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Neurite Orientation Dispersion and Density Imaging (NODDI) of Brain Microstructure in Adolescent Cannabis and Nicotine Use

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Abstract: ***Introduction:*** Despite evidence suggesting deleterious effects of cannabis and nicotine tobacco products (NTP) use on white matter integrity, there have been limited studies examining white matter integrity among users of both cannabis and nicotine. Further, updated white matter methodology provides opportunities to investigate use patterns on neurite orientation dispersion and density (NODDI) indices and subtle tissue changes related to the intra- and extra-neurite compartment. We aimed to investigate how cannabis and NTP use among adolescents and young adults interacts to impact white matter integrity microstructure. *Materials & Methods:* 221 participants between the ages of 16-22 completed the Customary Drinking and Drug Use Record (CDDR) to measure substance use and a magnetic resonance imaging (MRI) session. Participants were divided into NTP-control and NTP groupings and cannabis-control and cannabis groupings (≥26 NTP/cannabis uses in past 6 months). Tract-Based Spatial Statistics (TBSS) and two-way between-subjects ANOVA investigated the effects of NTP use group, cannabis use group, and their interaction on fractional anisotropy (FA) and NODDI indices while controlling for age and biological sex. *Results:* NTP use was associated with decreased FA values and increased orientation dispersion in the left anterior capsule. There were no significant effects of cannabis use or the interaction of NTP and cannabis use on white matter outcomes. *Discussion:* NTP use was associated with altered white matter integrity in an adolescent and young adult sample. Findings suggest NTP-associated alterations may be linked to altered fiber tract geometry and dispersed neurite structures versus myelination, as well as differential effects of NTP and cannabis use on white matter structure. Future work is needed to investigate how altered white matter is related to downstream behavioral effects from NTP use.

Keywords: Nicotine; Cannabis; Co-Use; Adolescence; Young Adulthood; White Matter; Neurodevelopment.

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1. Introduction

Adolescence and young adulthood mark periods of protracted neurodevelopment [1,2]. Subtle neurobiological processes associated with typical neuromaturation changes, such as synaptic pruning and white matter microstructure development, continue in humans until their mid to late twenties [3]. During this developmental window, neural substrates are sensitive to environmental influences that may alter health outcomes [4]. Substance use during this period is of great importance considering that use, including cannabis and nicotine, often starts and escalates during adolescence [5]. In 2022, 31% of high school seniors reported using cannabis and 25% reported using nicotine/tobacco products (NTP) in the past 30 days (Miech et al., 2023).

Due to the timing of these factors, studies investigating the effects of substance use on brain health using advanced neuroimaging approaches have increased over the last several decades. One key brain health outcome includes the examination of white matter tissue integrity, because white matter has the most prolonged period of development, with microstructural and architectural changes occurring well into late young adulthood [6]. White matter consists of myelinated axons of neurons that support fast communication within the brain [3]. Tracking of white matter volume and microstructural indices have shown differences not only within adult clinical populations [7–9] but across neurodevelopment [1,6], including in substance-using populations [10,11]. As adolescents age and their brains undergo neuronal pruning, white matter markers of tissue health, such as fractional anisotropy (FA) increase, suggesting better coherence and compactness of fiber tracts and, thus, better white matter integrity [12]. However,

neuroimaging studies in adolescents who use substances suggest that white matter may exhibit abnormal neurodevelopmental processes [11].

Cannabis has been noted as a potential exogenous factor that may have a deleterious impact on white matter development. Cannabis acts on the endocannabinoid system, which is thought to mediate synaptic and cellular changes that influence pruning and cellular migration during adolescence [13,14]. Cross-sectional and longitudinal studies have demonstrated an association between decreased white matter integrity and adolescent and young adult cannabis use in both association and projection white matter fiber tracts [10,15,16]. Although early evidence suggested that cannabis use leads to poorer white matter integrity (as evidenced by decreased FA values and changes in other common diffusion tensor imaging estimates) in adolescents and young adults, additional studies have found no relationship between cannabis use and white matter integrity [17–19], demonstrating that the nature of these relationships remains unclear.

