

# The impact of levamisole and alcohol on white matter microstructure in adult chronic cocaine users

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## Abstract

Previous brain imaging studies with chronic cocaine users (CU) using diffusion tensor imaging (DTI) mostly focused on fractional anisotropy to investigate white matter (WM) integrity. However, a quantitative interpretation of fractional anisotropy (FA) alterations is often impeded by the inherent limitations of the underlying tensor model. A more fine-grained measure of WM alterations could be achieved by measuring fibre density (FD). This study investigates this novel DTI metric comparing 23 chronic CU and 32 healthy subjects. Quantitative hair analysis was used to determine intensity of cocaine and levamisole exposure—a cocaine adulterant with putative WM neurotoxicity. We first assessed the impact of cocaine use, levamisole exposure and alcohol use on group differences in WM integrity. Compared with healthy controls, all models revealed cortical reductions of FA and FD in CU. At the within-patient group level, we found that alcohol use and levamisole exposure exhibited regionally different FA and FD alterations than cocaine use. We found mostly negative correlations of tract-based WM associated with levamisole and weekly alcohol use. Specifically, levamisole exposure was linked with stronger WM reductions in the corpus callosum than alcohol use. Cocaine use duration correlated negatively with FA and FD in some regions. Yet, most of these correlations did not survive a correction for multiple testing. Our results suggest that chronic cocaine use, levamisole exposure and alcohol use were all linked to significant WM impairments in CU. We conclude that FD could be a sensitive marker to detect the impact of the use of multiple substances on WM integrity in cocaine but also other substance use disorders.

## KEYWORDS

cocaine, diffusion-weighted imaging, levamisole

## 1 | INTRODUCTION

Mortality risk is significantly elevated among individuals who reported lifetime use of cocaine based on a recent study including

data from ~20.500 adults, yielding a hazard ratio of 1.27 (95% CI: 1.04–1.55).<sup>1</sup> Cocaine use is associated with several mechanisms of brain injury including ischaemic, haemorrhagic and metabolic changes.<sup>2–5</sup> In the last two decades, magnetic resonance imaging

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(MRI) techniques have provided insights into the complex structural brain abnormalities in cocaine users (CU). Chronic use of stimulants including cocaine is associated with both impairments in decision-making<sup>6-9</sup> and structural abnormalities in brain grey and white matter (WM).<sup>10-14</sup> Diffusion tensor imaging (DTI) studies reported that chronic exposure to cocaine commonly results in reduced fractional anisotropy (FA), increased mean diffusivity (MD) or increased diffusivity in directions perpendicular (radial) to the prominent diffusion direction in parts of the corpus callosum (CC).<sup>12,15-18</sup> Less reliably, DTI signs of abnormal WM have also been seen in frontal and parietal brain regions.<sup>15,19-24</sup> Variations in the pattern of abnormal DTI values associated with chronic cocaine use or addiction is partly accounted for by comorbid alcohol consumption and by route of cocaine administration with smoking cocaine associated with lower FA and higher perpendicular diffusivity than intranasal administration.<sup>16</sup> Length of abstinence of cocaine use appears to be related with larger FA and smaller MD values in the CC, frontal, parietal, temporal and occipital lobes as well as the cerebellum.<sup>19</sup> In the study by Bell et al, cocaine abstinent patients (abstinence duration ranged between 5 days and 102 weeks) showed initially lower FA in the left anterior callosal fibres, left genu of the CC, right lateral superior longitudinal fasciculus (SLF), right callosal fibres and the superior corona radiata bilaterally when compared against non-using controls.<sup>25</sup> Higher FA in the cocaine abstinent patients was observed in the splenium of the CC and in medial parts of the right SLF, a WM tract connecting frontal control areas to parietal regions. Differences between the cocaine abstinent groups were observed bilaterally in the inferior fronto-occipital fasciculus (IFOF), right anterior thalamic radiation and ventral posterolateral nucleus of the thalamus as well as in the left superior corona radiata, SFL bilaterally, right cingulum and the WM of the right precentral gyrus.

The tensor model, and therefore FA measures, is inadequate to illustrate the underlying tissue structure in regions with complex fibre geometries and multiple fibre populations.<sup>26-31</sup> Voxels capturing these anatomical complexities occur frequently (60%-90% of all WM fibre voxels) throughout the brain WM due to limited spatial resolution and partial volume effects between adjacent tracts.<sup>30</sup> Although a reduction of FA is often thought to reflect axonal degeneration and demyelination,<sup>32-35</sup> complex fibre configurations may critically confound the interpretation of changes in tensor-derived diffusion metrics and complicate a correct and quantitative interpretation of changes in DTI-related parameters.<sup>30,36-39</sup> Yet, diffusion acquisition techniques and data analysis pipelines have been improved significantly by addressing these inherent problems of the tensor model. Higher order diffusion models based on acquisition schemes with high number of diffusion directions and new reconstruction methods as the constrained spherical deconvolution technique<sup>40</sup> were developed. These advances enable the resolution of multiple fibre directions within a single voxel and can improve the performance of fibre tractography significantly.<sup>41-43</sup> Additionally, there is a growing literature on removing tractography biases,

which is a crucial prerequisite to derive absolute and quantitative measures from fibre tractograms.<sup>44,45</sup> However, the reliable extraction of quantitative measures from tractograms across different populations remains challenging. Recent developments in global top-down tractography optimizations enable the estimation of fibre contributions and compartment fractions,<sup>46-51</sup> whereby all of these optimization methods have their own pitfalls; for a comprehensive overview, see literature.<sup>52</sup> Numerous models based on diffusion-weighted data have been proposed to estimate parameters related to the restricted, intra-axonal compartment, commonly referred to as fibre density (FD).<sup>53,54</sup> Smith et al<sup>50</sup> as well as Daducci and colleagues determined an optimal weight for each streamline, according to a biologically motivated forward model and the measured diffusion signal.<sup>50,51,53</sup> By assigning a weight of zero, false positive or implausible connections can be eliminated. The FD is calculated by multiplying each streamline contribution (fibre weight) by the streamline length. Previously, we have demonstrated that WM alterations were more prominent in FD compared with FA in patients with amyotrophic lateral sclerosis<sup>55</sup> and schizophrenia.<sup>56</sup> However, the potential advantages of the aforementioned innovations in basic neuroimaging methods have not been established for clinical neuroscience research of pathologies in substance use disorders such as chronic cocaine use. It is thus unknown whether microstructural WM fibre alterations can be quantified with the new FD measure.

