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Ndlovu, Nhanisi

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The Specific Neuroanatomical Features of Nyaope Addiction and Associated Co-Morbid Psychiatric Disorders.

A full dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand in fulfilment of the requirements of the degree Master of Science in Medicine

Researcher: Nhanisi Ndlovu (854185)

Degree: MSc Med

Supervisors: Dr Tanya Calvey, PhD

Prof Martijn van den Heuvel, PhD

Johannesburg, 2021

Declaration

University of the Witwatersrand, Johannesburg

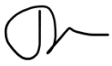
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Signature:  Date: 24 June 2021

Dedication

In loving memory of my beautiful mother, Gladys Ndlovu (1952-2020).

To my brother, Bakani, none of this is possible without you, thank you.

To my son, Phenyo, I look forward to reading your thesis one day.

To my beautiful sisters Tjawada and Nozizwe, thank you, I hope you are proud.

To Dr Calvey, thank you for being the greatest teacher.

To my extended family Mowapiwa, Phiwokuhle, Emmanuel and Katleho, thank you for your continued support and confidence.

Presentation and Publication from this work

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Abstract

Nyaope is the street name for what is believed to be a drug cocktail in South Africa although recent research suggests that it is predominantly heroin. Nyaope powder is most commonly smoked together with cannabis, a drug-use pattern unique to the region. Due to the increasing burden of this drug in low-income communities and the absence of human structural neuroimaging of combination heroin and cannabis use disorder, we initiated the first ever cohort study in order to identify the neuroanatomical features of the disorder. We also sought to characterize the neuroanatomical sequalae of various clinical features.

Twenty-eight male nyaope users and thirty healthy, matched controls were recruited from drug rehabilitation centers and the community, respectively. T1-weighted MRI images were obtained using a 3 Tesla General Electric Discovery and cortical and subcortical volumetric properties were examined and compared.

Nyaope users displayed extensive grey matter atrophy in the right hemispheric medial orbitofrontal, rostral middle frontal, superior temporal, superior frontal, and supramarginal gyri (two-sided t-test, $p < 0.05$, corrected for multiple comparisons). No significant cortical or subcortical effects were found in clinical features such as relapse rate or current depressive episode.

Our findings suggest cortical abnormality in nyaope users in regions involved in impulse control, decision making, social- and self-perception, and working memory. Importantly, affected brain regions show large overlap with the pattern of cortical abnormalities shown in heroin use disorder. Our findings suggest that treatment should include opioid agonist maintenance therapy according to the World Health Organization (WHO) guidelines.

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List of acronyms and abbreviations

1.	SUD	substance use disorder
2.	Nacc	nucleus accumbens
3.	VTA	ventral tegmental area
4.	CB1 and CB2	cannabinoid receptor 1 & 2
5.	$\Delta 9\ THC$	$\Delta 9$ tetrahydrocannabinol
6.	D1R	dopamine 1 receptors
7.	D2R	dopamine 2 receptors
8.	PFC	prefrontal cortex
9.	OFC	orbitofrontal cortex
10.	mOFC	medial orbitofrontal cortex
11.	lOFC	lateral orbitofrontal cortex
12.	ACC	anterior cingulate cortex
13.	dlPFC	dorsolateral PFC
14.	sMRI	Structural Magnetic Resonance Imaging
15.	VBM	voxel-based morphometry
16.	MMT	methadone maintenance therapy
17.	IPL	inferior parietal lobule
18.	MCC	middle cingulate cortex
19.	SBM	surface-based morphometry
20.	MDUT	multi drug urine test
21.	NDI	Nyaope dependent individuals
22.	HCs	Healthy controls
23.	FDR	False Discovery Rate
24.	dmPFC	dorsomedial prefrontal cortex
25.	DMN	default mode network
26.	rTMS	transcranial magnetic stimulation
27.	BLA	basolateral amygdala
28.	PLC	prelimbic cortex
29.	CeA	central nucleus of the amygdala
30.	BNST	bed nucleus of the stria terminalis
31.	HPA	hypothalamic-pituitary-adrenal axis
32.	CRF	corticotropin releasing factor
33.	PCC	posterior cingulate cortex

Chapter 1: Neuroanatomical features of nyaope use disorder

1 Introduction

1.1 Nyaope

1.1.1 The nyaope substance

Nyaope or whoonga is an illicit substance abused in South Africa. It is considered a polysubstance because it is comprised of various substances, but the primary component is heroin. It appears to be unique to each region in the country i.e. its composition differs region to region (1–4). Commonly it is a brown powder that is mixed with cannabis and smoked (1,4–6), however recent reports suggest that some individuals may be injecting it (1). One study analyzing the composition of nyaope from 12 different regions in Gauteng found that the nyaope powder was composed of several substances that included heroin and caffeine in all samples; and less consistently amphetamine/meth-metabolites, acetamorphine (paracetamol), opiates, dextromethorphan (cough suppressant), codeine, morphine/metabolites commonly, ARVs (efavirenz), cathine (b OH amphet), citroflex A, duracaine/lidocaine, thiofentanyl, benzitramide, benzodiazepines, phenobarbitone, pipradol, ,moramide and fenethylline less commonly (1,7).

Nyaope users report that the initial experience with the substance produces feelings of euphoria, peace or calm, pleasure and well-being, satiety, relaxation, absence of stress and ability to sleep (5,8). However, the pleasurable hedonic effects are soon replaced by intense cravings that are reported to appear anywhere from the fourth administration to after two weeks of regular use (5). Withdrawal symptoms reportedly include abdominal pains, seizures, vomiting, salivation, psychosis, diarrhea, joint and muscle pain, temperature changes, insomnia and flu-like symptoms (1,2,5,8). Compounding the highly addictive nature of the substance is the low cost of the drug. Nyaope has been reported to cost from about 20 to 45 rand a dose (1–3), but the cost rises as individuals soon require more than one dose a day.

1.1.2 Social and health repercussions of nyaope use disorder

The highly addictive nature of nyaope results in the users re-orienting their lives towards the acquisition of the drug which has several social and health repercussions for the user, family and community (5,8). Users have been described as having poor hygiene, poor appetite therefore low BMI and signs of malnutrition (5,8,9). A study into nyaope users in rehabilitation centers found that excluding for antisocial personality disorders, approximately 50% of the subjects had some form of psychiatric comorbidity that included major depression, generalized anxiety, and post-traumatic stress disorder (10). Additionally, approximately 69% had suicide ideations (10). One study that assessed organ and hematological integrity in users found metabolic acidosis, liver dysfunction that needed further investigations, aberrant gonadal function and variations in triiodothyronine 3 levels (9).

Nyaope users are said to threaten the family unit by causing feelings of misery, helplessness, fear and conflict amongst families (8). Most users report an inability to maintain relationships with families or spouses (5). Within the community, nyaope users are infamous for their petty theft activities to support their habits (1–3,5,8). Morgan and colleagues (2019) reported that from a sample of 300 nyaope users, approximately 70% had committed a crime to support their habit and 30% had committed a violent

crime. Nyaope users and/or dealers reportedly robbed or illicitly purchased antiretrovirals from health officials and HIV patients for their nyaope cocktail, which inadvertently resulted in patients defaulting on their treatment (11). Of additional concern is that nyaope is being sold to adolescents (8) and unfortunately this affects the level of education the individuals are able to attain which perpetuates poverty in South African communities (3).

1.1.3 Prevalence and treatment of nyaope use disorder

The prevalence of nyaope, much like other drugs, is hard to estimate accurately. Data about prevalence is acquired from rehabilitation centers mostly; but one study on methamphetamine users in Cape Town found that only 10% of the study subjects had accessed treatment, with less than 3% in the report accessing treatment within the previous year hence much of the existing data may profoundly underestimate the true extent of the problem (12). Dada and colleagues (13) found that from a population of users in rehabilitation facilities, nyaope was reported mainly in Gauteng (6%), the Northern Regions (Limpopo) (6%), and KZN (2%). What was noted as an issue of concern was the rising levels of nyaope consumption in Limpopo from 2% in the period of January-June 2014 to 6% in the period of July to December 2014 (13).

Nyaope is difficult to treat. It is estimated that treatment may take up to 12-15 months (2) indicating the severity of disease. In a study examining the locus of control in 221 nyaope users, from the interview process, the authors reported that 71.9% of their subjects reported trying to quit unsuccessfully with only 10.1 % being able to abstain for a year or longer (14) while Morgan et al. (2019) found that at the three month follow up interval, 65.5% had already relapsed.

According to the DSM V, an important feature of substance use disorder (SUD) is the underlying change to neural circuits that may persist beyond detoxification and manifest as repeated relapses (15). Therefore, the pathological behaviors observed in substance use disorders are a consequence of altered neural systems and it thus becomes imperative to understand the underlying neural mechanisms of nyaope use disorder.

1.2 Neurobiology of Substance Use Disorders

1.2.1 The reward system of the brain

All substance use disorders (SUD) are a result of changes in the reward system of the brain that is largely under the control of dopaminergic system (15–18). The reward system of the brain functions primarily to produce feelings of pleasure, motivation and plays a role in learning (17). Natural stimuli essential for survival such as food and opportunities for mating elicit a pleasure response by increasing dopamine levels that are interpreted as rewarding and used to reinforce and improve behavior in order to obtain the reward again (17). A number of substances such as opioids, cannabis, nicotine, alcohol and psychostimulants (cocaine and methamphetamines) are also able to directly activate and ‘highjack’ this system (19,20), and soon, normal or beneficial activities are neglected in pursuit of the pleasure derived from the drug reward (15). The pleasurable effects from the aberrant activation of the reward system initiates learning processes in the brain that lead to a consolidation of the hedonic effect, initiate learning cues that predict availability thus consumption of the drug, and assign value and motivational drive to procure the substance such that behavior is then directed towards obtaining the drug (17). Therefore, SUD result in changes of brain circuits that may persist despite cessation or detoxification of the substance (15).

1.2.2 The central role of dopamine in reward generation

The reward circuit is built around the stimulation and release of dopamine in key brain regions such as striatum (particularly the nucleus accumbens), the frontal cortex (particularly the prefrontal cortex), amygdala and hippocampus (17). Drug induced increases of dopamine in the nucleus accumbens (Nacc) have been shown to be associated with subjective descriptors of reward e.g. pleasure, high and euphoria (21); and the fast not slow delivery of dopamine being shown to be responsible for reward (22). The mechanisms of the stimulation of dopamine release/delivery by substances are not uniform but depend on the substance's chemical nature and its brain receptor action.

Opiates, including heroin, a key component of nyaope, increase dopamine levels in the Nacc by interacting with GABAergic interneurons of the ventral tegmental area (VTA) (23,24). Opiates are structurally similar to endogenous opioids and are able to bind to the mu-, delta- and kappa opioid receptors of GABAergic interneurons, but mainly the mu-opioid receptor (23,25). In vitro, heroin is metabolized into 6-monoacetylmorphine (6-MAM) and morphine; and the two are mu-opioid receptor agonists (23,26). Within the VTA, GABAergic interneurons tonically inhibit the activity of dopamine projecting neurons that project through the cortico-mesolimbic tracts to the striatum, amygdala and frontal regions (23,24). Opioid receptor binding inhibits GABAergic interneuron activity thus disinhibiting dopamine neuron activity resulting in a surge of dopamine release into the striatum (particularly the Nacc), frontal regions and amygdala (23,24). Nacc neurons also express mu-dopamine receptors that opiates are able to bind to directly and cause an increase of synaptic dopamine (17).