NTP use in adolescents is also thought to impact white matter development through chronic activation of nicotinic acetylcholine receptors [20]. It has been hypothesized that constant cholinergic stimulation may promote glial proliferation leading to changes in white matter integrity during development [10]. Yet, there are far fewer studies as compared to cannabis and the findings are mixed, with some demonstrating increased white matter integrity among adolescents with tobacco exposure compared to their non-using peers [21]. Yet others have found the inverse relationship [22], indicating that early nicotine use might be associated with deleterious white matter health trajectories during adolescence and young adulthood [23].

The use of both cannabis and NTP is increasingly prevalent, with up to 37% of young adults reporting both cannabis and NTP use [24], and may result in differing outcomes compared to use of either substance in isolation [25–27]. Despite these prevalence rates, few studies have examined the effects of cannabis and NTP use on neuroimaging outcomes [28]. The studies that have been completed from our laboratory show increased white matter tissue cerebral blood flow and poorer white matter integrity (i.e., decreased FA) among cannabis users without history of nicotine [29] and unique white matter profiles in nicotine and cannabis use groups; for example greater cannabis use was associated with greater FA in bilateral regions of the cingulum and the left fornix tracts, but only among those also reporting a history of nicotine [30]. These studies demonstrate that the interaction between cannabis and NTP use may lead to unique white matter morphometry in youth, and even introduce the possibility that NTP use may diminish or rescue the impact of cannabis use on the brain at an early age, prior to a long-term and chronic use history.

While outcomes such as FA and mean diffusivity (MD) have most commonly been used to measure white matter integrity, additional diffusion imaging techniques have been developed to help parse out the complicated structure of white matter [31]. Neurite orientation dispersion and density imaging (NODDI) is an approach to measure both the intra and extra-neurite water diffusion. NODDI provides important markers of neurite density, the concentration of tissue comprised by axons, and orientation dispersion index (ODI), which reflects the neurite structure (i.e., the bending and fanning of axons and dendrites in white matter; [32]. These measures provide greater specificity to microstructural features compared to broad strokes DTI measures such as FA [33] and evidence suggests that neurite density in particular may be more sensitive to changes that occur in early adolescence [34]. While some studies have utilized NODDI measures to investigate the pathology of diseases such as Alzheimer's Disease [35] or broader psychiatric disorders [36], their use to investigate the impact of substance use on white matter health in adolescent or adult populations is largely nonexistent, except for one study exploring the impact of binge drinking in adults [37].

The current aims of the study are to investigate how cannabis and NTP use interact among adolescents and young adults and relate to lesser-studied white matter tissue health metrics. While studies remained mixed, the majority of studies have found decreased white matter integrity among adolescent users of cannabis and increased white matter integrity in among adolescent NTP users, therefore, we hypothesize that cannabis use would be associated with decreased white matter integrity on measures of FA, ODI, and neurite density [16]. Inversely, we predicted that NTP use would demonstrate increased white matter integrity on all three measures based on the majority of nicotine-related findings to date [21]. Finally, we hypothesized that there would be an interaction between NTP and cannabis use on white matter health, as the strength of the relationship between cannabis and white matter integrity outcomes may be diminished (i.e., less deleterious) for those also using NTP.

2. Materials & Methods

2.1. Participants

Data for this report were culled from a recently completed study on the effects of nicotine and cannabis co-use on brain structure and function during adolescence/young adulthood. As previously reported [29,30,38,39], late adolescents/young adults (ages 16-22) were recruited through physical and electronic flyers at local high schools, community colleges, four-year universities, as well as social media sites. Potential participants completed a screener via phone call to determine eligibility and establish substance use group classification at study enrollment. Recruitment and enrollment eligibility groups were determined based on past six-month cannabis and NTP use episodes and were defined as 1) frequent cannabis use only (≥ 1 weekly average cannabis use episode), 2) frequent NTP use only (≥ 1 weekly average NTP use episode), 3) frequent cannabis and NTP use (≥ 1 weekly average cannabis and ≥ 1 weekly average NTP use episode), and 4) controls (≤15 cannabis and NTP uses in the past 6 months). Groups described here were for study enrollment purposes and not used for statistical analysis. Additional exclusionary criteria included current or past DSM-5 psychiatric disorder other than cannabis and/or tobacco use disorder, any lifetime illicit substance use > 10 times, acute influence of alcohol or cannabis use at study visit (confirmed with breathalyzer, urine, and oral fluid toxicology), major psychiatric or medical issues, use of medications affecting the brain, MRI contraindications (e.g., implanted metal, and metal braces), or history of developmental disability or prenatal substance exposure.

