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The Effects of Nicotine and Cannabis Co-Use During Late Adolescence on White Matter Fiber Tract Microstructure

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ABSTRACT. Objective: Co-use of cannabis and nicotine and tobacco products (NTPs) in adolescence/young adulthood is common and associated with worse outcomes than the use of either substance in isolation. Despite this, little is known about the unique contributions of co-use to neurostructural microstructure during this neurodevelopmentally important period. This study sought to investigate the interactive effects of nicotine and cannabis co-use on white matter fiber tract microstructure in emerging adulthood. **Method:** A total of 111 late adolescent (16–22 years old) nicotine (NTP; $n = 55$, all past-year cannabis users) and non–nicotine users (non-NTP; $n = 56$, 61% reporting cannabis use in the past year) completed demographic and clinical interviews and a neuroimaging session comprising anatomical and diffusion-weighted imaging scans. Group connectometry analysis identified white matter tracts significantly associated with the interaction between nicotine group and past-year cannabis use according to generalized fractional anisotropy (GFA). **Results:** Nicotine Group × Cannabis Use interactions were observed in the right and left cingulum and left fornix tracts (false discovery rate = 0.053), where greater cannabis use was associated with increased GFA in the cingulum and left fornix, but only when co-used with nicotine. **Conclusions:** This report represents the first group connectometry analysis in late adolescent/young adult cannabis and/or NTP users. Results suggest that co-use of cannabis and NTPs results in a structurally distinct white matter phenotype as compared with cannabis use only, although to what extent this may change over time with more chronic nicotine and cannabis use remains to be examined in future work. (*J. Stud. Alcohol Drugs, 83,* 287–295, 2022)

NICOTINE AND TOBACCO-PRODUCT (NTP) consumption is one of the leading causes of preventable disease and death (Warren et al., 2014), and a multitude of new nicotine delivery devices such as e-cigarettes and e-hookahs have gained popularity among adolescent populations and have substantially increased in prevalence in recent years (Johnston et al., 2020). Of note, consumption of e-cigarettes before age 18 is associated with a threefold increased likelihood of being a regular user of combustible cigarettes by young adulthood (Pierce et al., 2021), which increases the risk for nicotine dependence across the life span and for concurrent and problematic use of other substances such as cannabis (Hindocha & McClure, 2021; Hindocha et al., 2021). Cannabis use disorder is more common among tobacco users, and co-use of cannabis and nicotine is associated with poorer mental and physiological health outcomes as opposed to single substance use (Goodwin et al., 2018; Hindocha et al., 2021; Meier & Hatsukami, 2016). Although co-administration of nicotine and cannabis exacerbates negative health outcomes, co-use remains highly common (and often underreported) compared with the use of either nicotine or cannabis exclusively during adolescence and young adulthood (Cohn et al., 2019; Hindocha & McClure, 2021; Schauer & Peters, 2018). Between 18% and 52% of tobacco users are estimated to also use cannabis (Agrawal et al., 2012; Nicksic et al., 2020; Tucker et al., 2019), and vaping of both of these products has doubled or tripled from 2017 to 2018 for some high school grades (Johnston et al., 2020) and college students (Schulenberg et al., 2019). Despite this, the unique contributions of co-use on neurostructural microstructure have not been thoroughly investigated during peak developmental use periods (e.g., ages 16–22).

Protracted white matter tissue development from childhood to age 25 is important for efficient communication across the brain, supporting functional specialization and cognition (Baum et al., 2020); subtle alterations in this normative neurobiological process may result in enhanced vulnerability to psychopathology, including excessive drug-taking behaviors (Achterberg et al., 2016; Jacobus et al., 2013c; Paus et al., 2008; Squeglia & Gray, 2016). Diffusion-weighted imaging studies have largely evaluated users of cannabis and nicotine independently as single substance users. These studies demonstrate structural brain alterations in white matter microstructure compared with non-users, with youth cannabis users demonstrating vulnerability to lower fractional anisotropy (FA), a measure of anisotropic diffusion indicating poorer white matter health (Becker et al., 2015; Gruber et al., 2014; Jacobus et al., 2013a, 2013b; Orr et al.,

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2016). In contrast, regular nicotine use in adolescent and adult samples appears to be associated with an increase in FA (Hudkins et al., 2012; Jacobsen et al., 2007; van Ewijk et al., 2015; Yu et al., 2016), although not exclusively (Kangiser et al., 2020). However, it is likely that with a greater severity of nicotine use over the lifetime, decrements in white matter health would emerge as reduced FA (Hudkins et al., 2012).