Therefore, we applied a whole-brain and regional tract-based spatial statistics (TBSS) analysis approach<sup>57</sup> to test its potential in detecting microstructural alterations in FD between CU and healthy controls (HC). Based on the meta-analysis of DTI-based WM integrity studies,<sup>12</sup> we hypothesized to find lower FA and FD in CU in the CC as this region showed the highest effect size estimates across included studies. Moreover, two case reports of leukoencephalopathy in CU implicated a commonly added adulterant in street cocaine, levamisole, as a potential cause.<sup>58,59</sup> In fact, multifocal leukoencephalopathy has been shown previously in patients who received levamisole as a medication against ascariasis, recurrent aphthous ulcers and malignant melanoma and for adenocarcinoma of the colon.<sup>60-63</sup> Additionally, a dose-dependent effect of levamisole exposure on neurocognitive functions and thinning of distinct cortical areas of CU has been shown recently.<sup>64</sup> Recently, we could additionally show that the use of levamisole-adulterated cocaine is accompanied by an increased load of WM lesions.<sup>65</sup> Therefore, immune-related neurotoxicity related to the cutting agent levamisole may contribute to cocaine-associated brain injury. We thus examined if levamisole exposure is associated with WM integrity in CU. Further, we tested the impact of alcohol use on WM integrity, given that alcohol use disorders are highly prevalent chronic CU<sup>66</sup> and have a well-established impact on WM microstructure.<sup>67,68</sup> Henceforth, by choosing a within-patient group (see below) analytical approach, we aimed to disentangle the effects of cocaine, levamisole and alcohol use on local WM integrity in CU.

## 2 | METHODS

### 2.1 | Participants

We recruited chronic CU ( $n = 43$ ) from inpatient and outpatient units of the Psychiatric University Hospital in Zurich and from affiliated institutions.<sup>69</sup> Age-, sex- and education-matched HC ( $n = 42$ ) were recruited by online advertisements. For inclusion, CU had to report cocaine as primary substance of choice with a consumption level of  $>0.5$  g per month and an abstinence duration of  $<6$  months. Exclusion criteria for CU were the presence of DSM-IV Axis I adult psychiatric disorders—except for cocaine, cannabis, nicotine, alcohol abuse/dependence, attention deficit hyperactivity disorder (ADHD) and a previous depressive episode. Controls were excluded if a current or previous DSM-IV Axis I psychiatric disorder (except for nicotine dependence) or illegal substance use of  $>15$  lifetime occasions or during the past 6 months (except for cannabis) was assessed. Before the testing session, participants were asked to abstain from illegal substances for at least 72 h and not to consume alcohol for 24 h. Urine samples were collected to verify self-reports. When available, 6-cm hair samples were cut from the occiput, enabling to estimate substance use during the last 6 months. Hair samples were analysed with liquid chromatography–tandem mass spectrometry.<sup>70</sup> Data from these 6-month hair samples were used for (a) confirmation of regular cocaine use (cocaine hair concentration  $>500$  pg/mg)<sup>71,72</sup> and (b) confirmation that cocaine is the primary used illicit substance and (c) for the quantification of levamisole. The ethics committee of the Canton of Zurich approved the study, which was in accordance with guidelines from the Helsinki declaration. All participants gave written informed consent.

### 2.2 | Psychopathological and neuropsychological assessment

A trained psychologist conducted a clinical interview to determine the presence of DSM-IV Axis I diagnoses.<sup>73</sup> Subjective report of substance use including alcohol was assessed by the Interview for Psychotropic Drug Consumption.<sup>74</sup> Smoking habits were assessed by the use of the Fagerström Test for Nicotine Dependence.<sup>75</sup> The Barratt Impulsiveness Scale BIS-11<sup>76</sup> and the ADHD self-rating scale ADHD-SR<sup>77</sup> to estimate trait impulsivity and ADHS symptomatology, respectively, were applied. In addition, we applied the Beck Depression Inventory (BDI).

### 2.3 | MRI data acquisition

MRI data acquisition was performed on a 3T whole-body MR scanner (Achieva, Philips Healthcare, Best, the Netherlands), equipped with 80 mT/m gradients and a 32-channel receive head coil. Diffusion data were acquired using a diffusion-weighted single-shot spin-echo echo-planar imaging sequence with the following parameters: repetition

time (TR): 6.64 s, echo time (TE): 53.6 ms, field of view (FOV):  $240 \times 240$  mm<sup>2</sup>, 50 contiguous transversal slices, slice thickness: 2.5 mm, acquisition matrix:  $96 \times 96$ , SENSE factor: 2.5, partial Fourier encoding: 60%. The slices were positioned parallel to the anterior and posterior commissure defined on a T1-weighted midline sagittal survey image. Diffusion acquisition was performed along 32 directions with a b-value of 1000 s/mm<sup>2</sup> and two signal averages. Additionally, four non-diffusion-weighted  $b = 0$  s/mm<sup>2</sup> scans were acquired resulting in a scan time of 8 min 31 s. For structural reference and anatomical priors for the tracking algorithm, T1-weighted images were recorded using a three-dimensional magnetization prepared rapid gradient-echo (MP-RAGE) sequence with 1-mm isotropic resolution.

### 2.4 | Diffusion data preprocessing

Before any preprocessing steps, quality control of all acquired diffusion data was assessed based on several criteria: First, diffusion tensor residuals were calculated for every acquired diffusion direction, and the nine slices in the whole diffusion dataset with the highest residuals were identified for visual inspection. Plots were generated depicting the 12 slices (four sagittal, four axial and four coronal directions) with the highest noise level. Second, mean signal intensity plots for every diffusion direction and the non-diffusion-weighted image were derived and plotted slice by slice in sagittal, axial and coronal directions. Artefacts, such as signal dropouts due to head motion, can easily be spotted on these plots. A trained MR physicist inspected the data for artefacts and rated the signal courses and fitting residuals of every subject on a Likert-type scale. Preprocessing diffusion data followed a similar procedure previously described in our recent publication.<sup>56</sup> After denoising the raw data using the 'dwdenoise function' from the MRtrix3 software package, diffusion-weighted data were first corrected for eddy-current and motion-induced distortions by registration the diffusion-weighted images to the  $b_0$  image using the dwipreproc routine from MRtrix3 software package. This function makes use of the eddy tool implemented in FSL (FMRIB, Oxford, UK Version 6.0.0).<sup>78</sup> The Brain Extraction Tool (BET) from FSL was then applied to remove non-brain tissue and estimate the inner and outer skull surfaces. Next, the diffusion data were corrected for susceptibility-induced distortions using the bdp correction algorithm implemented in the BrainSuite software package (<http://brainsuite.org>).<sup>79</sup> Diffusion maps derived from the diffusion tensor, that is, FA, MD, radial diffusivity and axial diffusivity, were then calculated using the DTIFIT tool implemented in the FSL software package. In our study, we focus on FA and FD. Subsequently, constrained spherical deconvolution with recursive calibration of the response function<sup>40</sup> and fibre tractography was performed in MRtrix3 using the iFOD2 probabilistic tractography algorithm.<sup>80</sup> In order to apply biological tissue priors to the streamline generation, the 'Anatomically-Constrained Tractography' option was selected (MRtrix3 tckgen ACT option).<sup>45</sup> Tractography seed points were determined dynamically according to the spherical-deconvolution informed filtering of the tractogram model (MRtrix3 tckgen seed-dynamic option for