In order to investigate the effects of regular substance use, all participants were classified into two cannabis using groups: 1) regular cannabis uses (≥26 episodes of past 6-month cannabis use, or more than weekly, on average) and 2) cannabis controls (<26 episodes of past 6-month cannabis use). Additionally, all participants were also classified into two NTP use groups: 1) NTP use (≥26 episodes of past 6-month NTP use, more than weekly, on average) and 2) NTP controls (<26 episodes of past 6-month NTP use, less than weekly, on average). This re-grouping resulted in 221 subjects maintained for the present analyses. Past 6-month use patterns rather than past year were used to account for more recent use. Infrequent nicotine and cannabis use were included in both controls groups due to differences noted in casual substance use compared to regular use [40,41]. See Table 1 for substance use characteristics by group.

Table 1. Cannabis and Nicotine/Tobacco Product (NTP) Use Descriptives.

	Cannabis Use (CU)			**NTP Use**		
M(SD)/%	**Cannabis Controls (N=94)**	**Cannabis Use (N=127)**	**P-value**	**NTP Controls (N=127)**	**NTP Use (N=94)**	**P-value**
Age	19.2 (1.7)	19.7(1.5)	0.03	19.3 (1.6)	19.8 (1.5)	<0.01
Sex (% Male)	57.4%	63.0%	<0.01	48.0%	62.8%	0.04
Race (% White)	47.9%	52.0%	0.03	46.5%	55.3%	0.23
Years of Education	12.9 (1.7)	13.2 (1.4)	0.18	12.9 (1.6)	13.3 (1.4)	0.05
Substance Use						
Ever Used Cannabis	57.4%	100%	<0.01	68.5%	100.0%	<0.01
Past 6-month CU	3.8 (6.0)	252.5 (375.1)	<0.01	92.8 (151.2)	219.5 (431.6)	<0.01
Days Since Last CU	103.5 (187.3)[a]	2.7 (4.5)	<0.01	14.7 (30.6)	50.2 (152.0)	0.03
Lifetime CU Episodes	69.7 (186.3)[a]	1145.4 (2025.2)	<0.01	382.8 (757.8)	1100.1 (2277.5)	<0.01
Age of First Regular CU	17.2 (1.3)[b]	17.8 (1.7)	0.15	17.8 (1.6)	17.6 (1.7)	0.44
Ever Used NTP	46.8%	88.2%	<0.01	48.8%	100%	<0.01
Past 6-month NTP Use	507.7 (1477.1)	905.6 (2661.0)	0.16	2.2 (4.5)	1728.4 (3183.7)	<0.01
Lifetime NTP Episodes	69.7 (186.3)	1145.4 (2025.2)	<0.01	433.9 (1915.1)	7495.2 (14431.5)	<0.01
Age of First Regular NTP	17.9 (1.6)	18.3 (1.7)	0.28	18.2 (1.4)[c]	18.2 (1.7)	0.94
Ever Used Alcohol	77.7%	100%	<0.01	83.5%	100%	<0.01
Past Year Alcohol Use	34.7 (52.6)	56.2 (57.2)	<0.01	30.7 (44.1)	68.5 (63.0)	<0.01

Notes. CU=Cannabis Use. NTP=Nicotine/Tobacco Product. Bold denotes significant differences between cannabis use or NTP use groupings. Cannabis, NTP, and alcohol use are composites of total use derived from assessment of standard units of each substance (Cannabis = flower, concentrates, vaping, dabs, tinctures; Nicotine = cigarettes, e-cigarettes, cigars, pipe, hookah, smokeless tobacco; Alcohol = beer, wine, hard liquor); Regular Use defined as weekly use. [a]N=54 to only includes cannabis control participants who had used cannabis; [b]N=14 to only include cannabis control participants who had used cannabis regularly; [c]N=10 to only include control NTP participants who had used NTP regularly.