Given the seemingly opposing effects of cannabis and nicotine on white matter microstructure in younger populations, it is conceivable that NTP use may compensate for acute cannabis-related neural (Courtney et al., 2020) and cognitive impairments (Schuster et al., 2016; Viveros et al., 2006) in co-users during the initial years of substance use. Unique alterations in neural tissue microenvironments and metabolic integrity may result from the synergistic effects of recurrent co-stimulation of the endocannabinoid and nicotinic cholinergic systems that are still being shaped and refined during adolescence (Scherma et al., 2016; Somerville & Casey, 2010; Stiles & Jernigan, 2010; Wiers et al., 2016). A recent investigation by our team explored cerebral blood flow (CBF) values in white matter tissue among co-users of nicotine and cannabis (Courtney et al., 2020). We found that among adolescent/young adults who did not use nicotine, there was a positive relationship between CBF and cumulative cannabis use. More reported cannabis use was linked to greater blood flow in white matter tissue, yet the use of nicotine seemed to diminish this relationship. Of note, greater white matter CBF in the cannabis users without a history of nicotine use was linked to poor white matter microstructure (reduced FA) in the right superior longitudinal fasciculus/inferior fronto-occipital fasciculus/forceps major cluster. Thus, there may be greater metabolic demand in less healthy tissue compartments among cannabis-only users that is diminished among co-users.

The current study builds on our prior work by using a connectometry analysis of whole-brain white matter tracts to determine if the relationship between cannabis use and fiber tract microstructure is dependent on nicotine use among a sample of adolescents/young adults. Given our prior results in CBF markers of white matter health, it is hypothesized that the effect of cannabis use on generalized fractional anisotropy (GFA) estimates of white matter will be moderated by nicotine use in this sample. The combined, interactive effects of cannabis and nicotine co-use on neural health outcomes warrants greater consideration given the high frequency of co-use during this critical neurodevelopmental period, which may translate to differing early life health outcomes as compared with single substance use.

Method

Participants and procedures

Data for this report were taken from an ongoing project investigating the structural and functional neural effects of nicotine and cannabis co-use in adolescence/young adulthood. As previously reported (Courtney et al., 2020), participants (N = 112; ages 16–22) were recruited from San Diego County via electronic and physical flyers posted on social media sites and at high schools, 4-year universities, and community colleges. Inclusion criteria for the ongoing study include regular use of cannabis and/or nicotine in the past 6 months. For the purposes of this investigation, participants were grouped into NTP and non-NTP users, with NTP users reporting an average of 2 or more NTP use episodes per month and non-NTP users reporting less than 2 NTP use episodes per month during the previous year. A cutoff of 2 or more NTP use episodes per month was chosen because it ensures that all NTP group participants were engaging in at least monthly use, which is a metric commonly used by public health agencies (e.g., Centers for Disease Control and Prevention) and epidemiological studies (e.g., Monitoring the Future) to identify "current" nicotine users. NTP use was defined as the use of any of the following: electronic cigarettes (e.g., vape pens, Juul, e-hookah), tobacco cigarette, tobacco pipe, cigars (including blunts, spliffs), hookah with tobacco, smokeless tobacco, chew, snuff, snus, and/or nicotine replacement (e.g., patches, nasal sprays, inhalers, gum, lozenges). Participants were not selected for inclusion based on previous cannabis use. Participants were excluded from the study if they reported any lifetime illicit substance use more than 10 times, a current or past psychiatric disorder (other than tobacco and/or cannabis use disorder) based on criteria from the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5; American Psychiatric Association, 2013), acute influence of cannabis or alcohol use at testing (confirmed with urine, breath alcohol analysis, and oral fluid toxicology), use of medications affecting the brain, major psychiatric or medical issues, or history of prenatal substance exposure or developmental disability.