Cannabis contains the compounds $\Delta 9$ tetrahydrocannabinol ($\Delta 9\ THC$) and a number of cannabinols that interact with the endocannabinoid system of the brain. The endocannabinoid system contains two main receptor types, cannabinoid receptor 1 & 2 (CB1 and CB2) that are activated by the endogenous cannabinoids anandamide and 2-arachidonoyl glycerol, although the latter is mostly in peripheral tissues (25,27). CB receptors are located on presynaptic neurons and cannabinoids are produced by postsynaptic neurons. Receptor binding inhibits neurotransmitter release by the presynaptic neuron i.e. retrograde synaptic transmission (27). The rewarding effects of cannabis are mediated by $\Delta 9\ THC$ binding to CB1 receptors that are located on glutamatergic and GABAergic neurons throughout the brain (25,27). The exact mechanism of action on the reward system by the endocannabinoid system are not yet fully clear, but it may mediate the release of dopamine in the Nacc and VTA directly or indirectly, possibly by inhibiting GABAergic (similar, but less in magnitude to opiates) and glutamatergic neurons (24,27). CB1 receptor activation has also been shown to interact with other drugs of abuse such as opioids, nicotine, ethanol and cocaine (25). Studies suggest that CB1 receptor agonists (including THC) facilitate the rewarding effects of opioids and may augment opioid reward possibly through the mesolimbic pathway (25). This is intriguing for a substance like nyaope, whose key components include heroin that is smoked with cannabis; and how this would translate to the neuroanatomical deficits of the nyaope patient's brain is not yet known.

1.2.3 Neuropathology of substance use disorders

The Nacc is divided into a core and shell region and it is the fast release/delivery of dopamine in the shell region that is thought to be the source of reward generation(17,18,24,28). Nacc shell neurons expressing dopamine 1 receptors (D1R) are thought to activate the basal ganglia direct pathway which results in the hedonic pleasant feeling being produced by the frontal cortex and other limbic centers (28). Nacc shell neurons expressing dopamine 2 receptors (D2R) activate the basal ganglia indirect pathway which inhibit hedonic unpleasant feelings such as disappointment by the cortex (28). D1R have a much lower affinity for

dopamine compared to D2R, therefore, fast dopamine firing/delivery is required to substantially elevate dopamine levels in the Nacc to enable D1R binding (28). Drugs of abuse thus produce reward for a short time through D1R while inhibiting aversive responses (driving the motivation to take more drugs) for longer through the D2R meditated indirect pathway. Release of dopamine in the Nacc results in its neurons interacting with other emotion regulating centers of the limbic system such as the amygdala (including basolateral and central nuclei), ventral palladium/substantia innominate; and executive centers in the frontal cortex (which also receive direct dopamine stimulation) (17,18,24). Collectively these interactions produce the pathology of substance use disorder and repeated relapse (17,18,24).

Furthermore heroin neuropathological studies have shown that heroin is a neurotoxic substance that causes cell death in both neurons and supporting cells (23,26). Several ideas have been put forth and tested on how heroin cell death occurs, and it is more likely that they work simultaneously in producing cell death. Oxidative stress from the excessive delivery of dopamine has been reported as a cause of neuronal cell death. That is the because the metabolism of dopamine results in the formation of the superoxide anion (O_2^-) and H_2O_2 which may react with transition metals to form the highly toxic hydroxyl radical (OH) (23). Oxidative stress markers have been reported in a range of substance use disorders, including but less so, in heroin (23). Heroin metabolites in vivo have been shown to induce neuronal and microglial apoptosis and this was confirmed in rat cortical neurons in vivo; and the study showed that heroin-initiated apoptosis by activating caspase 3 (23). Interestingly, heroin did not induce apoptosis via opioid receptor binding, ionotropic glutamatergic receptors and oxidative stress (23). Lai and colleagues (29) found that heroin induced apoptosis through Caspase 3 was linked to the activation of c-Jun N-terminal kinases (JNK/c-Jun), a mitogen activated protein kinase (MAPK) that has been shown to play a role in cell death. Lastly heroin has been shown to induce hypoxic ischemic changes such as cerebral edema, neuronal damage and loss possibly due to heroin-induced respiratory depression (30).

Therefore the aberrant and protracted dopaminergic innervation and neurotoxicity due to drug use in the brain leads to long term changes to neuron and receptor profiles, neurochemistry and ultimately neuroanatomy- gray and white matter atrophy which produce and sustain substance use disorders characterized by compulsive drug seeking despite negative consequences (17,18,28).

1.2.3 Emergence of Substance use disorders

The emergence of SUDs requires a transition from voluntary and casual drug use to more compulsive and habitual drug use, a pathological overvaluation of the reward obtained from a drug above other beneficial rewards and lastly the disintegration of executive inhibitory control of behavior (24). These properties in the patient function synergistically to produce and sustain SUDs.

The shift gradual shift from voluntary/casual- to habitual/compulsive drug use is a reflection of the greater involvement of the dorsal striatum in drug seeking behavior (18). The locus of drug seeking behavior, in the absence of SUD, is under the control of the ventral striatum (Nacc core and basolateral amygdala) but protracted use causes changes that result in this function being increasingly controlled by the habit driving dorsal striatum (17,18,24,28). This is a consequence of changes to the existing spiraling circuit between the Nacc shell and core, VTA and dorsal striatum (18). The Nacc shell upon dopamine stimulation provides feedback to the VTA that then stimulates the Nacc core which then stimulates the dorsal striatum through the substantia nigra (18). The dorsal striatum has been shown to be the locus for habit driven behavior; and thus, the gradual overstimulation of the circuit by drugs results in greater recruitment and dominance of the dorsal striatum in drug seeking behavior (18). The propensity to escalate to habitual drug seeking has also been associated with an impulsive phenotype in animals and patients alike that can be predicted by low D2/3R availability in the ventral striatum (18). Therefore, the transitioning from voluntary to habitual drug use may be a consequence of the change in the mesolimbic spiraling circuit due to protracted drug use and a genetic predisposition reflected by low D2/3R availability in the ventral striatum.

The other two hallmarks- pathological overvaluation of the drug reward and a loss of top down inhibitory control- are both largely under the control of the prefrontal cortex (PFC) (28). Neurotoxicity, aberrant dopamine delivery from the VTA and alterations of dopamine receptor (1 and 2) signaling from the striatum in protracted drug use are thought to cause atrophy and changes of activity within the regions (28).

The orbitofrontal cortex (OFC) of the PFC assigns salience to stimuli by analyzing potential outcomes and providing predictive information about them which is used to shift behavior if the reward is deemed no longer beneficial or reinforcing in conjunction with other PFC regions (18,28). Changes to the region may result in an inability to analyze outcomes and shift behavior in SUDs even when the drug is causing social, physical and mental harm to the individual (17,28), a common trait in SUDs. Indeed, the OFC (and anterior cingulate cortex) were found to be hypoactive in addicts in the absence of drug stimuli and becoming hyperactive in the presence of said stimuli (28), which may explain how a drug reward becomes overvalued above other beneficial activities driving an addict to seek that drug neglecting all other responsibilities (17,24,28). The function of the OFC and its properties of analyzing outcomes and assigning salience are integral to the executive inhibitory circuit.

The executive inhibitory circuit relies on the lateral orbitofrontal cortex (lOFC), anterior cingulate cortex (ACC), the dorsolateral PFC (dlPFC) and the inferior frontal cortex (Brodmann area 44) communicating to suppress activity within the PFC that produces craving and in the striatum that drives habitual drug seeking (28). The ACC facilitates inhibitory learning by keeping tabs on predicted outcome versus actual outcome and conveying that information to the dlPFC, inferior frontal cortex and dorsal striatum (particularly the caudate) (28). Inhibitory function of this circuit- OFC, ACC, dlPFC and inferior frontal cortex is dependent on slow dopamine firing hence the protracted fast dopamine firing may alter the function (28). Indeed, the ACC was found to be hypofunctional with the OFC (*see above*); and the inferior frontal cortex was associated with the deactivation of the Nacc and ventral PFC upon successful suppression of craving in cocaine addiction while the dlPFC (and inferior frontal) was shown to be essential in suppressing dorsal striatal activity thus producing self-control (28).

Ultimately, changes to the neuroanatomy of the patient produce and perpetuate the substance use disorder. The patient gradually seeks drugs in a habitual automated way due to a shift from ventral to dorsal striatal control in drug seeking. The ability to analyze and predict outcomes (OFC and ACC) becomes increasingly erroneous and thus the drug reward becomes overvalued and takes precedence above other cognitive and behavioral function (OFC and other PFC regions). Simultaneously the intrinsic mechanism built to inhibit behaviors and impulses deemed to be detrimental to the well-being of the individual become increasingly ineffective (lOFC, ACC, dlPFC, inferior frontal cortex) and are unable to suppress activity in the PFC that produces craving and the dorsal striatum rendering the dorsal striatum uncontrolled in its compulsive habitual drug seeking behavior.

Drugs of abuse pose a threat to a system that is vulnerable to injury (23). Neurons do not divide thus damage resulting in death is irreversible for the most part (23). Therefore, defining neuroanatomical changes in SUDs is an important step in understanding the pathology, subsequently predicting outcomes and tailoring targeted therapy that address neuroanatomical deficits. Neuroimaging techniques are non-invasive techniques that have been used to study structural and functional changes in SUD patients.

1.3 Neuroimaging tools

Several tools can be used to study the brain in vivo. These include magnetic resonance imaging (MRI), Positron Emission Topography (PET) scanning, Magnetoencephalography (MEG) scanning and electroencephalogram (EEG). Each scan is used for a specific purpose for example MRI is used to obtain images of the brain while EEGs are used to obtain information about electrical activity produced by neurons.

MRI scanning is used to discern the structural and functional integrity of gray and white matter. An MRI is a strong magnet that introduces an electromagnetic energy that forces the protons in a patient to align along this field. A radiofrequency pulse is then passed through the patient that disrupts this alignment causing protons to spin out of equilibrium (31). The pulse is then removed forcing the protons back to alignment with the field and this process releases energy that sensors in the MRI can detect (31). The time it takes to realign, and the amount of energy released depends on the chemical nature of the environment (31). The intensity of the energy released is plotted on a grey scale and images are built up from this. Ultimately the sensors of an MRI produce T1-, T2- and diffusion weighted images, amongst others, that can be used to view the brain.

To discern the structural integrity of gray and white matter in patients, structural MRI (sMRI) analysis is used. sMRI is only concerned with the structural integrity of gray and white matter. Each T1-weighted image is quantified using voxels, a volume of $1mm^3$ (1mm x 1mm x 1mm). Volumetric data can then be collated and comparisons between patients and controls can be made in post processing techniques. The comparison using volumetric data is called voxel-based morphometry (VBM) (32). A newer technique, surface-based morphometry (SBM) analysis, uses 3D voxels to create 2D triangular vertices on the surface of the brain that too can be collated and compared between patients and controls. SBM has been suggested to be more sensitive to cortical thickness changes compared to VBM (32). sMRI in conjunction with VBM and SBM analysis have previously been used to identify gray and white matter changes in various SUDs (32-48).