2.2. Procedures

Participants completed a single, four-hour assessment and neuroimaging session. All participants completed informed consent protocols in adherence with the local university Institutional Review Board. Participants were asked to refrain from using cannabis and alcohol for > 12 hours prior to their research appointment, which was verified by urine, oral fluid, and breathalyzer testing. The Drager DrugTest® 5000 tested onsite oral fluid for recent Δ9-tetrahydrocannabinol (THC) use (≥ 5 μg/L THC). Urine samples were sent to a toxicology lab to quantify cotinine (nicotine metabolite) and THCCOOH (THC metabolite) and to confirm that participants were negative for other substance use. Participants were not required to abstain from NTP use to avoid any deleterious effects of nicotine withdrawal; however, self-report of last NTP use was collected. During the research visit, participants underwent a comprehensive demographic, mental health, and substance use interviews, a full neurocognitive battery, and a magnetic resonance imaging (MRI) scanning session.

2.3. Materials

Demographics. A psychosocial interview was conducted to obtain relative demographic variables such as age, sex assigned at birth, race/ethnicity, socioeconomic status, education, and medical history.

Substance Use. A modified version of the Customary Drinking and Drug Use Record (CDDR; Brown et al., 1998; Jacobus et al., 2018) was administered by a trained research assistant to obtain current and lifetime substance use including cannabis and NTP use. Participants were first asked whether they had ever tried a substance in their lifetime. If they had used a substance, participants were asked how many times that have independently used cannabis products (e.g., flower; concentrates, edibles, and tinctures) and NTP (e.g., cigarettes, cigars, vape, pipe, hookah, smokeless tobacco, and nicotine replacement). In this way, measures of past month, three-month, six-month, and past year cannabis and NTP independent use episodes were obtained.

2.4. Neuroimaging

Imaging studies were conducted on a 3.0 Tesla GE Discovery MR750 scanner with a Nova Medical 32-channel receive-only head coil. A high-resolution T1-weighted anatomical scan was acquired using an inversion-prepared fast spoiled gradient echo sequence with parameters TI/TE/TR = 1060/2/2500 ms, flip angle = 8°, field of view (FOV) = 256 mm, matrix = 256 × 256, 1.0 mm^3 voxels. Diffusion data were collected with a multi-shell 96-direction single-shot spin echo diffusion sequence with b-values (500, 1000, 2000, and 3000 sec/mm^2) and 6, 15, 15, and 60 unique diffusion directions, respectively, for each b-value (TE/TR = 81.9/4100 ms, 81 axial slices, FOV = 240 mm, matrix = 140 × 140, 1.7 mm^3 voxels). Acquisition parameters were modeled after those used in the Adolescent Brain Cognitive Development (ABCD) Study (Hagler et al., 2019).

All data were visually checked for artifacts and general image quality by a trained research team member (JJ or KC). FA values were obtained using FSL's FMRIB's Diffusion Toolbox ([42]. FSL's TOPUP program was used to correct susceptibility-induced distortions. FSL's eddy tool was used to correct for eddy current distortions and subject motion. FMRIB's Linear Image Registration Tool (FLIRT) was utilized for linear registration to standard space. Finally, DTIFIT in FSL was used at the subject level to derive FA values [43]. NODDI parameter maps were obtained using the NODDI MATLAB toolbox [32]. Resulting parameter maps were used to create ODI and neurite density outputs in standard space at the subject level.

2.5. Analyses

Between-subject comparisons of FA, ODI, and neurite density maps were completed using FSL's Tract-Based Spatial Statistics (TBSS; Smith et al., 2006). Nonlinear registration was used to determine alignment of subject level FA data to a standard-space image (FSL's FMRIB58_FA). FA data were then nonlinear transformed and merged into a single 4D image, which was used to create a mean FA tract skeleton. The FA skeleton threshold was set to 0.2 to exclude voxels containing grey matter. Each participant's ODI and neurite density data were projected onto the tract skeleton to create concurrent mean ODI and neurite density tract skeletons. Voxel-wise statistics were then run modeling a two-way between-subjects ANOVA investigating the effects of NTP use group, cannabis use group, and their interaction (representing NTP and cannabis co-use) while controlling for age and biological sex. Threshold free cluster enhancement (TFCE) was used to correct for multiple comparisons across space [45]. All statistical decisions were made at $p<.05$ and all significant clusters were extracted for data visualization purposes.