Participants underwent a single laboratory visit (approximately 4 hours) after providing written informed consent in accordance with the University of California, San Diego (UCSD), Human Research Protections Program. During this visit, participants completed a thorough demographic, psychological, and substance use interview, neurocognitive assessment, and magnetic resonance imaging (MRI) scan session. All participants were instructed to refrain from cannabis use for 12 hours and alcohol use for 24 hours before the appointment. Oral fluid, urine, and breath alcohol analysis (for alcohol) substantiated self-reported use and verified abstinence. The Dräger DrugTest 5000 (Dräger, Lübeck, Germany; Desrosiers et al., 2012) was used to examine Δ9-tetrahydrocannabinol (THC) using onsite detection of recent cannabis use (≥5 μg/l THC) in oral fluid samples. Urine samples were sent to Redwood Toxicology to quantify the THC metabolite 11-nor-9-carboxy-THC (THCCOOH) normalized to creatinine and the nicotine metabolite cotinine for all substance users and to confirm the absence of illicit sub-

stances. NTP use was not restricted before testing to avoid withdrawal effects during data acquisition. No participants screened positive for any illicit substances.

Measures

As described in Courtney et al. (2020), a demographic and psychosocial interview was conducted to assess background information on socioeconomic status, education, race/ethnicity, and medical history. A modified version of the Customary Drinking and Drug Use Record structured interview (Brown et al., 1998; Jacobus et al., 2018; Karoly et al., 2019a, 2019b) was used to assess detailed substance use and substance-related problem history. For the purposes of this report, current nicotine use was defined as the use of an NTP two or more times per month during the previous year. The 21-item version of the Depression, Anxiety and Stress Scale (DASS-21) was administered to assess depression, anxiety, and stress symptomology (Lovibond & Lovibond, 1995).

Neuroimaging acquisition and processing

Imaging studies were conducted at the UCSD Center for Functional MRI on a 3.0 Tesla GE Discovery MR750 scanner with a Nova Medical 32-channel phased array head coil. Participants were instructed to remain still and awake for the duration of the scan session. A high-resolution T1-weighted anatomical fast spoiled gradient echo scan was acquired with TI/TE/TR = 1060/2/2500 ms, flip angle = 8˚, field of view (FOV) = 256 mm, 256 × 256 matrix, 1.0 mm^3 voxels. Diffusion data were collected with a multi-shell 96-direction single-shot spin echo diffusion sequence with 4 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 ABCD Study (Hagler et al., 2019). As described in Courtney et al. (2020), data were collected with reversed phase-encode blips (A>P, P>A), which resulted in image pairs with distortions going in opposite directions. Using the TOPUP tool in FSL (Smith et al., 2004), the susceptibility-induced off-resonance field was estimated from these pairs (Andersson et al., 2003). The two images were combined to form a single image corrected for susceptibility-induced distortions (Graham et al., 2017). FSL's Diffusion Toolbox (FDT) was used to conduct linear registration to standard space (FLIRT) and to correct for eddy current distortion (EDDY; Andersson & Sotiropoulos, 2016).

Diffusion data were further processed within DSI Studio (http://dsi-studio.labsolver.org; Yeh, 2021). Similar to methods previously used by the authors (Sorg et al., 2021), data were reconstructed in the Montreal Neurological Institute space using q-space diffeomorphic reconstruction (Yeh & Tseng, 2011) to obtain the spin distribution function (Yeh et al., 2010). A diffusion sampling length ratio of 1.25 was used, and the output resolution used in analyses was 2 mm^3. Participant registrations were inspected for quality and goodness-of-fit using the R^2 statistic between the warped individual subject diffusion image data set and the template image (Yeh et al., 2013a).

Given that traditional FA provides poor anisotropy estimates in regions with crossing fibers (De Santis et al., 2014; Nilsson et al., 2012; Oouchi et al., 2007), GFA was computed as the primary index of white matter microstructure, as it can measure anisotropy across multiple diffusion directions (Tuch, 2004). Consistent with traditional studies of FA, lower GFA values are associated with undirected, isotropic diffusion, whereas higher GFA values signify more unidirectional diffusion, suggestive of healthier white matter.