1.4 Neuroanatomical changes in substance use disorders

1.4.1 Neuroanatomical changes in heroin use disorder

Several neuroanatomical studies into gray- and white matter changes in heroin use disorder have been carried out. Wide arears of cortical atrophy or volumetric reductions have been reported in the brains of heroin patients. Within the cortex, the frontal cortex is often reported to experience gray matter loss or reductions (32–37). The regions appear predominantly in the PFC in the dlPFC (32–36), dorsomedial PFC (dmPFC) (32–34,37) and the ACC (33,34). Other areas of the frontal cortex that were reported to have reduced gray matter in heroin were the gyrus rectus, the supplementary cortex and poste central gyrus (33,34,37). In other cortices, gray atrophy or volume loss was also reported in the insula (32,33,37), the middle or posterior cingulate cortices (35–37), the fusiform gyrus (33,35,37), the superior temporal gyrus (32,37), the middle or inferior occipital cortices (36,37) and in the inferior parietal lobule (35). Gray matter increases are seldom reported with only (32) reporting gray matter increases in the superior parietal lobule, temporal poles, lateral occipital cortex, inferior parietal lobule and cuneus regions.

Cortical gray matter reductions are prevalent in heroin use disorder but in variable regions. To resolve this variability, a metanalysis was conducted by Wollman and colleagues (38) to determine the commonly affected areas of gray matter loss across twelve different opioid studies, nine of which were heroin studies. The metanalysis found consistently reduced gray matter in in the bilateral fronto-temporal regions specifically in the right Heschl's gyrus extending to the right insula and superior temporal gyrus, the right gyrus rectus extending to the bilateral orbitofrontal regions, the right middle frontal gyrus and left temporal pole extending to the inferior frontal gyrus (38).

Changes to subcortical gray matter were found in the cerebellum, Nacc and thalamus of heroin users (33,37,39). White matter volumes do not appear to be affected with only Cheng and colleagues (37)reported increased white matter volume in the medial orbitofrontal cortex and supramarginal gyrus. Heroin appears to affect subcortical structures and white matter minimally.

There are several noticeable limitations that may influence the variabilities observed in previous literature. Firstly the heterogeneity in populations may impact the statistical power when running analysis. The lowest number of participants in this

literature review was eleven heroin users conducted by Yuan and colleagues (35) and the highest being thirty-three conducted by Cheng and colleagues (37). They both show similar regions of gray matter density reductions in the frontal, cingulate, temporal and parietal regions; but Cheng and colleagues (37) also reports gray matter reductions in the occipital region and thalamus and, found increased white matter in the OFC and supramarginal (parietal region) suggesting that Cheng and colleagues' (37) greater population sample therefore greater statistical power may have allowed them to discern greater areas of atrophy. Secondly, the period since last use of heroin during time of scanning may impact the extent of gray matter atrophy. A study by Wang and colleagues (36) reported some degree of reversibility in gray matter atrophy during an abstinence period from the first scan at three days of abstinence and at the second scan at thirty days of abstinence. They reported that gray matter atrophy was noticeably reduced in the superior frontal region and not others suggesting that reversibility may be restricted to the superior frontal gyrus and no other brain regions (36). However, metanalysis by Wollman and colleagues revealed that longer duration of abstinence appeared to be beneficial with damage in the frontal, temporal and insular regions being reversed to statistically insignificant atrophy except in the precuneus where atrophy persisted (38). The reversibility observed by Wang and colleagues (36) may be time dependent and greater areas may experience recovery at longer periods of abstinence as suggested by (38). Thirdly the presence of opioid substitution therapy may affect the areas of cortical atrophy and has been reported to increase volume in the precuneus (38). Lastly age has been shown to affect gray matter loss, particularly in the right insula and left temporal poles (38), but most studies averted this by regressing the effect of age out in their analysis.

Duration of use was shown to be negatively correlated with some neural regions (32,33,35,37,38). These areas are also located mostly in the PFC of users in the dlPFC (32,33,35,38); and in the ACC and gyrus rectus (33). Other areas reported are in the fusiform gyrus, insula and cerebellum (33,38). A metanalysis carried out by Wollman and colleagues (38) indicated that the duration of use was negatively correlated with gray matter loss in the right Rolandic operculum and left cerebellum. The greatest duration of use in a whole brain study was reported Li and colleagues (32) and the lowest duration was Yuan and colleagues (33); gray matter reductions were reported by both in the frontal, temporal and insular regions but Li and colleagues (32) also reported gray matter increases in parts of the parietal, temporal and occipital regions. It is worth noting that Li and colleagues (32) reported much greater areas of gray matter changes than Yuan and colleagues (33) possibly suggesting that protracted use exacerbates areas of gray matter changes.

1.4.2 Neuroanatomical changes in cannabis use disorder

Previous studies into cannabis use disorder are more equivocal on the effects of cannabis on gray matter integrity in users. Some studies reported increased gray matter density while others reported gray matter reductions. Increases in gray matter density have been reported in the cerebellum (40,41), in the precentral gyrus and putamen (42–44). Increases in gray matter density were also reported in the lingual gyrus and dlPFC but these effects did not survive multiple comparison corrections suggesting a minimal effect (42). In contrast Battistella and colleagues (40) reported gray matter density reductions in the in the orbitofrontal cortex, parahippocampal gyrus, insula, temporal poles.

Studies into the effects of cannabis use disorder share similar limitations: the concurrent use of tobacco use which is often overlooked or regressed out. Wetherill and colleagues (43) sought to address this limitation by recruiting pure cannabis users, cannabis with concurrent tobacco use and pure tobacco use. They found that pure cannabis users had increased gray matter in the right putamen; pure cannabis and concurrent cannabis and tobacco use had reduced gray matter in the thalamus; pure tobacco and concurrent cannabis and tobacco use had reduced gray matter volume in the cerebellum; and all the groups had greater gray matter volume in the putamen.

Evidently gray matter changes in cannabis use are not explicit, possibly owing to the complexity of the cannabinoid system of the brain. To resolve the equivocality in cannabis use studies Lorenzetti and colleagues (44) conducted a metanalytic study encompassing a total of 717 cannabis users compared to 778 healthy controls and found that cannabis commonly caused gray matter reductions in the hippocampus and the OFC. Lastly no studies reported indicated any volumetric changes in the white matter of cannabis users suggesting that the effects are largely limited to gray matter.

Correlational analysis in cannabis use disorder did not indicate explicit correlations between duration of use and gray matter changes. Cousjin and colleagues (41) found that there was an inverse relationship between the severity of cannabis dependence and the gray matter volume of the right amygdala and that the bilateral hippocampal volumes were inversely correlated to the weekly consumption of cannabis quantity.

Previous studies into heroin and cannabis use disorders show that the two substances cause detectable changes in the neuroanatomy of their users particularly they gray matter. It thus becomes pertinent, to identify and characterize these changes in nyaope users as no neuroanatomical data exists on combination heroin and cannabis use. Furthermore, recent research and advocacy has classified nyaope as a form of heroin use disorder and called for greater attention to the problem and increased access to opioid maintenance therapy. The study could also provide neural templates for some of the pathological behaviors reported by (1–3,5,8), and possibly aid in the development of more tailored treatment (28).

2 Study aims, objectives and expected outcomes

2.1 Specific aim 1: Identify the neuroanatomical features of nyaope use disorder

We aimed to characterize the structural morphology of the brains of nyaope dependent individuals (NDIs) using sMRI scanning protocol in NDI compared to healthy controls (HC).

We expected to find similar structural irregularities to what has been found previously in heroin (33,34,38,39), polysubstance (45) and cannabis abuse (44), such as gray matter atrophy in prefrontal regions, cingulate cortices, supplementary motor cortex, posterior central gyrus, gyrus rectus (orbitofrontal cortex), temporal regions, the insula cortex and the cerebellum.

3. Methods

3.1 Sample size

Principled power calculations are known to be difficult in neuroimaging analysis frameworks. Previous similar studies (33,37,39,41) that have been able to reject the null hypothesis in one or more neuroanatomical regions of interest used test and healthy control (HC) groups of n=30.

3.2 Recruitment

This study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand and was carried out in accordance with the Declaration of Helsinki. All participants signed a written informed consent prior to participation.

3.2.1. Participant recruitment

Male nyaope dependent individuals (NDI) (n=28) were screened and recruited from Life Randfontein and Empilweni rehabilitation centers in Gauteng, South Africa. Screening and assessment included the Mental State Examination (MSE), the Mini-International Neuropsychiatric Interview (MINI), full drug-use history and the Multi Drug Urine Test (MDUT), amongst others (10,46). NDI were selected based on their last nyaope or methadone administration being more than one week prior to recruitment as well as their MDUT being positive for opioids and cannabis. Patients with a history of co-morbid psychiatric disorders or presenting with symptoms of psychiatric comorbidity on the MSE were excluded.
NDI were followed up at three months and nine months for further psychiatric assessment.

Healthy, age-, and sociodemographically- matched male control participants (n=30) were recruited from the community of Soweto, South Africa. The control group was screened for history of psychiatric, neurological, and substance use disorders using our biological questionnaire (see appendix).

NDI and HC were excluded if they were HIV positive or had not undergone an HIV test with a negative result in the six months prior to recruitment. Recreational alcohol and nicotine use patterns were similar in both groups.
Further exclusion for MRI scanning included inserted metal devices, claustrophobia, and a history of head injury that resulted in loss of consciousness.

3.2.2. MRI scanning protocol

NDI were transported from the rehabilitation centers to the Nelson Mandela Children's hospital for MRI scanning. Control participants were transported from the community to the hospital. T1-weighted MRI images were obtained using a 3 Tesla General Electric Discovery under the guidance of experienced radiologists and radiographers. Images were obtained with dimensions 256mm x 256mm x 156mm, voxel size 0.937500, 0.937500, 1.200000, flip angle 8° field of view (FOV) 240 and repetition time (TR) 600 msec, time to inversion (TI) 600 msec, time to echo (TE) 2.4 msec covering the whole brain.

4. Data analysis

4.1 Population demographics and drug use

Statistical differences between NDI and the control group (means, standard deviations, unpaired t-test) were calculated in Microsoft Excel.

4.2 Software analysis

We converted the raw MRI DICOM format data to NIfTI format using MRIcron convert dicom to nifti(dcm2niix) interface. MRIcron was accessed from (https://www.nitrc.org/frs/download.php/11539/mricron_macOS.dmg).

4.2.1 SBM analysis using FreeSurfer

T1 NifTI images from MRIcron were processed using FreeSurfer accessed from (https://surfer.nmr.mgh.harvard.edu/). We first ran *recon-all* (a pre-processing automatic reconstruction command) on each subject's T1 images. *recon-all* registered all images to Talaraich space, segmented brain tissue, performed cortical ribbon reconstruction and estimated the thickness of the cortex using the inbuild Desikan-Killiany (DK) atlas. This process produced a number of data containing files on the cortical thickness of the left and right hemispheres: (. thickness) and labels (aparc. annot) that we used for group analysis.