determining seed points based on the SIFT model).<sup>49</sup> Due to this dynamic seeding strategy within the whole WM, the distribution of streamlines is already approximating the apparent FD, and therefore, intrinsic tractography biases are reduced. In total, five million fibres were generated per subject. The resulting streamlines were optimized using the COMMIT framework<sup>51</sup> applying the parameters described elsewhere.<sup>81</sup> The derived intracellular compartment fraction of the COMMIT optimization corresponds to FD.

## 2.5 | Intracranial volume

The intracranial volume (ICV) measure, sometimes referred to as total intracranial volume, refers to the estimated volume of the cranial cavity as outlined by the supratentorial dura matter or cerebral contour when dura is not clearly detectable. To extract the ICV for each subject, the following steps were conducted with FreeSurfer software (v5.3.0, <http://surfer.nmr.mgh.harvard.edu/>): motion correction, skull-stripping<sup>82</sup> automated Talairach transformation, with subsequent segmentation of the WM,<sup>29,83</sup> correction of intensity variations due to magnetic field inhomogeneities and placement of grey/white and grey/cerebrospinal fluid borders based on intensity gradients.<sup>84,85</sup>

## 2.6 | Statistical analysis

To evaluate differences between the groups, voxel-wise (whole-brain) TBSS analysis based on a general linear model was performed using FSL's randomize tool<sup>86</sup> with 5000 permutations to correct for multiple comparisons ( $p < 0.05$ , corrected). All results included threshold-free cluster enhancement (TFCE).<sup>87</sup> The TFCE correction method is somewhat similar to cluster-based thresholding, but generally more robust and avoids the need for the arbitrary initial cluster-forming threshold. Two contrasts were computed, testing for positive and negative differences of the FA and FD parameters between the HC and CU. To probe for the impact of cocaine, levamisole or alcohol on WM integrity, we computed *three* statistical models:

- **Model 1:** Testing for the influence of cocaine use (pg/mg) on WM integrity. Here, we adjusted for age, sex, ADHD, nicotine (cigarettes per day), 3,4-methylenedioxymethamphetamine (MDMA) (hair concentration, pg/mg), ICV, alcohol (weekly use; pure ethanol g per week) and levamisole (hair concentration, pg/mg).
- **Model 2:** Testing for the influence of levamisole on WM integrity. Here, we adjusted for age, sex, ADHD, nicotine, MDMA, ICV, alcohol and cocaine use.
- **Model 3:** Testing for the influence of alcohol on WM integrity. Here, we adjusted for age, sex, ADHD, nicotine, MDMA, ICV, levamisole and cocaine use.

Consistently, we included—apart from age, sex, ADHD and ICV—MDMA and nicotine in all models, as it has been shown that MDMA

can alter GM and WM integrity,<sup>88</sup> whereas nicotine alters WM microstructure.<sup>89,90</sup>

These models are likely to illustrate WM alterations by the particular substances compared with HC. Yet, as HC did not consume cocaine or levamisole, we could not enter all three variables (alcohol, cocaine and levamisole) in one statistical model to assess the strongest impact on WM integrity by a particular substance by this comparison. We therefore applied a within-patient group analysis. Here, we first computed track-specific (48 tracts; based on the JHU CBM-DTI-81 WM labels atlas<sup>91</sup>) Spearman rank-order correlations between FA and FD with substance-specific values while controlling for the same covariates (apart from cocaine, levamisole and alcohol) as for the between-group models (i.e. age, sex, ADHD, nicotine, MDMA and ICV). For significant ( $p < 0.05$ , uncorrected) correlations, we further compared the coefficients of the particular tracts between substances by means of the 'corr\_rtest' function implemented in Matlab. By this analysis, we could assess which of the substances lead to the strongest WM impairment (e.g. seen as a significantly stronger negative correlation with FA and/or FD for a particular substance over the other substances).

## 3 | RESULTS

### 3.1 | Data quality

From the original sample ( $n = 85$ ), seven (three CU and four HC) participants did not receive a DTI scan.<sup>69</sup> For these remaining participants ( $n = 38$  HC and  $n = 40$  CU), we had to exclude data from six HC and 17 CU, due to insufficient data quality caused by excessive head motion or the lack of information on cocaine and levamisole levels due to missing hair samples. Thus, the final included sample consists of 32 HC and 23 CU.

### 3.2 | Demographic and clinical values

Participants were mostly right-handed (90.9%), and there was no group difference in handedness (chi-square:  $p = 0.220$ ). Groups did not differ regarding age, IQ, education and ICV (see Table 1). Although the original sample was matched for sex, after data quality-related exclusions, the two groups slightly but non-significantly differed in sex distribution. As expected, CU had significantly higher ADHD scores, alcohol intake, nicotine intake and MDMA hair concentration.