Statistical analysis

Group differences in demographic variables were investigated with independent chi-square or *t* tests with a $p < .05$ statistical significance threshold. Diffusion MRI connectometry (Yeh et al., 2016) was conducted within DSI Studio, which essentially provides "correlational tractography" to map the exact segment of pathways correlated with a given variable. The interaction of nicotine group (coded 0 = Non-NTP, 1 = NTP) and cannabis use episodes (total past year) on GFA tractography values was estimated through examination of the fiber pathways that correlate with this interaction term (i.e., correlational tractography), controlling for age and sex (main effects of nicotine group and cannabis also included) (Yeh, 2021; Yeh et al., 2016). Given the significant nicotine group differences in past-year cannabis use episodes and the associated collinearity between the nicotine group and cannabis use variables, the cannabis use variable was mean centered within group and these centered values were used to calculate the interaction term (Chen et al., 2014). An outlier with cannabis use episodes >4,000 in the past year was excluded ($n = 1$). A total of 111 subjects were included in the analysis (non-NTP = 56, NTP = 55). Given the relatively high presence of zeros in the cannabis use variable and to enable consideration of the nonlinear relationship between the interaction term and the GFA outcome, the nonparametric covariate-adjusted Spearman rank-order correlation option was selected in DSI Studio to derive the correlation for the whole brain tractography analyses (Yeh, 2021; Yeh et al., 2016). A T-score threshold of 2 was selected and tracked using a deterministic fiber tracking algorithm (Yeh et al., 2013b) to obtain the correlational tractography. Topology-informed pruning (Yeh et al., 2019) with four iterations was used to filter the tracts. A length threshold of 40 voxel distance was used to select tracts. A total of 4,000 randomized permutations were applied to obtain the null distribution of

Table 1. Sample demographics

Variable	Group *M* (*SD*) or %		
	Non-nicotine (*n* = 56)	Nicotine (*n* = 55)	*p*
Age	18.95 (1.69)	19.29 (1.40)	.24
% Male	55.4%	65.5%	.28
% White	53.6%	45.5%	.29
Education years completed	12.64 (1.53)	12.80 (1.40)	.57
DASS-21 Depression Standard Score	3.18 (3.75)	3.95 (4.30)	.32
DASS-21 Anxiety Standard Score	2.66 (2.62)	3.84 (3.12)	.03
DASS-21 Stress Standard Score	3.43 (3.52)	4.95 (3.68)	.03
Days since last nicotine use (*n* = 32)	96.84 (255.50)	6.22 (10.71)	.01
Nicotine use episodes previous year	5.02 (6.72)	1,485.02 (2,822.39)	<.001
Days since last cannabis use	14.15 (24.96)	33.77 (92.73)	.23
Cannabis use episodes previous year (*n* = 34)	86.66 (124.27)	301.00 (363.19)	<.001
% Positive for THCCOOH	39.3%	67.3%	.002
Days since last alcohol use (*n* = 43)	40.67 (199.16)	13.49 (32.12)	.11
Alcohol drinks per drinking day previous year	3.63 (5.14)	4.91 (3.89)	.14
Alcohol drinking days per month previous year	1.85 (2.64)	5.47 (5.66)	<.001

Notes: DASS-21 = 21-item version of the Depression, Anxiety and Stress Scale; THC = Δ9-tetrahydrocannabinol; THCCOOH = THC metabolite 11-nor-9-carboxy-THC.

the tract length to enable estimation of the false discovery rate (FDR). Mean GFA data across the resultant tract set, as well as split by hemisphere, were extracted for each participant and imported into SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY) for follow-up analyses. Spearman rank-order correlations were also conducted for all follow-up analyses.

Results

Participants

Demographic information is presented in Table 1. The sample consisted of approximately 60% males ($n = 67$), and 49% of the sample self-identified as White ($n = 55$), with both sex and race roughly balanced across groups. The NTP group included individuals reporting a range of low (at least twice monthly) to heavy (>38 per day) NTP use episodes over the previous year (range: 25–14,199 episodes). All NTP participants reported past-year cannabis use, as did 61% of non-NTP participants. When modeled as a continuous variable, past-year NTP use episodes was found to significantly and positively correlate with past-year cannabis use episodes in the full sample ($r = .591$, $p < .001$). The NTP group reported an average of 1.17 and 1.52 more points on the DASS anxiety and stress subscales, respectively, as compared with the non-NTP group ($p < .05$); however, the NTP group means still fell within the normal ranges per the cutoff scores provided by the scale authors (Lovibond & Lovibond, 1995). As expected, the groups differed significantly on nicotine recency and past-year use of nicotine, as well as past-year use of cannabis (NTP > non-NTP). The groups also differed on drinking days per month (NTP > non-NTP), yet group differences in the number of drinks per drinking day did not meet statistical significance (Table 1).