3. Results

3.1. Demographics & Substance Use

Cannabis use groupings. Cannabis use groups consisted of 94 cannabis controls and 127 individuals who used cannabis regularly. Past 6-month cannabis use consumption in the cannabis use group was predominately smoked flower (smoked flower=100%; concentrates=94%; edibles=86%; tinctures=17%). Cannabis use groups significantly differed by age (t=-2.14, p=0.03), sex (χ2=8.29, p<0.01), and race (χ2=10.49, p=0.03). They did not significantly differ by level of education (t=-1.32, p=0.19) (see Table 1). Of the 94 cannabis controls, 57.4% of participants had used cannabis in their lifetime and 48.9% had used cannabis at least once in the past 6 months. Cannabis control participants that had used cannabis in the past 6 months had, on average, only four standard cannabis use episodes in the past 6-months (M=3.79, SD=6.02). As expected, this was significantly lower than the average regular cannabis use group past 6-month use (t=-7.47, p<0.01; M=252.49, SD=375.11). The regular cannabis use group also predominately reported lifetime use of NTP (χ2=42.58, p<0.01) with 88.1% of individuals in the regular cannabis use group having used NTP compared to 46.8% of cannabis controls. Despite this, cannabis use groupings did not significantly differ by past 6-month NTP use episodes (t=-1.42, p=0.15).

Nicotine use groupings. NTP use groupings consisted of 127 NTP controls and 94 individuals who used NTP. Past 6-month NTP use consumption was predominately through vaping (vape=94%; cigarettes=55%; Hookah=25%; cigars=23%; smokeless tobacco=17%; tobacco pipe=6%; nicotine replacement 6%). NTP use groups significantly differed by age (t=-2.62, p=0.01), sex (χ2=4.15, p=0.04), and years of education (t=-1.98, p=0.04). Groupings did not significantly differ by race (χ2=5.59, p=0.23). In the NTP control group, 48.8% reported NTP use in their lifetime with 35.4% having used NTP in the past 6-months. All participants in the NTP use group had tried cannabis in their lifetime (100%) with 91.5% having used cannabis in the past 6-months. Further, the NTP use group had used significantly more cannabis in the past 6-months (M=219.53, SD=431.61) compared to NTP controls (t=-2.73, p<0.01; M=92.80, SD=151.23). See Table 1 for more details.

3.2. White Matter Integrity

Fractional Anisotropy. There was a main effect of NTP use groupings on FA values within the left posterior limb of the internal capsule (p<.05, TFCE corrected) showing decreased FA values within the NTP use group compared to NTP controls, controlling for age and biological sex (see Figure 1 and Table 2). There were no significant effects of cannabis use groups or the interaction between NTP and cannabis use groupings.

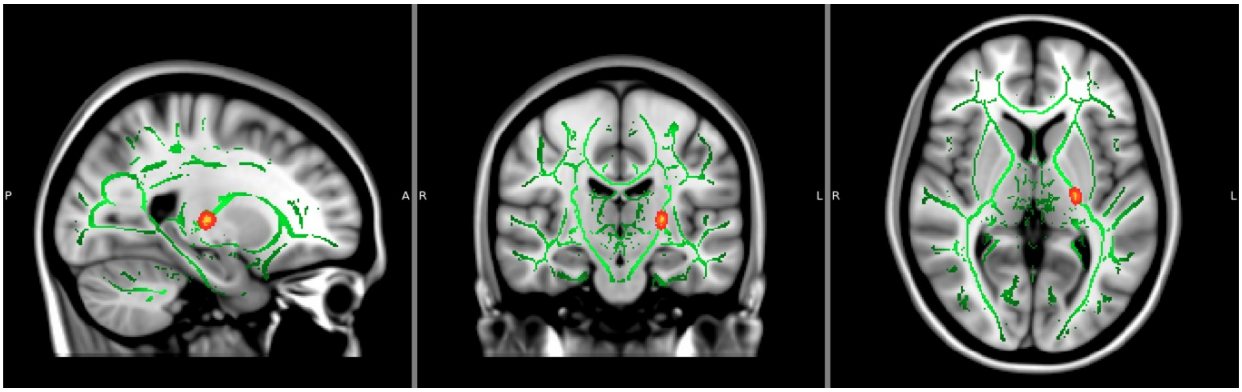

Figure 1. Significant FA Cluster (CON>NTP, p<.05, corrected).

