.049**</td>
<td>.029</td>
<td>.044</td>
<td>.053</td>
<td>.049</td>
<td>.317**</td>
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<td>.535</td>
<td>.652</td>
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**: Correlation is significant at the 0.01 level (2-tailed).

As Table 2 indicates, the participant’s number of times used is highly correlated with both duration ($r^2 = 0.498$, meaning that ~50% of the variance in times used can be explained by duration) and age of first use ($r^2 = 0.299$).

Discussion and Conclusions

As discussed above, the scientific literature on the effects of marijuana use on various subcortical structures has not yet found a consensus to whether marijuana use correlates with morphology changes in mediotemporal structures. While some studies have concluded that no reduction is taking place -- in the hippocampus, for example (2,12,15) -- other studies will conclude that the region is in fact expanding in shape (46), while other studies will conclude that no significant volume or shape change is occurring (2,12,14,15,45). Such mixed results strongly imply that additional factors exist beyond what have been so far considered. For example, it was discussed earlier that while it has historically been very common for marijuana studies to control for alcohol use, only recently have the majority of studies controlled for tobacco use.

The current study controlled for both tobacco and alcohol, and framed the question of marijuana impact in three ways: the effect of early age of onset, duration of marijuana use, and total number of times of marijuana use. Interestingly, we found that age of onset and number of
times used did not have a significant relationship with subcortical shape or volume. In fact, only the metric of duration was seen to significantly interact with subcortical shape.

These results suggest that marijuana does have an impact on the morphology of the subcortical regions considered, but only in terms of duration of use, which was seen to correlate with significant morphological deflections in the right hippocampus, left amygdala, and left nucleus accumbens. These duration-dependent results are interesting, as few studies have looked specifically at the number of years over which a marijuana-using participant has been smoking, tending to focus more on number of times used and age of first use. As discussed, a recent study by Weiland and colleagues (14) performed a stringent analysis of adult and adolescent daily marijuana users matched in alcohol and tobacco use, as well as other metrics, against their marijuana-free peers and found no significant morphological differences in same the regions of interest as considered in our study. While both the current study and the Weiland and colleagues’ studies controlled for comorbid alcohol and tobacco use, Weiland’s study only compared current daily users with non-users. The current study considers not just a “times used”-dependency in marijuana’s effect on morphology, but also an age-of-onset and a duration-of-use dependency. It is possible that the significant effect of duration revealed in our study was simply not explored in the Weiland study or previous studies that have found no effect of marijuana of subcortical morphology (45, 14,15,12,2).

The current study also reports specific regions of significant deflection associated with marijuana use. As stated, prolonged duration of marijuana use, defined as the time from first use to present age, was shown to correlate with the hippocampus being more convex in the ventral region of the right hippocampus and the left amygdala being more concave in the dorsomedial region. Though the significant deflections were not ipsilateral, axon projections from the dorsal-anterior region of the hippocampus are known to link to the basomedial and medial nuclei of the amygdala (72). This potential connectivity correspondence between regions with significant shape deflection of the hippocampus and amygdala may imply a bilateral impact of marijuana on the connectivity of the hippocampus and amygdala. Future diffusion tensor imaging (DTI) studies could determine whether such implied connectivity differences exist between these regions based on marijuana use. Moreover, by reporting the deflections of specific regions, particular receptor types (which are dispersed in a fairly region-specific way across the hippocampus) more responsive to marijuana consumption may be targeted for further study. Genetic assay has identified multiple discrete gene expression domains in the hippocampus that govern the expression of adhesion molecules and ion channels (72; marijuana’s repeated interaction with these specific biomolecules may be the cause of the differences in the morphology observed in the hippocampus due to long-term marijuana use.
Lastly, this current study found that with increased duration of marijuana use, the nucleus accumbens was also more concave. While the nucleus accumbens’ significant deflections occurred in its dorsal and ventral regions, it remained unaffected along its lateral circumference. The locations of concavity may be significant as the shell and core of the accumbens are known to express different receptors and communicate with different regions of the brain (73). Here as well, marijuana-susceptible mechanisms unique to different regions of the nucleus accumbens could be identified with future histology studies guided by high-resolution shape deflection data, thus clarifying the mechanism of impact of marijuana. The current study also observed that the shape deflection in the nucleus accumbens was only significant in the left hemisphere and just trend-level in the right. Given the strong link between marijuana consumption and adult-onset schizophrenia, the fact that the accumbens significantly deformed on the left side only may be significant as reductions in the left nucleus accumbens have been linked to linguistic thought disorders (a core feature of adult onset schizophrenia) (74). Additionally, this result seems to corroborate findings that marijuana consumption is linked to a significant reduction in verbal memory tasks and verbal IQ (10,18,75).