Connectometry findings

An interaction between nicotine group and cannabis use was found to correlate with GFA from the right and left cingulum tracts (and left fornix to a lesser extent; FDR = 0.053), whereby the NTP group exhibited greater positive cannabis and GFA correlations than the non-NTP group in these tracts. No significant negative correlations were observed (Figure 1).

Correlations performed on the extracted GFA within nicotine groups separately revealed a significant positive correlation between cannabis use and mean GFA in the bilateral, $r(53) = .293$, $p = .030$; right, $r(53) = .283$, $p = .036$; and left, $r(53) = .318$, $p = .018$, cingulum for the NTP group, yet the correlations did not reach statistical significance for the non-NTP group, overall: $r(54) = -.035$, $p = .796$; right: $r(54) = -.014$, $p = .917$; left: $r(54) = -.152$, $p = .263$; see Figure 2 for a visualization of the relationships.

Exploratory analyses

Correlation analyses between extracted cingulum mean GFA estimates and DASS-21 subscale scores revealed no significant relationships across, $r(109)$: -.077 to .096, $p > .318$, or within nicotine groups, non-NTP $r(54)$: -.131 to -.006, $p > .334$; NTP $r(53)$: -.069 to .174, $p > .204$. Correlation analyses between extracted cingulum mean GFA estimates and alcohol drinks per drinking day and days drinking

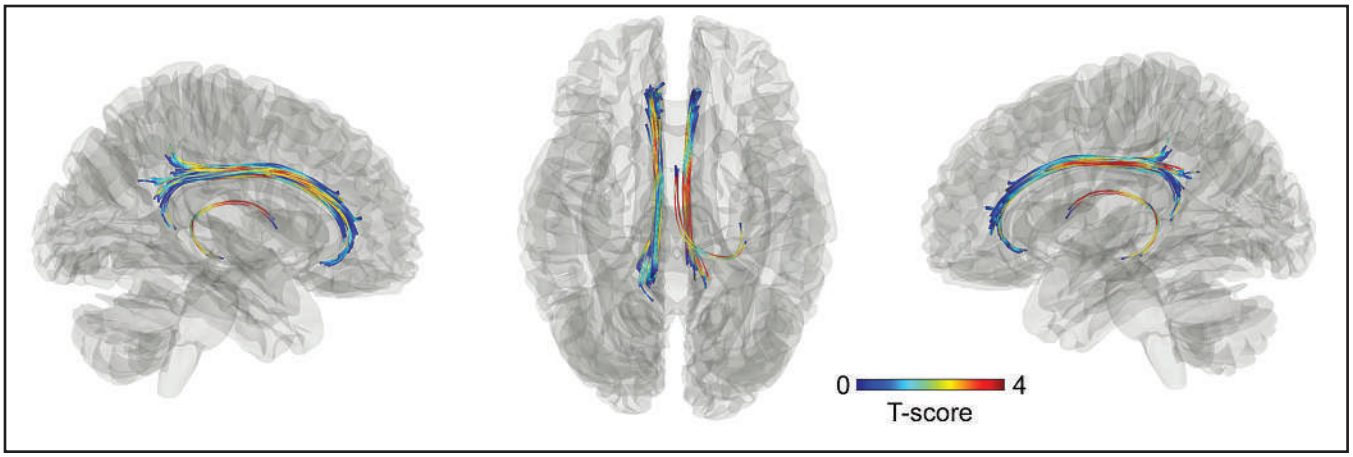

FIGURE 1. Results of connectometry analysis depicting fiber pathways where the nicotine and tobacco product (NTP) group exhibited stronger positive correlations between generalized fractional anisotropy (GFA) and past-year cannabis use, compared with the non-NTP group, controlling for age and sex. Tracts include the right and left cingulum and left fornix. Results are corrected for multiple comparisons via permutation testing (4,000 iterations), with a false discovery rate = 0.053.

also revealed no significant relationships across groups, $r(109)$: .121 to .166, $p > .082$, or within nicotine groups, non-NTP $r(54)$: .049 to .087, $p > .524$; NTP $r(53)$: -.036 to .217, $p > .111$. An additional diffusion MRI connectometry analysis controlling for drinking days revealed results consistent with the primary findings (i.e., an interaction between nicotine group and cannabis use positively correlating with GFA from bilateral cingulum was still present).