Group analysis was done through vertex wise analysis through group linear modelling (GLM) and a cluster-wise correction (CW) for multiple comparisons using permutation simulation. All cortical reconstructions were aligned and normalized to the average FreeSurfer brain (fsaverage) using the FreeSurfer functions *mri_preproc* and *mri_surf2surf.* Next, a vertex-wise group analysis was performed using a general linear model (GLM) using *mri_glmfit* testing for group differences in cortical thickness ($P<0.001$) at each point (vertex) along the cortical mantle, with group as main factor and gender and age taken as covariates. Cortical atrophy was taken as a difference in cortical thickness between patients and controls, with permutation testing by means of *mri_glmfit-sim* used for statistical evaluation of each of identified subclusters of vertices. Permutation testing was set at a threshold of $p<0.01$.

4.2.1 Regional analysis

For all 68 cortical regions of the DK atlas, metrics of average cortical thickness was extracted from the FreeSurfer *aparc* stats files. In addition, metrics of total gray matter volume, white matter volume and subcortical volume for 14 subcortical areas (7 per hemisphere, including hippocampus, thalamus, caudate nucleus, putamen, lateral ventricle, cerebellar cortex, cerebellar white matter) were extracted from the aseg.stats file. As all the subjects' cortical thickness maps were aligned to a common surface template, the intracranial volume was not used as a covariate (47) and there was no significant difference in intracranial volume between NDI and controls ($p=0.57$). Cortical atrophy was computed as the difference in thickness between nyaope users and the group of healthy controls. Statistical testing was performed using two-sample t-tests using MATLAB accessed from (http://www.mathworks.com). Effects reaching an FDR threshold of $p<0.05$ were taken as significant.

4.2.2 Risk of relapse neuroanatomical features analysis

We used the follow up data collected nine months after rehabilitation of nyaope patients to divide them into abstinent- and relapse patients. We then ran regional analysis on their neuroanatomical (gray and white matter volumes) using regional analysis (*see chapter 2*).

4.2.3 Psychiatric co-morbidities neuroanatomical analysis

We used the data from the MINI to divide our NDI into those with current psychiatric features and those without and then ran regional analysis (*see chapter 2*) to compare the neuroanatomical features (gray- and white matter) of those with current psychiatric features to those without it.

5. Results

5.1 Demographics

5.1.1 Socio-demographic characteristics

Socio-demographic characteristics are presented in Table 1. The mean age of NDI was 26.75 SD± 3.79 years (range 21-34 years), and the mean age of the controls was 25.7 SD± 4.25 years (range 21-37). There were no significant differences in mean age (p=0.3).

Handedness was comparable between the two groups. In both groups 25 of the participants were right-handed. In NDI, 3 were left-handed; and in HC, 4 were left-handed and 1 was ambidextrous.

5.1.2 Substance use history and profiles

Substance use history and drug use profiles are presented in Table 1.

All NDI were diagnosed with nyaope use disorder by a psychiatrist. The mean duration of nyaope use was 6.54 ±2.97 years. The mean age of first use of nyaope was 20.21 ± 3.78 years (range 14-28).

Analysis of urine samples indicated that 26/28 NDI had only opioids and cannabis present in the urine. Two participants had recently consumed cocaine and one participant had recently consumed amphetamines and methamphetamines, although they reported infrequent use of these substances.

The majority of NDI smoked nyaope together with cannabis 23/28, 6/28 injected nyaope and 3/28 snorted nyaope.

Table 1: Sociodemographic and substance use profiles of the nyaope addicted individuals (NDI) and healthy controls (HC).

Demographics		
Nyaope dependent individuals (n=28)	Healthy controls (n=30)	p-values

Max age	34	37	-
Min age	20	21	-
Mean age	26.75 ±3.79	25.7 ±4.25	0.3
Age of first use	20.21 ± 3.78	-	-
Duration of use	6.54 ±2.97	-	-

	Handedness		
	Right	Left	Ambidextrous
NDI (n=28)	25	3	0
HC (n=30)	25	4	1

5.2 Neuroanatomical Imaging results

5.2.1 Cortical atrophy

Several regions in the brains of NDI showed reduced cortical thickness when compared to HC ($p<0.001$) and are presented in Table 2 and Figures 1 & 2. They were located bilaterally. In the right hemisphere, NDIs presented with reduced cortical thickness in the frontal cortex: medial- and lateral orbitofrontal, rostral middle frontal, superior frontal, pars opercularis and pars triangularis of the inferior frontal gyrus; and in the temporal cortex: banks superior temporal sulcus, middle temporal, superior temporal and transverse temporal; in the parietal cortex: inferior parietal, post central, precuneus, and supramarginal; and in the insula (Figure 1). In the left hemisphere, NDIs presented with reduced cortical thickness in the frontal cortex: superior frontal and inferior frontal's pars opercularis and pars orbitalis; in the transverse temporal cortex and in the lateral occipital cortex (Figure 2).

Permutation simulation generated two clusters that were less than the cluster-wise threshold $p<0.01$ in the right hemispheric medial-orbitofrontal ($p=0.002$) region and superior temporal cortex ($p=0.002$) and one in the left hemisphere, the rostral middle frontal gyrus ($p=0.002$).

5.2.2 Regional testing

We further examined cortical thickness from each of the cortical regions and compared values between groups (using a two-sided t-test and False Discovery Rate (FDR) detection) (Table 2). Regional analyses confirmed reduced cortical thickness between the two groups ($p< 0.05$) in the right medial orbitofrontal cortex, right rostral middle frontal cortex, right superior frontal cortex, right superior temporal cortex, and right supramarginal cortex. Affected regions showed right hemisphere lateralization, however, post-hoc analysis showed that all homotopic counterparts in the left hemisphere showed a similar pattern of atrophy (left medial orbitofrontal, reduction -2.7%, $p=0.0752$; left rostral middle frontal, reduction -2.76%, $p=0.0721$; left superior frontal, reduction -3.13%, $p=0.0155$; left superior temporal, reduction -2.69%, $p=0.0722$; left supramarginal, reduction -1.97%, $p=0.1617$ but effects did not reach significance following FDR correction.

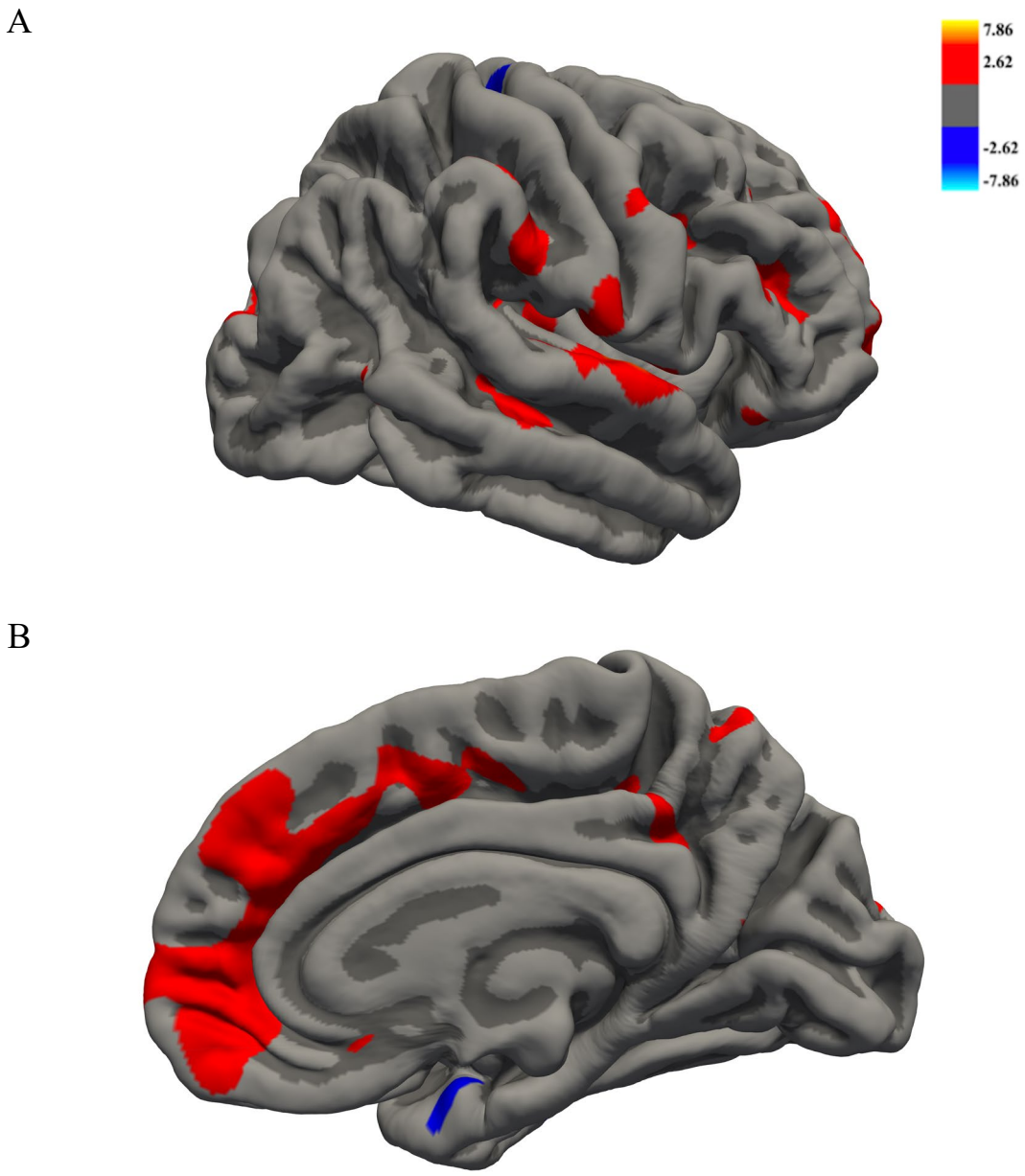


Figure 1: Vertex-wise map of cortical atrophy in NDI compared to healthy controls in the right hemisphere.

Panel A shows the lateral pial surface, panel B shows the medial pial surface. Threshold was set at $p<0.001$ for each cluster. Cortical atrophy (indicated in red and yellow) was most pronounced in the medial orbitofrontal, superior frontal, paracentral, precuneus, rostral middle frontal, caudal middle frontal, lateral orbitofrontal, pars triangularis, precentral, superior temporal, insula, postcentral, supramarginal, middle temporal, inferior parietal, lateral occipital, and superior parietal regions. The color bar represents T score.