#### 3.2.1 | Whole-brain between-group analysis

- **Model 1 (cocaine):** The comparison between HC and CU revealed reduced FA in CU in the external/extreme capsule (IFOF), sagittal stratum (IFOF), centrum semiovale (callosal fibres, corona radiata, SLF I), internal capsule (posterior limb) and perisylvian region. For FD, reductions were seen in the external/extreme capsule

**TABLE 1** Demographics

Measure	Group (HC = 32, CU = 23)	p-value (t-test/chi-square)
Age (years)	HC: 31.43 (STD: 6.6) CU: 32.91 (STD: 7.2)	0.45
Sex (female/male)	HC: 16/16 CU: 9/14	0.22 <sup>a</sup>
Education (years)	HC: 10.5 (STD: 1.5) CU: 10.4 (STD: 1.4)	0.63
Verbal IQ	HC: 108.6 (11.2) CU: 102.5 (11.7)	0.07
Handedness	HC: 32 R, 0L CU: 20 R, 3L	>0.05 <sup>a</sup>
ICV (mm <sup>3</sup> )	HC: 1592550 (STD: 142211) CU: 1585372 (STD: 145853)	0.86
BDI score	HC: 2.3 (5) CU: 9.1 (9.8)	<b>0.0016</b>
ADHD-SR score	HC: 6.2 (STD: 5.9) CU: 15.4 (STD: 7.9)	<b>&lt;0.001</b>
Alcohol (pure ethanol in g/week)	HC: 56.5 (STD: 45.7) CU: 253.9 (STD: 353.1)	<b>0.0033</b>
Nicotine (cigarettes per day)	HC: 3.7 (STD: 5.2) CU: 7.9 (STD: 9.1)	<b>0.04</b>
MDMA hair concentration (pg/mg)	HC: 0.99 (STD: 5.5) CU: 322.2 (STD: 787.5)	<b>0.03</b>
Levamisole hair concentration (pg/mg)	HC: 0 CU: 4265.4 (STD: 6443.8)	n.a.

Note: Bold means  $p < 0.05$ .

Abbreviations: ADHD-SR, Attention Deficit Hyperactivity Disorder Self-Rating Scale; BDI, Beck Depression Scale; CU, cocaine users; HC, healthy controls; ICV, intracranial volume; L, left-handed; MDMA, 3,4-methylenedioxymethamphetamine; R, right-handed.

<sup>a</sup>Chi-square test.

(IFOF and uncinate fasciculus) and centrum semiovale (callosal fibres) (Figure 1, row on the top).

- Model 2 (levamisole): We found lower FA in CU in the CC (*callosal fibres* running through the *genu, body, isthmus* and *splenium*), *centrum semiovale* (callosal fibres, corona radiata, SLF I) and perisylvian region. Similar results were obtained for FD (Figure 1, row on the bottom).
- Model 3 (alcohol, Table 2): FA reductions in CU in the external/extreme capsule (IFOF), centrum semiovale (callosal fibres, corona radiata, SLF I) and perisylvian region. Similar results were obtained for FD.

All results are summarized in Table 2.

### 3.2.2 | Within-patient group analysis

The results are illustrated in Figure 2 and summarized in Table 3.

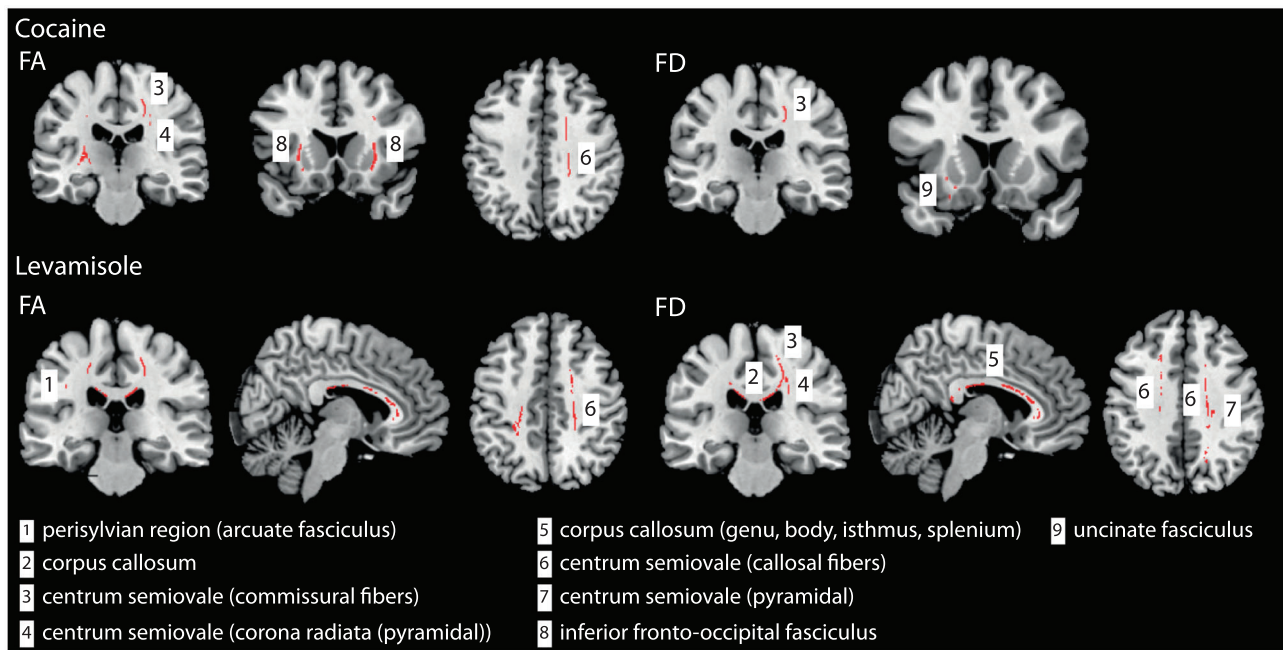
Cocaine (pg/mg) showed a positive correlation of FA with WM of the right posterior limb of the internal capsule ( $r = 0.48$ ,  $p = 0.050$ ;

Figure 2a), as well as a positive correlation with FD in posterior limb of the internal capsule ( $r = 0.57$ ,  $p = 0.017$ ), right cingulate gyrus ( $r = 0.49$ ,  $p = 0.045$ ) and in superior fronto-occipital fasciculus ( $r = 0.49$ ,  $p = 0.045$ ; Figure 2a). For levamisole (pg/mg), negative correlations with FA (Figure 2b) were seen in the genu of the CC ( $r = -0.54$ ,  $p = 0.025$ ). In addition, FD negatively correlated with the genu of the CC ( $r = -0.57$ ,  $p = 0.018$ ).

Negative correlations between alcohol (pure ethanol in g/week) and FA (Figure 2c) were seen with the right posterior limb of the internal capsule ( $r = -0.58$ ,  $p = 0.015$ ), right retrolenticular part of the internal capsule ( $r = -0.73$ ,  $p < 0.001$ ), right superior corona radiata ( $r = -0.54$ ,  $p = 0.027$ ) and left SLF ( $r = -0.50$ ,  $p = 0.040$ ). The correlation of FA and right retrolenticular part of the internal capsule survives multiple comparison correction ( $p$  corrected =  $0.05/49$ , i.e.  $0.001$ ). For FD, negative correlations with alcohol were observed for the right retrolenticular part of the internal capsule ( $r = -0.70$ ,  $p = 0.002$ ).