Discussion

This report sought to identify differences in white matter microstructure related to cannabis and NTP co-use versus cannabis only use in a sample of adolescents/young adults. Results suggest the presence of an interaction between substances on white matter microstructure, where greater cannabis use is associated with increased GFA in the cingulum and left fornix, but only when co-used with nicotine. This is consistent with previous findings from our group (Courtney et al., 2020), which also note a seemingly compensatory effect of nicotine on cannabis-related alterations to white matter CBF in late adolescents.

Cannabis use in adults has been associated with reductions in microstructural integrity and volume in a number of brain regions, particularly those rich in cannabinoid CB1 receptors (e.g., insula and orbitofrontal cortex, Battistella et al., 2014; medial temporal cortex, Matochik et al., 2005; and hippocampus and amygdala, Lorenzetti et al., 2015; Schacht et al., 2012). Consistent with the current findings, cannabis use is also associated with altered volume of the cingulate cortex in adults (Hill et al., 2016; Rapp et al., 2013), impaired axonal connectivity within the fornix in adults (Zalesky et al., 2012), as well as integrity of the cingulum in adults and adolescents (Becker et al., 2015; Jakabek et al., 2016; Wade et al., 2020). Reduced integrity of the cingulum (particularly the angular bundle; Ezzati et al., 2016) and fornix (Benear et al., 2020) have been previously linked with poorer memory function in adults, and thus may represent structural mechanisms underlying the observed memory impairments associated with chronic cannabis use (Broyd et al., 2016; Solowij & Battisti, 2008).

Although cannabis use has been largely associated with decreases in FA in adolescent samples (Becker et al., 2015; Gruber et al., 2014; Jacobus et al., 2013a, 2013b; Orr et al., 2016), nicotine use is often associated with increases in FA in youth (Hudkins et al., 2012; Jacobsen et al., 2007; van Ewijk et al., 2015; Yu et al., 2016). It is possible nicotine stimulation of acetylcholine receptors (nAChRs) promotes certain neurodevelopmental processes during emerging adulthood such as glial activity (Garrido et al., 2003; Liu et al., 2005), which support white matter maturation (Dwyer et al., 2009). These altered processes may then interact with cannabis-induced changes to result in a structurally distinct phenotype for co-users versus cannabis-only users. Alternatively, it is also possible that adolescents with preexisting increased FA may be more likely to engage in greater nicotine and cannabis co-use for reasons currently unknown. Longitudinal studies are greatly needed to uncover the temporality and causality of these relationships.

This study has many strengths, including the use of a well-characterized sample of late adolescent/young adult cannabis and NTP users despite inherent difficulties related to the measurement of nicotine and cannabis use in this population. The inclusion of users of various NTPs, as opposed to limiting the inclusion to tobacco cigarette use only, also allows for greater generalizability of these findings to the majority of adolescent/young adult nicotine users (Hindocha & McClure, 2021). Furthermore, this study represents the first group connectometry analysis in cannabis and NTP co-users. This approach allows for the characterization of