Notes. Significant findings of the left internal capsule are displayed. Significant clusters bolded using FSL's TBSS fill command. Axial and coronal view are in radiological view (Left = Right).

Table 2. Significant Clusters.

FA Values	**Voxels**	**MAX**	**MAX X (vox)**	**MAX Y (vox)**	**MAX Z (vox)**
	22	0.95	-23	-19	2
OD Values	**Voxels**	**MAX**	**MAX X (vox)**	**MAX Y (vox)**	**MAX Z (vox)**
	39	0.958	-16	-11	-5
	27	0.962	-16	-5	5

	17	0.956	-21	-16	1

Notes. Voxels represent the number of voxels in each significant cluster. Max represents the maximum beta value within the cluster. MAX X/Y/Z the location of the maximum intensity voxel. FA=Fractional Anisotropy. OD=Orientation Dispersion.

Orientation Dispersion Index. There was a significant main effect of NTP use groupings on ODI values in three distinct clusters, all within the left posterior limb of the internal capsule (*p*s<.05, TFCE corrected), controlling for age and biological sex (see Figure 2 and Table 2). Findings showed larger ODI estimates in NTP use groups compared to the control group (see Figure 3; figure is only for visualization purposes to help with interpretation of results). There were no significant effects of cannabis use groupings or the interaction between NTP and cannabis use groupings.

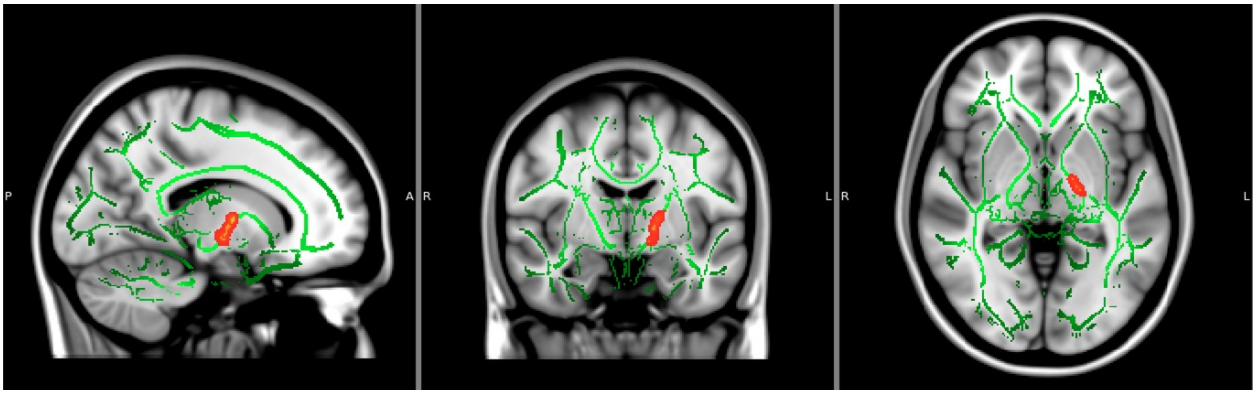

Figure 2. Significant OD Clusters (CON<NTP, *p*<.05, corrected).

Notes. Significant findings of the left internal capsule are displayed. Significant clusters bolded using FSL's TBSS fill command. Axial and coronal view are in radiological view (Left = Right).