Next, we compared correlations strengths for significant WM clusters (summarized in Figures S1–S3). For example, we found that FA (of the right posterior limb of internal capsule) was inversely correlated to alcohol consumption (Figure S2) and that this correlation





**FIGURE 1** Between-group differences for the cocaine and levamisole model. Cocaine use (top row) and levamisole exposure (bottom row) reduce FA and FD values in multiple WM tracts (for details, we refer to Table 2) comparing patients to controls. Results were adjusted for age, sex, ADHD, alcohol, cigarette use, MDMA, ICV, levamisole exposure (cocaine model) or cocaine use (levamisole model). All results are shown at  $p < 0.050$  (corrected using threshold-free cluster enhancement [TFCE] correction)

significantly differed from the correlation of alcohol with levamisole or cocaine (all between-substance correlation comparisons are summarized in Table 3).

As displayed in Figure 3, longer cocaine use (in years, normalized values in the figure) resulted in a significant positive correlation of FA with the right superior cerebellar peduncle ( $r = 0.51, p = 0.037$ ) and a negative correlation of FD with the right cingulum hippocampus ( $r = -0.55, p = 0.022$ ).

## 4 | DISCUSSION

In this cross-sectional DTI study, we found consistent WM alterations in CU compared with HC using FA and FD. Thus, apart from FA, FD appears as sensitive marker to identify WM changes specifically associated with cocaine, levamisole and alcohol in chronic CU. Some of these impairments were related to a particular substance, detected as significantly stronger negative correlations. Negative correlations were only observed for levamisole exposure and alcohol use. However, we demonstrated that at least a longer use of cocaine is related to greater WM impairments, which is, however, also inevitably associated with a longer levamisole exposure.

### 4.1 | HC versus patients: The role of cocaine use on WM integrity

A WM tract that showed consistently lower FA in CU across all examined models was the SLF-I. The SLF-I originates from the

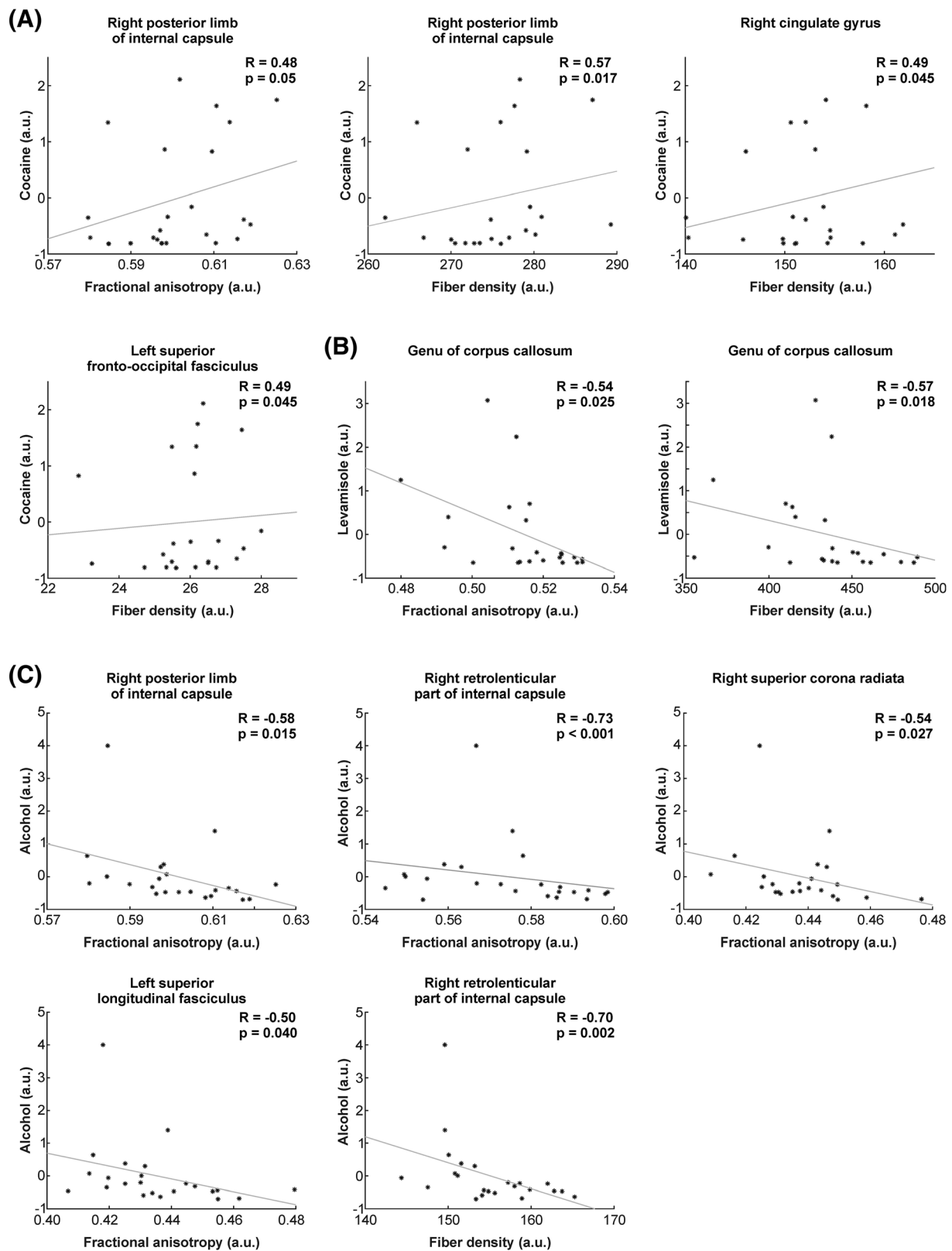
superior parietal lobe, which projects (ventrally) along the cingulate gyrus, to the superior frontal gyrus (dorsal spatial/motor network<sup>92</sup>), terminating within the supplementary motor and premotor areas in the frontal lobe. This bundle plays a role in attention, response inhibition, proprioception and motor movement.<sup>93,94</sup> This finding is in line with previous behavioural data in rodents and humans showing specific impairments in response inhibition and flexible decision-making in chronic CU.<sup>21,95,96</sup> In addition, the study by Lebel et al reported abnormal WM microstructure in adolescents with prenatal cocaine exposure in the arcuate fasciculus, cingulum and CC. The arcuate fasciculus contains both long and short WM fibres that connect the frontal, parietal and temporal lobes.<sup>97</sup> It plays a key role in visuospatial processing and some aspects of language processing, such as prosody and semantics.<sup>97</sup> Here, we have not assessed tests related to language and visuospatial processing, but previous work has shown considerable impairments of chronic CU in visuospatial working memory and visuospatial paired associates learning<sup>98</sup> as well as in perception of speech, verbal fluency and other language functions.<sup>99</sup>