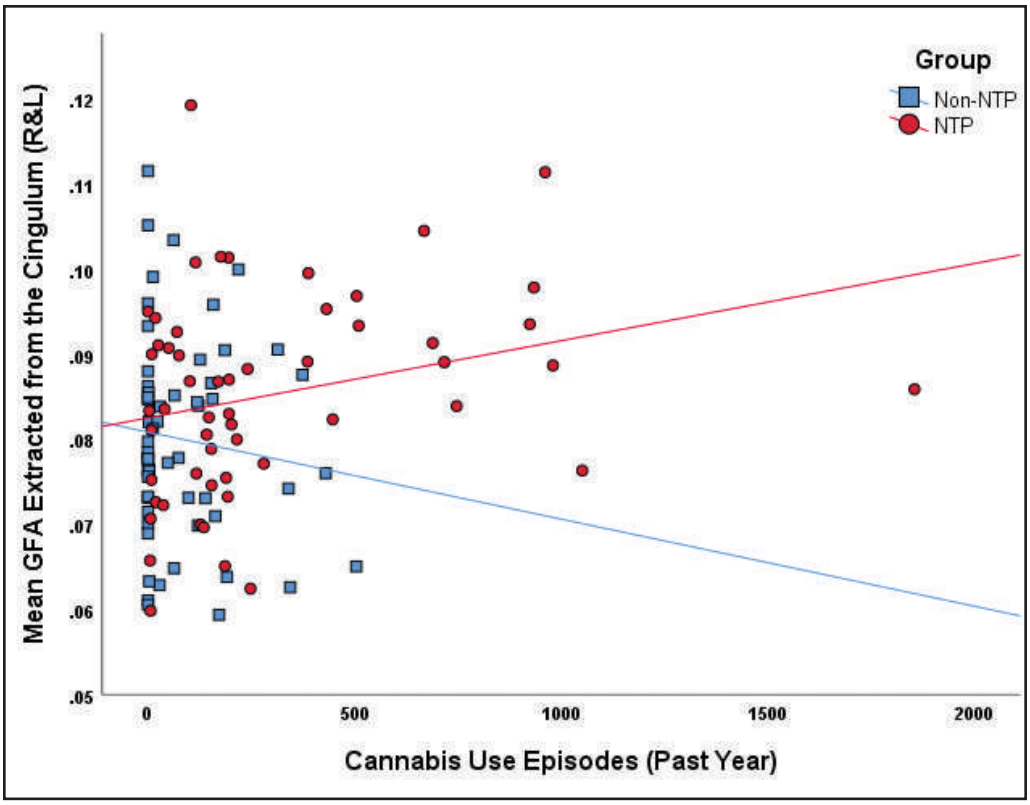

FIGURE 2. Scatter plot depicting the association between mean extracted generalized fractional anisotropy (GFA) from the cingulum tract identified in the connectometry analysis against past-year cannabis use episodes for each nicotine group. NTP = nicotine and tobacco product.

variability within white matter tracts in the context of local connectomes, thus providing a more focal analysis of structural differences within connected pathways that are related to the study variable of interest (i.e., cannabis use; Yeh et al., 2016).

The present study is also not without limitations. The nicotine groups were found to differ on measures of anxiety and stress, as well as frequency of alcohol use, despite attempts to match the groups on demographic and other substance use variables. Follow-up analyses revealed little support for the involvement of these additional factors on the main outcome; yet the possibility of their subthreshold influence remains. Although explicitly modeled in the analyses, differences in cannabis use between the groups were also large, which could have implications for the results that were not delineated here. Thus, additional investigations including "pure" cannabis use, "pure" NTP use, and non-using "control" groups are needed to fully appreciate the individual and combined contributions of cannabis and NTP use on white matter health. Further, the NTP group included a range from relatively infrequent (approximately two times per month) to frequent (>30 per day) NTP use episodes over the previous year; however, the majority of the sample reported non-daily use (median use = 16 use episodes per month). This limits the generalization of the results to heavier NTP users; yet this use pattern is more reflective of typical NTP use in the adolescent/young adult population, and the observance of effects while including individuals with lower NTP use frequencies suggests heavy nicotine use may not be necessary to impact estimates of white matter health. Last, causal inferences relating cannabis and nicotine co-use to future brain health could not be evaluated given the cross-sectional nature of the data.

This study represents the first report of whole-brain white matter microstructure in late adolescent/young adult co-users of nicotine and cannabis. The results support emerging evidence that co-use of these substances is associated with differential white matter profiles versus cannabis use in isolation (Courtney et al., 2020), which may translate to differing behavioral and psychological consequences for co-using adolescents/young adults. As data collection is ongoing, further investigation on associated neuropsychological and functional outcomes is planned for the larger study. In addition, longitudinal studies that map the impact of these substances on white matter development trajectories during this neurologically vulnerable period will help elucidate ca-

sual mechanisms by which the use of these substances, alone or in combination, relate to various health and psychological outcomes.

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