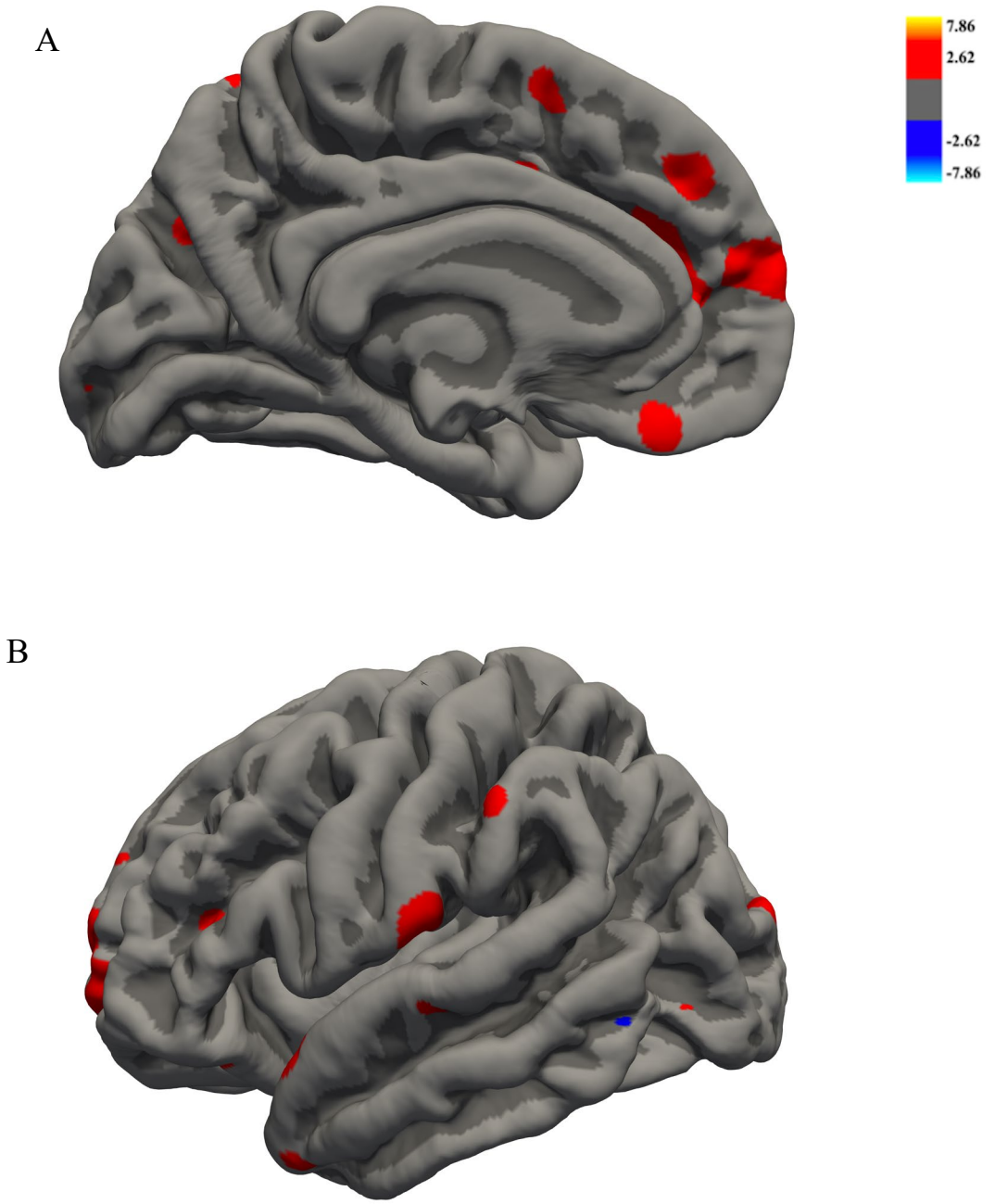


Figure 2: Vertex-wise map of cortical atrophy in NDI compared to healthy controls in the left hemisphere.

Panel A shows the medial pial surface, panel B shows the lateral pial surface. Threshold was set at $p<0.001$ for each cluster. Cortical atrophy (indicated in red) was most pronounced in the frontal cortex: superior frontal and inferior frontal pars opercularis and pars orbitalis; in the transverse temporal cortex and in the lateral occipital cortex. The color bar represents T score.

5.3. Duration of drug use and cortical thickness

Duration of drug use was not found to be particularly related to the pattern of atrophy. Duration of drug use was marginally associated with cortical atrophy in the left superior temporal sulcus, bilateral caudal-anterior cingulate, left fusiform, bilateral middle temporal, left superior parietal, left superior temporal, right inferior parietal, right pars opercularis, and right posterior cingulate ($p<0.05$), but these effects did not reach significance following FDR correction.

Table 2: Significant reductions in cortical thickness between NDI and controls corrected for multiple comparisons

Hemisphere	Regions reaching p<0·05 (uncorrected)	Percentage reduction in cortical thickness	Regions reaching p<0·05 FDR corrected
Right	medial orbitofrontal (p=0.001)	4.22	medial
	rostral middle frontal (p=0.002)	4.15	orbitofrontal
	superior frontal (p=0.0002)	4.62	rostral middle
	superior temporal (p=0.0005)	4.83	frontal
	supramarginal (p=0.002)	4.46	superior frontal
	banks superior temporal (p=0.04)	4.25	superior temporal
	inferior parietal (p=0.04)	2.65	supramarginal
	lateral orbitofrontal (p=0.02)	3.35	-
	middle temporal (p=0.003)	3.37	-
	pars opercularis (p=0.02)	3.36	-
	pars triangularis (p=0.02)	3.41	-
	precuneus (p=0.02)	3.09	-
	transverse temporal (p=0.03)	4.46	-
	insula (p=0.02)	3.43	-
			-
			-
Left	lateral occipital (p=0.03)	2.81	-
	pars opercularis (p=0.04)	2.50	-
	pars orbitalis (p=0.01)	4.41	-
	superior frontal (p=0.009)	3.13	-
	transverse temporal (p=0.02)	5.45	-

6. Discussion

6.1 General discussion

Our results show that nyaope use disorder reduces cortical thickness in large areas of the patients' brains that include the frontal, temporal, parietal, occipital and insular cortices. Subcortical and white matter analysis showed bilateral atrophy of the cerebellar white matter and atrophy of the left hemispheric thalamus proper; but none of the subcortical and white matter effects survived FDR correction indicating minor changes in these brain regions of the patients.

We found reduced gray matter in the medial and lateral orbitofrontal, rostral middle frontal, superior frontal, pars opercularis and triangularis of the inferior frontal gyrus of the frontal cortex. Frontal cortical damage in substance use disorders is a common finding and is consistently reported in heroin use disorders (33–38). It is less consistently reported in cannabis use disorder with some studies finding no changes (41). From previous literature, it is evident that frontal cortical damage is consistently reported in heroin and is diffuse throughout the region while in cannabis use disorder it is less consistently reported and when reported appears to be confined to the OFC (40,44) and in other instances conferring greater gray matter to its users (43). Therefore, we could suggest that the extensive frontal cortical damage seen in nyaope patients may largely be due to heroin content.

We found reduced cortical thickness in the banks of the superior temporal sulcus, middle temporal-, superior temporal- and transverse temporal- cortical regions of the temporal cortex. Temporal cortical damage in substance use disorders has been noted previously; and is commonly, but not consistently, reported in heroin use disorders (32,33,37,38). In a metanalysis study of opiate-induced cortical changes, Wollman and colleagues (38) reported that the temporal region was consistently damaged while Lorenzetti and colleagues' (44) metanalysis study into changes in the brains of cannabis users found no change in the temporal regions. We could, suggestively, ascribe the cortical atrophy in the temporal regions to heroin content in the present cohort.

We found reduced cortical thickness in the inferior parietal lobule, posterior central cortex, precuneus, and supramarginal gyrus of the parietal cortex. Cortical damage to the parietal cortex has previously been reported in substance use disorders. It has been reported in heroin use disorders (33,37), although Li and colleagues (32) found gray matter to be elevated in the region. To our knowledge, it has not been reported in cannabis use disorder. Wollman and colleagues (38) in their metanalysis did not find consistent changes in the parietal cortex of opiate users and there appears to be inconsistences in the reporting of changes to the region in heroin use disorders. However, compared to the absence of parietal cortical changes reported in cannabis, heroin content in nyaope may be the agent causing atrophy in the region.

We found reduced cortical thickness in the right hemispheric insula cortex. The region has been reported to be damaged in other substance use disorders. It is commonly, but not consistently reported in heroin use disorder (32,33,37,38). It is worth noting that Wollman and colleagues (38) in their metanalysis reported that the insula was commonly damaged in opiate use disorders while Lorenzetti and colleagues (44) did not find it to be consistently damaged in a cannabis use disorder metanalysis study. We could suggest, based on frequency of reporting, that the damage to the insula may be largely mediated by heroin in nyaope use disorder.

We found reduced cortical thickness in the left hemisphere lateral occipital cortex. Cortical damage to the occipital cortex has previously been reported to be damaged in some substance use disorders including heroine (36,37,48,49). In heroin use disorder, gray matter in the region was reported to be elevated by Li and colleagues (32). To our knowledge it has not been reported in cannabis use disorder. It is possible that heroin may be the causative agent to occipital cortical atrophy.

Subcortical structures are often not reported, possibly due to inherent limitations in segmenting the small structures during software analysis (39). In our analysis, we found that the left hemisphere thalamus proper was atrophied and that the cerebellar white matter volume was atrophied bilaterally in nyaope users using regional analysis. Cheng and colleagues (37) found reduced gray matter volume in the right hemisphere thalamus but increased white matter volume along the pons-midbrain-thalamus axis.

We had recruited a similar number of participants to Cheng and colleagues (37) compared to other studies which may have had an improved statistical power in detecting subcortical gray matter changes but in contrast to Cheng and colleagues (37), we used a vertex wise approach which has been suggested to be better at detecting gray matter atrophy than their voxel based approach (32,42). Gray matter atrophy has also been reported in the thalami of cannabis alone and cannabis with concurrent tobacco use, although they were unable to discern which of the two were the causative agent of the atrophy and may both contribute to the atrophy (43). Gray matter thalamic atrophy was also found in polysubstance use disorder (45); and their cohort had users of both heroin and cannabis amongst other substances.

White matter volume changes are seldom reported in substance use disorders but have been found in heroin, where Cheng and colleagues (37) found elevated white matter volume in the medial OFC and supramarginal gyri of heroin users. Changes in the cerebellum have been reported in heroin-, cannabis-, nicotine- and polysubstance use disorders. Cerebellar gray matter was found to be reduced in heroin users (33); although it has mostly been discovered as a correlational measure mediating duration of use (38) and the sensation seeking trait (37). Interestingly it has been reported to be elevated in cannabis use disorder (40,41).

Our initial results indicated very large and diffuse areas of cortical atrophy however most of these regions do not survive permutation simulation and regional analysis corrected using FDR suggesting that they are minor changes. Major/significant gray matter changes were revealed by further statistical correction (using permutation simulation) and are restricted to the right hemispheric medial-orbitofrontal (mOFC) region and superior temporal cortex; and in the left hemispheric rostral middle frontal gyrus (a region of the dlPFC). Using regional FDR analysis, we found a similar pattern of gray matter atrophy but restricted to the right hemispheric mOFC, rostral middle frontal cortex, superior frontal cortex (a region of the dmPFC), superior temporal cortex and supramarginal cortex (a region in the inferior parietal lobule). The cortical atrophy we found in the superior temporal cortex and supramarginal gyrus corresponds with the inferior parietal lobe/temporoparietal junction (IPL/TPJ), a region that is increasingly implicated in a range of cognitive functions (50). Our results are consistent with damage seen in heroin use disorder with fronto-temporal damage being consistently reported (38) suggesting that heroin is the causative agent in nyaope use disorder's neuroanatomical changes.