Lower FA and FD values were also seen in the IFOF. The IFOF consists of two layers: The first one is superficial and antero-superiorly directed, terminating in the inferior frontal gyrus. The second layer is deeper and comprises three portions: posterior, middle and anterior.<sup>100,101</sup> The posterior component ends in the middle frontal gyrus and dorsolateral prefrontal cortex. The middle component terminates in the middle frontal gyrus and lateral orbitofrontal cortex (OFC). The anterior one is directed to the OFC and frontal pole. Generally, the IFOF acts as a 'multifunction' bundle, with each anatomical subcomponent subserving different brain processing. The superficial

**TABLE 2** Summary of whole-brain between group differences (HC > CU) for the three statistical models (cocaine, levamisole and alcohol)

<b>Model 1 (cocaine)</b>		
DTI measure: FA		
Region	Fibre system	Hemisphere
External/extreme capsule	IFOF	Right and left
Sagittal stratum	IFOF	Left
Centrum semiovale	- Callosal fibres - Corona radiata (pyramidal tract) - SLF (I)	Right
Internal capsule (post. limb)	(Pyramidal tract)	Left
Perisylvian region	Arcuate fasciculus	Right
DTI measure: FD		
Region	Fibre system	Hemisphere
External/extreme capsule	- IFOF - UF	Left
Centrum semiovale	- Callosal fibres	Right
<b>Model 2 (levamisole)</b>		
DTI measure: FA		
Region	Fibre system	Hemisphere
Corpus callosum	Callosal fibres running through the genu, body, isthmus and splenium	n.a.
Centrum semiovale	- Callosal fibres - Corona radiata (pyramidal tract) - SLF (I)	Right and left
Perisylvian region	Arcuate fasciculus	Left
DTI measure: FD		
Region	Fibre system	Hemisphere
Corpus callosum	Callosal fibres running through the genu, body, isthmus and splenium	n.a.
Centrum semiovale	- Callosal fibres - Corona radiata (inclusive but not restricted to the pyramidal tract) - SLF (I)	Right and left
Perisylvian region	Arcuate fasciculus	Left
<b>Model 3 (alcohol)</b>		
DTI measure: FA		
Region	Fibre system	Hemisphere
External/extreme capsule	IFOF	Right and left
Centrum semiovale	- Callosal fibres - Corona radiata (pyramidal tract) - SLF (I)	Right and left
Perisylvian region	Arcuate fasciculus	Left
DTI measure: FD		
Region	Fibre system	Hemisphere
Corpus callosum	Callosal fibres running through the isthmus and splenium	n.a.
Centrum semiovale	- Callosal fibres - Corona radiata (inclusive but not restricted to the pyramidal tract) - SLF (I)	Right and left
Perisylvian region	Arcuate fasciculus	Left

Notes: All results are corrected for age, sex, ADHD, nicotine, MDMA, cocaine and intracranial volume. For Model 1, we additionally corrected for levamisole exposure and alcohol. For Model 2, we additionally corrected for cocaine and alcohol. For Model 3, we additionally corrected for cocaine and levamisole exposure. Differences are shown at  $p < 0.05$  (corrected). Labelling is based on ICBM-DTI-81 WM labels atlas (48 WM tract labels). Abbreviations: IFOF, inferior fronto-occipital fasciculus; SLF, superior longitudinal fasciculus; UC, uncinate fasciculus.



**FIGURE 2** Relationship between different substances and indices of white matter integrity (i.e. FA and/or FD). (A) Relationship between indices of white matter integrity and cocaine exposure. (B) Relationship between indices of white matter integrity and levamisole exposure. (C) Relationship between indices of white matter integrity and alcohol consumption. For display purposes, the different individual values (i.e. cocaine, levamisole and alcohol) were normalized across patients. a.u., arbitrary units. The  $R$  and  $p$ -values correspond to correlation analyses, including the six regressors as covariates (i.e. age, sex, ADHD, nicotine, MDMA and ICV)



**TABLE 3** Summary of significant correlations between FA and FD with cocaine, levamisole and alcohol

Cocaine					
FA					
Between substance comparison					
Name	COC and FA	ALC and FA <sup>a</sup>	LEV and FA <sup>a</sup>	Comparison COC and ALC	Comparison COC and LEV
Right posterior limb of the internal capsule	$R = 0.48$ 0.05	$R = -0.58$ 0.02	$R = 0.28$ 0.28	$p < 0.001$	0.44
FD					
Right posterior limb of internal capsule	$R = 0.57$ 0.017	$R = -0.44$ 0.08	$R = 0.04$ 0.17	$p < 0.001$	0.4
Right cingulum (cingulate gyrus)	$R = 0.49$ 0.045	$R = -0.12$ 0.65	$R = 0.25$ 0.35	$p < 0.037$	0.4
Left superior fronto-occipital fasciculus	$R = 0.49$ 0.045	$R = -0.09$ 0.75	$R = 0.30$ 0.23	0.048	0.5
Levamisole					
FA					
Between substance comparison					
Name	LEV and FA	ALC and FA <sup>a</sup>	COC and FA <sup>a</sup>	Comparison LEV and ALC	Comparison LEV and COC
Genu of corpus callosum	$R = -0.54$ 0.025	$R = 0.26$ 0.30	$R = -0.26$ 0.32	0.006	0.27
FD					
Name	LEV and FD	ALC and FD*	COC and FD*	Comparison LEV and ALC	Comparison LEV and COC
Genu of corpus callosum	$R = -0.57$ 0.018	$R = 0.25$ 0.33	$R = -0.31$ 0.23	0.005	0.30
Alcohol					
FA					
Between substance comparison					
Name	ALC and FA	LEV and FA <sup>a</sup>	COC and FA <sup>a</sup>	Comparison ALC and LEV	Comparison ALC and COC
Right posterior limb of internal capsule	$R = -0.58$ 0.015	$R = 0.28$ 0.28	$R = 0.48$ 0.05	0.003	$p < 0.001$
Right retrolenticular part of internal capsule	$R = -0.73$ $p < 0.001$	$R = -0.05$ 0.85	$R = 0$ 1	0.006	0.004
Right superior corona radiata	$R = -0.53$ 0.027	$R = 0.09$ 0.72	$R = 0.22$ 0.39	0.029	0.009
Left superior longitudinal fasciculus	$R = -0.50$ 0.040	$R = -0.16$ 0.537	$R = -0.04$ 0.89	0.22	0.10
FD					
Name	ALC and FD	LEV and FD <sup>a</sup>	COC and FD <sup>a</sup>	Comparison ALC and LEV	Comparison ALC and COC
Right retrolenticular part of internal capsule	$R = -0.70$ 0.002	$R = 0.09$ 0.72	$R = 0.32$ 0.21	0.002	$p < 0.001$