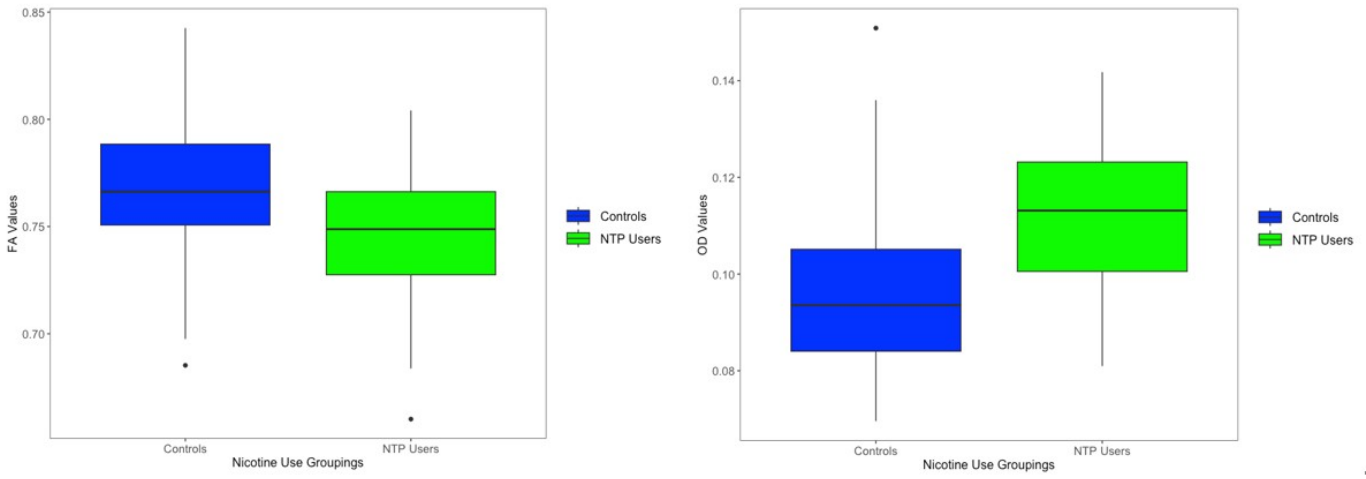

Figure 3. Significant FA and ODI Cluster Beta Values (For Data Visualization).

Notes. Boxplot represents the average beta values between both significant clusters.

Neurite Density. There were no significant effects of cannabis use groupings, NTP use groupings, nor their interaction on neurite density values.

4. Discussion

We aimed to investigate the relationship between NTP use group status, cannabis use status, and their interaction on white matter integrity, including white matter microstructure. Adolescent and young adult NTP use groups, compared to NTP controls, had lower FA and higher ODI values in left regions of the internal capsule. There were no significant differences in neurite density between any substance use groupings. Further, there were no significant differences in white matter integrity between cannabis use nor an interaction between cannabis and NTP use groupings.

The differences between NTP use and no use in white matter integrity within left regions of the internal capsule have previously been noted; although, the directionality of these FA values are a departure from some findings in the literature which suggest that adolescent NTP use (mean ages 16-18) may have higher FA values compared to no NTP use [21,46–48]. Yet, one study has also observed lower FA values in a similar age range of young adults (mean age 21) who use NTP [22], suggesting our result may be uniquely related to later adolescence/young adulthood given the slightly older age of our sample (mean age 19). Our findings are also similar to research demonstrating lower FA values in adult NTP use compared to non-NTP use [10]. It is possible that the relationship between FA and NTP use varies by age and use patterns [23], perhaps contributing to mixed findings in the late adolescence and early adulthood literature. Indeed, previous studies compared individuals who used NTP against no NTP use[46,48], while our study made comparisons between regular NTP use and NTP controls that included light to no NTP use. Nevertheless, individuals who used NTP demonstrated lower FA values within our sample, and decreasing FA values have been linked to poor brain and behavioral outcomes across medical conditions in the developmental literature [49]. This is particularly noteworthy as FA values have been shown to increase as individuals undergo healthy neurodevelopment [12]. NTP use may continue to interrupt white matter in these areas resulting in altered tissue integrity and increased vulnerability to addiction and pathology. Targeted and longitudinal work is needed to help decipher the exact age during neurodevelopment that NTP results in high risk of white matter integrity disruption as well as the functional outcomes related to that disruption.

Closer investigation of white matter microstructure demonstrated higher ODI values in individuals who use NTP compared to controls. ODI may provide a better index of the biological characteristics, such as intra- versus extra-cellular change, that are different among groups compared to the traditional DTI metrics (e.g., FA) that are less specific. Similar to our FA findings, the ODI differences were observed in left regions of the internal capsule. While this relationship aligns with previous literature demonstrating that FA values are more strongly influenced by ODI compared to neurite density and show a negative correlation from childhood to adulthood [32,34], the absence of neurite density results may also be due to the age group under study as neurite density is thought to be sensitive to younger age-related changes in myelin and the intracellular neurite compartment (e.g., ages 12-14; [34]). The presence of ODI differences and absence of neurite density differences in our sample also suggests that the observed differences in ODI may be due to geometrical fiber tract changes as opposed to myelination and tract packing. Together, the higher ODI, lower FA, and null neurite density findings suggest that NTP use may show increases in dispersions of fiber tracts projections (more complex bending and branching, and possibly less axonal alignment and coherence) during late adolescence and early adulthood [50]; however, whether this is related to poorer or better health outcomes is still unclear [35,36,51].