6.2. Prefrontal cortical atrophy

The PFC is the large portion of the brain located in the anterior pole of the brain, anterior to the premotor cortex (51). The PFC can be parcellated according to cytoarchitecture (a method used by Brodmann to define his areas) and more recently according to neuroimaging studies focusing on functional localization: dorsolateral-, dorsomedial-, ventromedial-, ventrolateral- and orbitofrontal prefrontal cortical regions (52). It has extensive connections with the thalamus (particularly the mediodorsal nucleus) and these connections are sometimes used to define the functional parcellations of the PFC that correspond with neuroimaging functional organizations and in this model the anterior cingulate cortex is included as a part of the PFC (51,52).

The PFC is involved in a range of functions that include visual, gustatory, auditory, motor, memory, and language processing through its extensive connections with other neural regions (51,53,54). Its individual components function together to produce what are termed higher executive-or cognitive functions, extremely complex actions and outcomes that are much greater than the sum of the individual parts (52). These functions include reasoning, emotional salience, decision-making, creativity, learning and reverse learning, goal setting and representation and inhibitory control (52,55,56).

Prefrontal cortical damage has long been suggested to underlie the neuropsychological deficits observed in heroin use disorder such as altered decision making and increased impulsivity (57–59). Heroin addicts were also noted to have some cognitive

deficits that included loss of sustained attention and conceptual thinking, reduced cognitive flexibility, and an inability of managing real life interference (57,59) and this may be attributable to prefrontal cortical damage. Our study shows that there is extensive PFC atrophy in nyaope patients. Atrophy was recorded in the mOFC, dlPFC (rostral middle frontal gyrus) and dmPFC (superior frontal cortex).

6.2.1 Orbitofrontal cortical atrophy

Damage to OFC gray matter (lateral and or medial) was previously reported in heroin- and cannabis use disorders (33,38,40,44). Metanalytic studies indicate that the region is consistently damaged in both substances use disorders (38,44). Other neuroimaging techniques have revealed aberrations in the region. Studies into white matter integrity in heroin and cannabis users did not reveal significant change in OFC connectivity, except one cannabis study that found improved white matter integrity in the region (60). We found no gross changes to white matter in the region, however DTI studies in the future may reveal some white matter structural changes

Insight into the functions of the OFC are revealed by its connectivity. The mOFC receives projections from the pyriform cortex (primary olfactory cortex) and carries out the task of processing olfaction (51,54). The lOFC was shown to be the secondary taste cortex and receives afferents from the primary taste cortex, and the caudal OFC (cOFC) receives strong inputs from the olfactory area (51,54). Visual inputs are projected from the inferior temporal cortex, superior temporal sulcus and temporal poles to the OFC (51). The different sensory modalities are integrated by the OFC to produce its functions: associative learning and stimulus reinforcement, reward valuation and reversal learning (51,54,61). OFC neurons have been shown to code for reward value in macaque neurophysiological studies (61).

The fact that OFC gray matter is consistently reported to be atrophied in substances suggests that damage to the region is necessary in producing non-specific substance use disorders. This is in line with the theory that OFC damage/dysfunction is necessary for the development of substance use disorders (28); and that the incurred damage results in a pathological overvaluation of a drug reward. Gray matter atrophy in nyaope users may explain some pathological behavioural phenotypes in nyaope users. Nyaope patients may, as a consequence of OFC gray matter atrophy, overvalue the reward from the substance above other beneficial behaviours. This is collaborated by Masombuka (8), Mokwena and Huma (5) and Aye and colleagues (9) who reported that the users neglected personal hygiene, relationships and food for the substance. The negligence may be perpetuated by an inability to evaluate the detrimental outcomes of using nyaope for instant gratification and an inability to reverse learn the detrimental reward when their health and relationships are compromised. Furthermore, Fernandes and Mokwena (14) reported that nyaope users reported a need to quit using but were unable to do so and they interpreted this as an external locus of control, however our study suggests that it is cortical atrophy in the OFC that mediates this behaviour as ventromedial brain lesions result in patients verbalizing intentions but being unable to carry out tasks (51). Future studies would benefit from conducting neuropsychological tests in conjunction with neuroimaging to elucidate the OFC's role in some pathological behaviours seen in addiction.

The OFC appears to be chiefly responsible for reward valuation and the subsequent pathological overvaluation in substance use disorders, but other neural regions mediate additional pivotal characteristics such as the loss of executive "top down" inhibition of behavior in substance use disorders. An area implicated is this function is the dlPFC, a region we found to be significantly atrophied in nyaope users.

6.2.2 Dorsolateral prefrontal cortical atrophy

Damage to the dlPFC has been consistently reported in heroin use disorder (32–36,38). Thayer et al. (42) reported reduced gray matter in the dlPFC of cannabis users but this effect was not strong enough to survive FDR testing. Therefore damage to the dlPFC in the present study appears to be a heroin signature.

White matter integrity, functional and connectivity studies have revealed abnormalities in heroin patients. Studies investigating white matter integrity in heroin users have reported reduced axonal integrity in the frontal subgyral regions (inferior to the dlPFC) (62–64). Studies into fMRI in heroin users at rest found decreased activity and dysfunctional connectivity in the dlPFC (64,65). Changes in functions mediated by the dlPFC have been suggested to be necessary for the development of substance use disorders (28). The connectivity of the dlPFC provides insight into some of its functions that include visuospatial information processing, working memory, reward anticipation and planning of behaviour. The dlPFC can be divided into ventral, dorsal, caudal and rostral regions using the principal sulcus of the frontal cortex. The caudal- and rostral dlPFC are preferentially connected to the posterior parietal cortex and the auditory cortex of the temporal cortex respectively (56). The dorsal dlPFC is linked to the caudal posterior parietal cortex and the posterior cingulate cortex (56,66). The ventral dlPFC is connected to the rostral inferior parietal lobule (includes the supramarginal gyrus), opercular part of the parietal lobule and the anterior cingulate cortex (56,66). The ventral and dorsal dlPFCs are also extensively connected to the promotor cortex (56).

Neurophysiological studies in macaques have shown that there is an overlap between the OFC and dlPFC in mediating reward value, location of objects and identity of objects. While the OFC processes information about the identity of the object and its reward value, the dlPFC processes information about the location of objects and reward anticipation (61). fMRI and connectivity strength studies indicate that information or cues related to an expected reward enter solely through the dlPFC and these signals substantially increased the dlPFC's influence on the VTA and Nacc, regions that correspond to drug expectancy and motivation (67). This is important because studies have shown that it is the anticipation of a reward that increases dopamine neuronal activity in the ventral tegmental area (VTA) driving the urge to take a drug in substance use disorders (28). However, VTA neurons are incapable of obtaining information about reward cues from the environment; and this function of reward anticipation and thus driving of motivation to obtain a reward is thought to be controlled by the dlPFC. This was confirmed by Liu and colleagues' (68) metanalytic study that found that the dlPFC was consistently activated in mediating reward anticipation and negative rewards. Reward anticipation is possible because the dlPFC is extensively connected with regions that convey information about reward value (OFC), compare reward values (OFC and ACC), sensory stimuli (parietal cortex and frontal oculomotor regions) (61,67) and is able to integrate that information to predict the value of the reward thus influence motor and subcortical regions (VTA) to produce the state of reward anticipation.

A loss executive "top-down" inhibition of behaviour has been suggested as an important hallmark in the development of substance use disorders (28). This function is carried out by the anterior/ventral dlPFC- ACC and the posterior/dorsal dlPFC-posterior parietal cortex networks. fMRI studies have shown that the anterior dlPFC-ACC network was involved in reward error calculation, conflict monitoring and resolution to sustain attention and enact action inhibition while the posterior dlPFC-parietal cortex network was involved the use of working memory in order to control motor behavior (56,66). Thus, there appears to be an anterior-posterior functional gradient in the executive "top-down" control of behavior. The anterior dlPFC-ACC network appears to control abstract processes such as the monitoring of performance and adjusting the behavior of other structures while the posterior network controls more basic cognitive processes of enacting control over behavioral action.

Our nyaope patients showed damage to their dlPFCs. The dlPFC has been implicated in important behavioral tasks that include executive 'top-down" inhibitory control, reward anticipation therefore motivated behavior, working memory and decision making, and initiation of motor behavior by using visuospatial integration. In a study classifying 20 substances using three criteria: physical harm, dependence and social harm, heroin scored the highest score in all three (69). Importantly it scored 3/3 in dependence (encompasses physical and psychological dependence) (69). The high dependence score may in large part be mediated by the damage it causes to the dlPFC. Aberrant dlPFC activity may result in diminished decision making due to a reduced working memory, aberrant reward anticipation driving motivation to obtain the drug and an inability to inhibit impulses that drive the incessant need to obtain nyaope. Mokwena and colleagues as well as Masomubuka (5,8) reported that the dependence on nyaope is overwhelming and that users dedicate their lives to obtaining the drug, at times resorting to crime to support their habit (10,11). This may be reflective of the enormous dependence produced by dlPFC dysfunction and increased impulsivity that too has been associated with dlPFC dysfunction (37).

6.2.3 Dorsomedial prefrontal cortical atrophy

Damage to the dorsomedial prefrontal cortex (dmPFC) has been consistently reported in heroin use disorder (32,33,36–38) and Wang and colleagues (36) reported that this damage persisted in users after thirty days abstinence. To our knowledge damage to the dmPFC gray matter has not been reported in cannabis use disorder. This may again suggest that it may be a heroin signature in nyaope use disorder. Additional neuroimaging techniques have revealed changes to the dmPFC in substance use disorders. Studies into white matter structural integrity showed that axonal integrity was compromised in the dmPFC of heroin users (63,70). Nodal connectivity studies showed abnormal connectivity in the dmPFC of heroin users (71), and fMRI studies have implicated the dmPFC in heroin use disorder (65,72)

Eickhoff and colleagues (73) used connectivity studies to parcellate the dmPFC into four clusters: rostral (ventral and dorsal), and caudal (left and right hemispheric). They then used experimental- and resting state fMRI to examine the connections of the four clusters. They found widely distributed connectivity of the dmPFC with the ventromedial PFC (vmPFC), the anterior- and posterior cingulate cortices, inferior parietal cortex, the inferior frontal gyri, amygdala, hippocampus, and with parts of the dlPFC (73). There was a great deal of overlap regarding connectivity, for example all four were connected to the other frontal and parietal regions bilaterally. There was also evidence for preferential connectivity: the right caudal dmPFC was alone connected to the right dlPFC, and the rostroventral cluster showed preferential interaction with other core regions of the default mode network (post central gyri and inferior parietal lobule) (73).