Limitations

As this study is not longitudinal, the cause of the significant shape deflections cannot be precisely determined nor the possibility of genetic predisposition to prolonged marijuana use be ruled out. This study was also limited by the drug history information collected from each participant by the HCP. As noted in the methods section, the age of first use and number of times used data was collected using arbitrary ranges. In particular, the number of times used score presented widely different categories for participants to select, ranging from 1-5 times used to “more than 1000.” This substantial difference in the mathematical range of each category presents a difficulty in the analysis, as a score of 1 (1-5 uses) is not as far from a score of 2 (6-10 uses) as a score of 2 may be from a score of 3 (11-100 uses). A similar difficulty and limitation was encountered in the age of first use scores.

Additionally, while tobacco and alcohol were controlled using scores (FTND and the “binge score,” respectively) believed to best represent the impact of comorbid substance use, it is possible that alternative metrics would change the representation of variance due to these substances. As it stands, the current alcohol and tobacco use scores presented significant co-variance with duration, age of first use, and times used, highlighting both the need to control for these factors and the importance of a data-set large enough to separate the effects of each variable.

Lastly, while the volume and shape analyses provide powerful ways to non-invasively understand the anatomical changes occurring with a brain, they are limited. Specifically, they
cannot elucidate the microscopic changes responsible for the more macroscopic GM and WM impacts. For example, macroscopic morphological changes could be caused by neuronal loss or changes in cytoarchitecture such as neuronal size, dendritic spine density, dendritic length, or synaptic protein levels (16). As such, morphology studies may strongly inform where such changes are occurring, but cannot pinpoint the microscopic causes of these structural changes.

Future Directions

As an exploratory analysis not reported here, the impact of marijuana use on cognitive functions, including emotion recognition, working memory, and verbal and episodic memory, was tested. Initial results seemed to implicate marijuana in an inability to recognize particular emotions, namely happiness. Further inquiry into the effect of marijuana on cognitive tasks and their correspondence with shape and volume changes of the subcortical structures analyzed in this study would be interesting, and potentially provide insight into the cognitive effects of morphological changes caused by marijuana.

It would also be worthwhile to explore how the connectivity is or isn’t changing with marijuana consumption, and Diffusion Tensor Imaging (DTI) data is available for download and analyses through the Human Connectome Project. Connections between the amygdala and hippocampus and nucleus accumbens would be of particular interest to me, as the shape analysis results of this current study implied the possibility of an altered connectivity between these regions. In terms of the scientific literature about marijuana’s effect on brain tractography, a 2013 meta-analysis by Batalla and colleagues found that two of four studies using DTI to quantify morphological changes from marijuana usage cited increased mean diffusivity (MD) in the corpus callosum and in the right genu (46, 47), with reductions in fractional anisotropy (FA) of the left frontal tracts (46, 55). This FA reduction and increased MD may correlate to decreased axon density in these tracts (greater and more spherical movement due to a lack of axon impedance). The review also noted that all studies which looked at connectivity of the frontal control network in relation to marijuana use found greater connectivity between right frontal control network and substantia nigra/subthalamic nucleus (8,10). From the literature and the results of this study, there does seem to be a likely impact of marijuana use on brain connectivity and a logical next step is to take a look at the HCP’s tractography data.
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References