While no other studies have investigated the effects of NTP use on microstructure such as ODI, similar findings of increased ODI within the posterior limb of the internal capsule have been demonstrated in individuals diagnosed with schizophrenia [52] and major depressive disorder compared to controls [53], suggesting a link in dendritic complexity and psychopathology. Studies examining NODDI parameters among multiple sclerosis patients have found higher ODI values during acute inflammation stages[54]. Further, a study investigating adults who binge drink showed higher ODI findings compared to controls in ventral striatal and parietal grey matter regions [37]. It is possible that less aligned and more dispersed neurite structures, or dendritic complexity, among young adult NTP-users is related to acute inflammatory processes and/or neural vulnerability for psychopathology and addictive disorders, both of which can also have downstream neurocognitive consequences [55], particularly for motor and sensory functioning given the involvement of the posterior limb of the internal capsule in our findings [56]. However, more work is needed to investigate the direct association between white matter microstructure and cognition before further relationships can be elucidated.

Interestingly, there were no significant relationships between cannabis use or cannabis and NTP co-use on white matter integrity observed in the present sample. Although studies of cannabis' impact on white matter structure have provided mixed results, significant effects are typically only found with heavy cannabis use [10]. Since our sample compared individuals who regularly use cannabis (at least weekly) against individuals who engaged in light to no cannabis use (less than weekly), it is possible that heavier cannabis use in this sample has yet to significantly impact white matter structure during this particular window of neurodevelopment. Cannabis use during neurodevelopment is complex with variables such as age of regular use [57] and duration of cannabis use [58] being important predictors of health outcomes. Further, studies from our laboratory investigating the relationship between cannabis and NTP use have suggested

that individuals who use both substances may have distinct white matter phenotypes compared to cannabis use only during the 16-22-year-old age range [30].

As with all studies, there are some limitations. Our study was cross-sectional in nature which limits the ability to determine directionality and, therefore, causation between substance use and white matter integrity. Utilizing longitudinal datasets such as the Adolescent Brain Cognitive Development (ABCD) Study [59] will be important for investigating causality of substance use and brain health relationships. Ongoing longitudinal data collection in our laboratory utilizing NODDI-derived estimates will be examined in future investigations. Additionally, despite the fairly tight age-range and restricted demographic characteristics of the full sample, it is possible that demographic and contextual differences within substance use groupings that were not measured may have influenced findings. Inclusion of a pure nicotine and tobacco product use group (i.e., without use of any other substances) may yield different findings, although the vast majority of youth nicotine product use also report cannabis use[60,61]. Similarly, due to power concerns, past year alcohol use was not controlled for in the sample. Further, while comparing individuals with regular cannabis and NTP use to a combined sample of individuals with light (less than weekly) and no substance use was done intentionally to explore the unique effects of heavier and more regular use from light use, follow-up studies investigating cannabis and NTP-only groupings against individuals who do not use substances would be important for determining if even light substance use plays a role in white matter development. Similarly, future studies modeling co-use episodes (i.e., episodes that capture simultaneous use as compared to single substance use) will help better understand if use at the same time has different brain health outcomes.

This is the first known study to investigate the role of cannabis and NTP co-use on ODI and neurite density estimates, in addition to FA. Our study found that NTP use, but not cannabis-only or cannabis and NTP co-use, impacted white matter integrity estimates within left regions of the posterior limb of the internal capsule in a sample of late adolescents/young adults. These findings were found not only with FA markers but ODI as well, suggesting reduced white matter integrity at the microstructural level. These results are found within an adolescent and young adult cohort, which is still undergoing neuromaturation; thus, continued changes may occur with ongoing substance use. Future longitudinal work will be important for determining the relationship between brain development and substance use as well as additional factors that may better explain the impact of substance use on white matter integrity.

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Data Availability: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to confidentiality.

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