Functions of the dmPFC are reflected by its connectivity and it appears to be an important hub for a number of networks. The right caudal dmPFC appears to mediate reward tasks (connected to the dlPFC), the left caudal dmPFC is involved in visual perceptual and spatial discriminatory tasks, the rostroventral dmPFC showed specific involvement in language tasks (semantic discrimination and covert reading), and the rostro-dorsal dmPFC appears to perform functional associations related to different emotions (73). As an important network hub centre, the dmPFC is part of the default mode network (DMN), salience network and frontoparietal networks. The rostroventral clusters showed connectivity to the DMN (73). The DMN is composed of the mid- and posterior cingulate cortices, dlPFC, inferior parietal cortex, precuneus, middle- and inferior temporal gyrus and the left cerebellum and is responsible for internal mental states, such as self-reflection, autobiographical memory retrieval, imagining the future and interoception (73,74). The right caudal was a part of the fronto-parietal network (with the dlPFC and inferior parietal lobule), a network that is involved in higher executive function such as attentional modulation of bottom up processing of visuospatial information (73). The left caudal was connected to the salience network (with the middle cingulate cortex and insula), a network

that mediates interoceptive awareness, pain detection, and empathy (73). Importantly the salience network is thought to maintain priority cognitive sets and behavioural goals; and is hypothesized to mediate between the endogenously oriented DMN and the exogenous frontoparietal networks (73) as well as the switch between the DMN and the central executive network (network active in cognitively demanding tasks) that has the dlPFC as a central node (75).

The dmPFC has been shown to be one of the highest associative centers of the brain and is active in a range of cognitive processes, social judgements, conflict resolution and response inhibition, decision making and reward mediated behavior (54,72,73,76–78). In reward, the dmPFC has been shown to mediate the nucleus accumbens' response to reward predictive cues (78). dmPFC neurons have been shown to be excited by discrete stimuli/reward cues and through glutamatergic projections that influence the Nacc neurons (78). They have been shown to drive the phasic firing of Nacc neurons in response to reward related cues which drives operant behavior (behavior that translates to obtaining the reward); and selective lesioning of dmPFC has been shown to significantly attenuate Nacc activity in response to reward predictive cues (78). It is worth noting that the dmPFC works in conjunction with the basolateral amygdala glutamatergic- and VTA dopaminergic neurons to carry out this function. It is hypothesized that a circuit exists whereby the dmPFC and BLA glutamatergic neurons, VTA dopamine neurons excite the Nacc neurons in the presence of reward cues (78). BLA neurons signal that a potentially significant affective event may be occurring, VTA neurons signal that a predictive cue may be present and dmPFC indicate that a reward cue is present and what actions to select; and these signals converge on the Nacc neuronal subpopulations that mediate the behavioral response selected to obtain the reward (78). Therefore, the dmPFC may play a big role in drug seeking behavior and has been hypothesized to be responsible for drug reinstatement of drug seeking behavior months after cessation of the substance (78). Interestingly Sun and colleagues (71) and Zhang and colleagues (79) found increased connectivity and nodal activity in the dmPFC of heroin addicts and which may aide in increasing glutamatergic delivery to the Nacc neurons.

Gray matter atrophy in the dmPFC may mediate drug seeking behavior in response to drug related cues through its glutamatergic inputs to the Nacc. In fact, studies suggest that in the presence of reward cues, the dmPFC (and basolateral amygdala) glutamatergic influence may be the primary stimulus to the Nacc neurons that drive reward operant behavior, and that the VTA dopaminergic neurons merely augment the glutamatergic input (78). Furthermore, atrophy to the dmPFC gray matter may alter the decision-making capabilities of the region. Nyaope patients may have reduced capabilities to resolve behavioral conflicts, whereby they are unable to inhibit behavior that is contrary to their greater goals (school, employment and social). Fu and colleagues (72) confirmed through fMRI that protracted abstinent heroin users had reduced inhibitory function which was suggested to underlie patients hyperresponsiveness to drug cues, therefore drug seeking and continuous relapse. This is in line with the difficulty in nyaope cessation reported by Fernandes and Mokwena (2) and Morgan and colleagues (10) even after protracted abstinence.

6.3 Superior temporal cortex

Damage to the superior temporal cortex was previously reported in heroin use disorder (32,37,38). It is less consistently reported in cannabis users, with only one study reporting a reduction of gray matter in the temporal poles (part of the superior temporal cortex) of cannabis users (40). Gray matter atrophy in the superior temporal cortex appears to be a common finding in substance use disorders and from this study we could suggest that it may a consequence of heroin and cannabis use of our cohort. White matter integrity studies have shown decreased white matter integrity in heroin-, cannabis- and polysubstance use disorder (70,80,81). Connectivity and fMRI studies into heroin users have not shown much alteration to the superior temporal cortex which may suggest that damage to the region may be mediated largely by gray matter loss. Interestingly Bernstein and colleagues (82)

found elevated levels of nNOS neurons in the temporal lobes of post mortem heroin users and it is thought that elevated levels of nitric oxide from these neurons causes greater neuronal cell death in the temporal lobe.

Studies have shown that the superior temporal lobe may be responsible for a number of sensory integration functions such as audio-visual integration, spatial awareness, motion processing, speech perception, phonological processes, face processing, semantic processing, theory of mind and social perception (83–88). Temporal lobe gray matter atrophy is a consistent finding in heroin studies and has been suggested to account for memory and spatial deficits as well as reduced verbal ability in heroin users (33,38). It would be worth examining the neuropsychological, spatial perceptive and motor dysfunctions brought about by gray matter loss in nyaope users. This may have major implications in their ability to maintain occupations and possibly prevent relapse that may be driven by an inability to find or maintain a job.

In the context of substance use disorders, it is preferential to consider the superior temporal cortex as part of the inferior parietal lobule/temporoparietal junction (IPL/TPJ), a region that is a major hub for a number of networks and thus mediating a number of functions (50) (see below).

6.4 Inferior parietal lobule (Supramarginal gyrus)

We found reduced cortical thickness in the supramarginal gyrus (a region located in the inferior parietal lobule) of nyaope users. Heroin has been shown to reduce gray matter in the parietal regions (33,34,36). Gray matter atrophy has been shown specifically in the inferior parietal lobule (IPL) by Yuan and colleagues (35), while Cheng and colleagues (37) showed atrophy in the post central gyrus of heroin addicts with white matter volume being increased in the supramarginal gyrus. Li and colleagues (32) in contrast reported elevated gray matter volume in the superior and inferior parietal lobules. Li and colleagues (32) employed a vertex wise surface-based morphometry approach compared to Cheng and colleagues' (37) and Yuan and colleagues' (35) who both employed a voxel-based approach. We also employed an SBM approach in determining cortical thickness but in contrast to Li and colleagues (32) we found reduced cortical thickness in the inferior parietal lobule. This may be due to differences in our sample sizes, we managed to recruit more nyaope users (twenty-eight) compared to their eighteen heroin users which would suggest greater statistical strength on our part. Furthermore, significant atrophy was found using regional FDR testing and not permutation simulation. This may suggest that the tests have different strengths when testing for cortical atrophy but also that the atrophy in the parietal cortex although significant, may not be as strong as the other regions.

Other neuroimaging studies showed changes to the white matter integrity and connectivity associated with the parietal cortex. The superior longitudinal fasciculus, a long association fiber that connects the parietal, frontal and occipital fibers was consistently reported as damaged in heroin use disorder (63,71,89) with Lin and colleagues (89) reporting damage to this tract specifically in the parietal cortex. Through connectomic studies, the IPL has been identified as a component of the DMN, and in these studies was shown to have abnormal (increased) connectivity within the DMN (71,90). fMRI studies have found abnormal activation of the IPL at rest and during cognitive tasks (35,65,72,91). Jiang and colleagues (65) found that the IPL had increased spontaneous activity at rest, while Yuan an colleagues (35) reported decreased functional connectivity between the dlPFC and IPL. The region is reportedly active during response inhibition tasks (72,91). Fu and colleagues (72) found that it was activated during an inhibitory control task in controls compared to heroin users while Lee and colleagues (91) reported its hyperactivity in a similar task in the brains of heroin users. The results are different because Fu and colleagues (72) reported activity in the supramarginal gyrus while Lee and colleagues (91) reported activity in the angular gyrus, but nevertheless they both implicate the IPL in inhibitory control of behavior.

The DMN is the primary network that controls internal states of the mind during non-task periods when the mind is allowed to wander (50,74). In addition to mind wandering, the DMN is thought to be responsible for autobiographical memory retrieval, or imagining the future (50). As part of the DMN, the IPL is connected to the medial PFC (including the dmPFC), posterior cingulate/retrosplenial cortex, lateral temporal cortex, anterior temporal lobe , medial temporal system and cerebellum (50). It is usually switched off by the frontoparietal network when presented with tasks but may co-activate with it in tasks that are internally directed. The DMN appears to be divided into two: a dmPFC system (dmPFC, lateral temporal cortex, temporal poles and posterior TPJ) and an MTL subsystem composed of the TPJ, ventromedial PFC, posterior midline- and medial temporal lobe. The dmPFC subsystem is active when subjects make judgements concerning their present situations and the MTL subsystem is engaged when individuals respond to hypothetical autobiographical events such as imagining their futures. Alterations to the DMN have been shown to cause pathology through the DMN interference hypothesis that suggests that the DMN may become active during task related goal driven behavior and the individual may wander into self-referential states resulting in an inability to sustain tasks during goal driven behavior (74). Within the DMN, we have found reduced gray matter in two of its components: the IPL and dmPFC. Aberrant, enhanced connectivity of the DMN has previously reported in heroin use disorder (71,90), and our results may be the first step in uncovering a disintegrated DMN in nyaope users, which may translate to an inability to maintain attention therefore employment/occupation by nyaope users. This may in part be the neural basis of Morgan and colleagues' (10) results that found that only 48/300 nyaope users completed matric and 282/300 were presently unemployed or were informally working.

The IPL may mediate reward through its role in decision making during times of uncertainty (92). When faced with decisions without certainty, our brains integrate action-reward associations, opponent and environmental modelling to make a final decision. A fMRI study that assessed this modality found that the IPL was highly active in decision making when using feedback (i.e. past experiences and rewards) while the dmPFC was reportedly active during tasks that required greater attentional effort not decision making per sé (92). The IPL was particularly active when using information about the past to determine future decisions, while high attentional tasks suppressed the IPL and activated the dmPFC. This establishes the IPL as a domain for using past experiences to mediate goal driven behavior (92), something that seems to be lost in nyaope users who will continuously use nyaope regardless of previous detrimental outcomes.

7. Limitations and Recommendations

Our study shows gray matter atrophy but does not indicate specifically whether the death of neurons or death of supporting cells is the cause of atrophy. One study found reversibility of cortical atrophy in heroin users limited to the superior frontal region alone (36) which may suggest that cortical atrophy may be a consequence of a loss of supporting cells more than neurons as neuroglia regenerate while neurons display less regenerative capabilities. This is speculative and is more likely that the atrophy is a consequence of both cell types being lost. Future studies could benefit in contrasting nyaope induced gray matter atrophy at different stages of abstinence. In a scenario whereby supporting matter is the primary cause of atrophy, one would find extensive reversibility with protracted abstinence. However, to deduce neuronal loss as a driving cause, specific neuronal studies would be required, for example molecular and genetic neuroimaging could be used to characterize receptor profiles that are specific to neurons hence a deficiency could be used to suggest that nyaope use disorder largely causes neuronal cell death. Previously it had been suggested that the dopamine receptor profiles change in the direct and indirect basal ganglia pathways which may result in reduction of cortical stimulation via these pathways further leading to atrophy in some regions (18,28). Therefore, characterizing

genetic neuroimaging may show which cortical regions may have reduced receptor profiles implicating specific neuronal populations that are responsible for cortical atrophy.