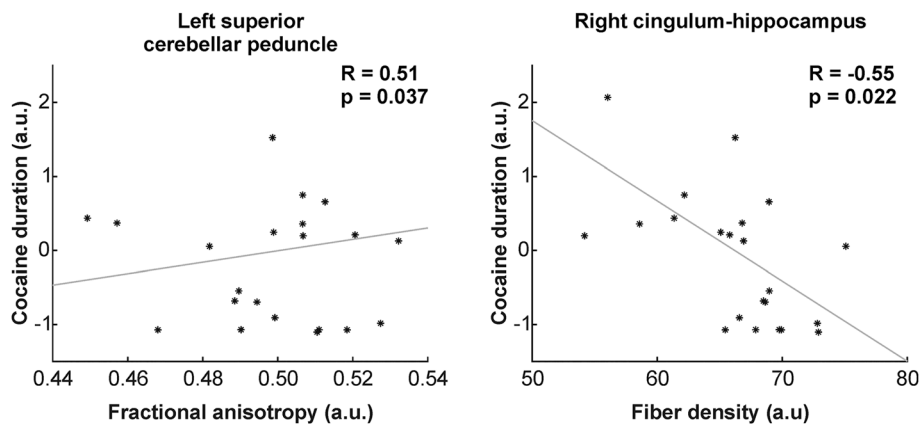
Note: The comparison of the correlation strength between two substances is listed in the last two columns of each table.

Abbreviations: ALC, alcohol; COC, cocaine; LEV, levamisole.

<sup>a</sup>Correlations are reported for completeness.

layer and the posterior component of the deep layer, which connects the occipital extrastriate, temporo-basal and inferior frontal cortices, might be relevant semantic processing. The middle component of the

deep layer could play a role in multimodal sensory-motor integration. Lastly, the anterior component of the deep layer might be involved in emotional and behavioural aspects (e.g. anxiety and depression). We



**FIGURE 3** Relationship between the duration of cocaine consumption and indices of white matter integrity (i.e. FA and/or FD). A positive relationship between the duration of the cocaine consumption and the FA was observed for the left superior cerebellar peduncle. A negative correlation was seen with the right cingulum hippocampus. For display purposes, the individual duration of cocaine consumption were normalized across patients. a.u., arbitrary units. The  $R$  and  $p$ -values correspond to correlation analyses, including the six regressors as covariates (i.e. age, sex, ADHD, nicotine, MDMA and ICV)

cannot examine layer-specific impairment by our DTI approach, but our results indicate the overall (mean) WM of the IFOF seem to be consistently disturbed in CU compared with HC.

The observed lower FA in the uncinate fasciculus in the CU is in line with two recent studies. One DTI study demonstrated lower FA in polysubstance users (including cocaine), compared with controls, in the body of the CC, anterior cingulate, uncinate fasciculus and retrolenticular part of the internal capsule. Duration of cocaine abstinence was positively correlated with FA in the uncinate fasciculus, posterior cingulate and fornix striatum. In the context of cocaine polysubstance use, chronicity of cocaine use was therefore likely to be associated with lower FA in the CC and chronicity of alcohol use with lower FA in the frontal-striatal and frontal-limbic tracts. Longer abstinence was associated with greater FA in frontal-striatal and frontal-limbic tracts. In a more recent study, it was shown that long-term abstinence resulted in higher FA values (compared with current users) in the uncinate fasciculus as well as in the bilateral CC, SLF, inferior longitudinal fasciculus, uncinate fasciculus, left IFOF and the left ventral and dorsal medial frontal regions.<sup>102</sup>

## 4.2 | HC versus patients: The role of levamisole and alcohol on WM integrity in CU

The presence of higher levels of levamisole in hair and more weekly alcohol intake was related to stronger WM alterations (Model 3; Table 2), indicating that not only CU but also the cocaine-adulterant levamisole and the additional use of alcohol impair WM microstructural integrity. Strikingly, association, commissural and projection fibres demonstrated WM impairments related to levamisole and alcohol. It has been shown that sex-dependent alterations occur in the commissural tracts (callosal body), CC, SLF (association fibres), IFOF and uncinate fasciculus in children and adolescents with prenatal alcohol exposure.<sup>103</sup> In a 'binge' model of early prenatal alcohol

exposure in sheep, Watari and co-workers reported WM alterations in further WM regions, including the frontal gyral WM, temporal gyral WM, optic radiation, CC, septum pellucidum, fasciculus subcallosus and capsule externa.<sup>104</sup> Seigneurie et al reviewed the brain abnormalities that might underlie the risk towards alcohol dependence.<sup>105</sup> With respect to WM, the authors reported local WM volume deficits in the CC and in the right OFC and lower FA in the left IFOF and in the right optic radiation. However, in our study, we did not assess if our CU came from families with multiple cases of alcohol dependence or experienced prenatal alcohol exposure. We also did not record if intense alcohol consumption started prior to cocaine use or was intensified later during cocaine use episodes. A previous study found FA alterations in the optic radiation as well as in the different parts of the corona radiata, CC and capsule interna in chronic cocaine users.<sup>15</sup> However, authors conclude a number of variables within and between the cocaine and control groups could have biased the DTI results, including alcohol use, level of education, smoking status and (within the cocaine group) age of onset of cocaine use. In Model 3, we corrected for age, sex, ADHD, nicotine, MDMA, ICV, levamisole and cocaine use and still observed significant alterations in association, commissural and projection fibres. We conclude that our study is the first, to our knowledge, that identifies seen as alterations in FA and FD of alcohol in CU users. The CC showed alterations in FA and FD for Models 2 and 3. Related to Model 3, this finding is in line with our initial hypothesis, as it has been shown that impairments in CC (and other brain regions) can be reversed by alcohol abstinence.<sup>19,25</sup>

A previous case report reported reduced FA and increased RD in a woman who developed multifocal inflammatory leukoencephalopathy after the treatment with levamisole for adenocarcinoma,<sup>106</sup> a result that is in line with our finding that levamisole contributes to WM integrity alterations in CU. Of note, none of our CU displayed the full picture of a multifocal leukoencephalopathy, as seen in levamisole-medicated patients.<sup>60,63</sup> However, we have

recently shown in an overlapping sample that levamisole-exposed CU display more WM hyperintensities,<sup>65</sup> which are considered more subtle, chronic ischemic lesions in the WM caused by constriction and damage of subcortical small vessels.<sup>107</sup> Moreover, high levamisole exposure due to the use of contaminated cocaine was also going along with stronger impairment of executive functions and reduced cortical thickness in various region of the prefrontal cortex.<sup>64</sup> Consequently, the present data again confirm that levamisole—also as an adulterant of cocaine—might be neurotoxic specifically for WM structures.

### 4.3 | Within-patient group analysis

Surprisingly, we were not able to statistically support the hypothesis that higher cocaine hair concentrations also went along with lower FA or FD values. Yet, it seems that subjects with longer use of cocaine exhibited similar WM alterations as seen for levamisole exposure. This might relate to the change from cocaine to levamisole-contaminated cocaine use along subjects' personal history of cocaine use. Furthermore, there was one WM region—the (superior) cerebellar peduncle—that contains the afferent and efferent tracts of the cerebellum, which showed a positive correlation of FA and cocaine use and duration. The tracts of this region connect the cerebellum with other parts of the central nervous system such as the pons, thalamus and prefrontal cortex.<sup>108</sup> The cerebellar peduncle supports refining motor movements or learning new motor skills, that is, a damage in this region most often results in imbalance and lack of proprioception.<sup>109–111</sup>

Multifocal leukoencephalopathy associated with cocaine use, especially with the use of levamisole, leads to increasing abnormal MRI signal lesions with patchy restricted diffusion and heterogeneous enhancement deep in both hemispheres, including internal and external capsules, putamen, corona radiata and periventricular WM as well as in the cerebellar peduncle and midbrain, that is, the pons.<sup>59</sup> Yet, based on these results, we would have assumed a negative correlation between cerebral peduncle WM and cocaine use. However, a study by Todd et al demonstrated that abnormal (increased) substantia nigra morphology was linked to regular stimulant use, including cocaine.<sup>112</sup> As the substantia nigra is in close spatial proximity to the cerebral peduncle, it might be that the increase of FA is associated with abnormal morphology of the substantia nigra and surrounding tissue including the cerebral peduncle. Future studies are required that might examine the interaction of WM integrity of the cerebral peduncle and substantia nigra and cocaine use.

The multimodal imaging study<sup>113</sup> emphasizes the tight link between WM alterations seen in several WM regions, including regions for which we found a negative correlation of FA/FD with elevated alcoholic intake (i.e. anterior corona radiata, body of the CC, cingulate gyrus, external capsule, fornix, IFOF, posterior corona radiata, retrolenticular limb of internal capsule and SLF). Specifically, Monning et al reported negative correlations of FA with the fMRI signal of regions of the frontoparietal and corticolimbic networks, which the authors interpreted as a loss of control over alcohol consumption. All

our study participants were adults, but the onset of alcohol was likely during adolescence, which might affect even more WM structure than adult-onset alcohol use as demonstrated recently.<sup>114</sup> All our results were corrected for age (as well as for sex, ADHD, nicotine, MDMA and ICV), which contribute differently on alcohol consumption on onset. Yet, nutrition might be another factor that might be considered when modelling human alcohol consumption and its link to WM alterations.<sup>115</sup>

## 5 | LIMITATIONS

Our study has some limitations. The sample size was relatively small specifically in the CU group. Unfortunately, our CU showed more agitation (i.e. more movement) in the scanner, likely explained by higher burden with ADHD symptoms, resulting in a strong dropout rate because of our strict imaging quality reasons. Moreover, we only used a cross-sectional design, and future longitudinal studies will be necessary to test the long-lasting impact of levamisole and alcohol on DTI metrics in CU. Hence, our results rely only on a correlation approach, and we could not examine the data in a causal manner. Another limitation is that we used a constrained spherical deconvolution algorithm for the tractography, for which the 32 acquired directions are few. The number of directions determines the L MAX, the maximum degree of harmonics that can be used; typically,  $L = 8$  is used, and for this, at least 45 directions are needed. With 32 directions, we are limited to  $L = 6$ . In addition, most of our correlations do not survive a proper statistical correction and should therefore be taken as trends and interpreted with caution. Furthermore, the group comparison with the exclusion of specific covariates in the different models informs only partially about the true impact of each separate substance (i.e. cocaine, alcohol, levamisole and substances not considered in our models) on WM differences between controls and patients. Moreover, other factors, such as more pronounced affective psychopathology, may have impacted WM integrity results in our patients, warranting future investigation on the impact of such symptoms on WM integrity in CU.

## 6 | CONCLUSION

Firstly, our results indicate that FD is a valid DTI marker that can detect (next to FA) structural alterations in CU. Secondly, cocaine-, levamisole- and alcohol-exposed CU demonstrated impaired WM structure. However, based on the correlation analysis comparing substances, alcohol and levamisole exposure were associated with stronger multiregional WM impairments than cocaine use itself. Thus, the reduction of levamisole in street cocaine should be a significant aim of current drug policymaking worldwide given that refraining from levamisole-contaminated cocaine likely attenuates WM disease and related symptoms. Nevertheless, longer use of cocaine resulted in a similar reduction of WM integrity, even though these reductions occur in different WM regions.

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## CONFLICT OF INTEREST

The authors declare that they have no known competing financial interest.

## AUTHOR CONTRIBUTIONS

BQ and SH designed and conceptualized the study. SH performed data recording and data preprocessing. MM, PS, and LM analysed the imaging (DTI) data. WS helped with white matter labelling and data interpretation. MB supported the study by performing the hair sample analysis. ES provided additional funding. LM and BQ wrote the manuscript. All co-authors read and corrected the original version of the manuscript. All authors approved the final version of the publication.

## DATA AVAILABILITY STATEMENT

The data associated with this publication can be shared upon reasonable request.

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