Our study showed gray matter atrophy in regions that mediate large parts of behaviour. Previously, a few studies (2,3,5,8) reported some behavioural traits in nyaope addicts such as a disregard for personal care, disintegration of interpersonal relationships and a complete reorientation of the patients' lives towards the acquisition of the drug. We are able to suggestively implicate gray matter atrophy in the OFC, dmPFC and dlPFC to some of these behavioural phenotypes. For example, damage to the OFC may mediate the pathological overvaluation of the reward obtained from nyaope above other beneficial functions such as personal well-being. Simultaneously the dysfunction in the dlPFC and dmPFC may cause pathological bias towards drug-reward predictive cues and increase reward anticipatory firing of subcortical structures, increasing the motivation and driving drug seeking behaviour. Furthermore there may be a loss of executive "top down" inhibitory control (exerted by the dlPFC and ACC) to subcortical structures and patients are unable suppress drug urges or to resolve the conflict that exists between obtaining the drug or maintaining focus to accomplish their greater goals which is controlled by the dmPFC. This however is speculative and future studies would benefit from administering neuropsychological tests (37,57–59) and correlating them to neuroimaging results in nyaope users. This would make for great translational behavioural therapy, as we would be able to tailor or recommend specific behavioural therapies to patients during and post rehabilitation.

Suggesting that gray matter atrophy may mediate the pathological behavioural phenotypes observed in nyaope addiction directly i.e. gray matter loss therefore diminished function of said region is overly simplistic. To fully understand the pathology, we would need to characterize white matter tract integrity (diffusion weighted imagery), examine connectivity strengths of different nodes and networks (connectomics), and lastly use fMRI in conjunction with neuropsychological tests to discern functionality or a lack thereof in nyaope use disorder. White matter structural studies in heroin have showed diminished axonal integrity in frontal subgyral and medial frontal regions (63), which would support gray matter atrophy in nyaope (heroin) use disorder. However some connectivity studies have shown to increase white matter connectivity or nodal efficiency in the OFC, dlPFC, temporal cortex, ACC and inferior parietal regions of heroin users (64,71,79); and this seems to contradict the gray matter atrophy we have found in the same regions. Abnormal fMRI activations, elevated and decreased, have previously been reported in heroin users at rest in the mOFC, dlPFC, temporal regions, inferior parietal cortex (65). Abnormal functional activity has also been reported in heroin users in the frontal, parietal and temporal regions and cingulate cortices during cognitive tasks; and they were implicated in diminished inhibitory control and impulsivity (72,91). Therefore it becomes evident that gray matter atrophy alone is insufficient towards predicting neurological and cognitive deficits in nyaope patients hence future studies would benefit from using diffusion weighted imaging to examine white matter integrity and using connectomics to reveal changes in connectivity across the brain. Administering fMRI during cognitive tasks and at rest would reveal functional changes to the nyaope users' brains.

Understanding (in addition to gray matter) white matter integrity, connectivity and nodal strength, and fMRI at rest and when engaged in cognitive tests may show a holistic picture of the underlying neural mechanisms. This would been beneficial in introducing more novel treatments such as repetitive transcranial magnetic stimulation (rTMS) that has been shown to increase or suppress excitability in neuronal populations whose aberrant structural and functional integrity would have been defined and correlated to neuropsychological deficits in nyaope patients (93). rTMS has been shown to be an effective and promising treatment in substance use disorders and would be of great benefit in treating the highly addictive and detrimental nyaope use disorder (93,94). More novel genetic neuroimaging techniques are being used to understand the distribution patterns of receptor profiles and potential predispositions to substance use disorders (95). Genetic neuroimaging may enhance tailoring of

pharmacological agents that may target overactive or underactive receptors, neurotransmitters or regions in nyaope/heroin use disorder (96).

8. Conclusion

Our neuroimaging study was successful in characterizing the structural changes induced by nyaope use disorder. We found wide areas of cortical atrophy, that when corrected were limited to the prefrontal cortex, supramarginal gyrus of the IPL and the superior temporal cortex. Within the prefrontal cortex we found cortical atrophy in the mOFC, middle frontal gyrus of the dlPFC and in the superior frontal gyrus of the dmPFC. Collectively these regions are suggestive of a heroin neuroanatomical signature i.e., fronto-temporal damage.

Previous chemical studies have indicated the dominant presence of heroin in nyaope substance (1,7) and our study agrees with previous studies by showing that nyaope patients have a neuroanatomical signature overwhelmingly similar to heroin. As such, we echo the call to recognize nyaope as heroin use disorder (10). This is important because heroin is widely regarded as the most severe substance use disorder (69), therefore if officials recognize nyaope as heroin, there may be greater urgency in availing methadone maintenance therapy to nyaope patients in state-funded rehabilitation centers.

Chapter 2: Clinical neuroanatomical features of Nyaope use disorder

1. Introduction

1.1 Neurobiology of relapse in substance use disorders

Neuroanatomical changes in the brain can also be used to predict or describe features of the disease such as relapse probability. Treating SUDs has as a primary objective: the patient becoming permanently abstinent. Rarely is that the result, even less so in nyaope use disorder (2,10). Relapse of an individual who has undergone rehabilitation may be brought about by a number of factors such as financial difficulty or the individuals' well-being. Accumulating evidence suggests that ultimately, relapse is a consequence of an altered neurobiology of the abstinent patient that persists long after detoxification (15). In contrast to healthy individuals, patients have an altered neurobiology that results in an inability to cope with socioeconomic, environmental and personal challenges that results in the patient seeking alleviation of these challenges by taking drugs. As discussed, there appears to be two key processes that culminate in relapse: craving and lack of inhibitory control (28,97,98).

Craving can be defined as the subjective feeling of an intense desire to obtain and consume the drug culminating in reinstatement of drug use or relapse. Often it is a consequence of re-exposure to the drug (drug induced cues), exposure to previous environmental stimuli and or affective states/ stress (cue induced relapses) (28,97–99). Drug taking, in addition to activation of the dopaminergic system, initiates learning of cues (environmental and interoceptive) known as associative learning that provides incentive salience to the cues (17,28,96,97). This is also known as conditioning. Stimuli such as the environment and internal thoughts associated with the drug become conditioned and over time will trigger phasic VTA dopamine neuron firing which is interpreted as reward expectation by the Nacc and drives the motivation to obtain the drug (28). Associative learning of drug related cues is dependent on a number of cortical and limbic structures that include consistently the BLA, hippocampus, Nacc, the dorsal striatum (caudate and putamen), the OFC, the ACC and the prelimbic cortex (97,98).

Craving can be brought about by an altered preoccupation/anticipation state, altered interoceptive state, and stress related states which are in part a consequence of altered neuroanatomy (96). An altered preoccupation/anticipation state is whereby drug seeking is induced by a drug stimulus and is paired with drug taking. This model implicates the medial PFC, Nacc, and ventral striatum and glutamatergic innervation (96). The PFC performs executive function such as analyzing stimuli, their outcomes, their value eliciting hedonic feelings that are associated with drugs and this subjective state of drug craving has been shown to activate the OFC, ACC and temporal lobe (including the amygdala) (96). Executive function in craving may be mediated by dopaminergic input from the VTA to the PFC, and the PFC exerts executive function through glutamatergic innervation to the Nacc and subsequent interactions with GABAergic neurons in the ventral striatum that results relapse (96). The system is linked to cue induced relapse (stimuli associated with drugs e.g. environment) via the BLA that mediates contextual stimulus relapse by influencing the hippocampus (96,97) and exerts its influence on the PFC as well presumably through glutamatergic innervation (96).

Interoceptive states are mediated by the insula and its reciprocal connections to limbic and frontal regions is also suspected to cause craving (96). The insula integrates autonomic and visceral information with emotion and motivation to produce conscious awareness of self and environment to influence behavior (96). The insula has a number of reciprocal connections with the ventromedial PFC that mediates emotional decision making and connections with limbic structures such as the amygdala and ventral striatum that mediates motivation to take the drug (96). The ACC is thought to play an important role in basal preoccupation/anticipation craving by inhibiting the habitual impulses in the striatum elicited by craving either through frontal regions or interoception from the insula (96).

Withdrawal and or negative affective states are thought to contribute to stress related relapse. Neurotransmitters and brain regions have been implicated in negative reinforcement of the withdrawal state. For example, in acute cocaine withdrawal, there are decreases of endogenous opioids that contribute to irritability, malaise, and dysphoria; and in protracted withdrawal, imaging studies have indicated a decrease of dopaminergic innervation reflected by a downregulation of dopamine 2 receptors which may contribute to anhedonia and amotivation (96). Simultaneously, during protracted withdrawal there is an increased sensitivity to conditioned cues (96,97). The change in affective mood (anhedonia and amotivation) and increased sensitivity to drug cues may drive relapse. Furthermore, imaging studies have showed disrupted activity of frontal regions including the dlPFC, cingulate cortices and OFC that underlie impaired inhibitory control and contributing to relapse due to negative affective states in withdrawal (96).

There is an interaction between the negative affective states and stress-arousal systems in the brain that exacerbates the subjective feeling of craving and contributes to relapse. The two systems are linked via the extended amygdala that is composed of the central nucleus of the amygdala (CeA), bed nucleus of the stria terminalis (BNST) and a transition zone in the medial shell subregion of the Nacc (96). The extended amygdala receives afferent fibers from the BLA and hippocampus and sends efferent fibers to the hypothalamus and ventral palladium connecting the limbic system to the output extrapyramidal system (96). The hypothalamic-pituitary-adrenal axis (HPA) and brain stress/aversive system are mediated by corticotropin releasing factor (CRF) and are activated during withdrawal resulting in an increase in adrenocorticotropin hormone, corticosterone, and amygdala CRF (96). Ultimately withdrawal produces aversive/ anxiety like states that are modulated by CRF and other stress related systems such as noradrenergic systems (96). Furthermore, there is an aberration of the PFC-amygdala circuitry that results in heightened anxiety (100). These systems likely act together to produce craving and subsequent relapse.

1.1.1 Neuroanatomical changes in relapse patients

There is less literature investigating neuroanatomical changes that influence risk of relapse or predict it compared to literature investigating the neuroanatomical changes brought about by substance use. This is possibly due to most researchers adopting a cross sectional approach and not longitudinal or following up on their cohorts after the investigation. In our literature review, we found no studies that focused on using gray and white matter changes in heroin or cannabis use to predict risk of relapse. Nevertheless, there have been studies that have examined these changes in other substance use disorders.

Beck and colleagues (101) examined the structural and functional neuroanatomical changes between sex matched forty-six abstinent alcohol dependent subjects (AD) and forty-six healthy controls (HCs). Follow up data was collected on abstinent- and relapse AD. There were thirty relapse patients and sixteen abstainers. Relapse group showed more pronounced gray matter atrophy in the bilateral medial prefrontal cortex, ACC, OFC and ventral striatum; and in the left hemisphere's amygdala and VTA compared to healthy controls. The abstinent group showed similar patterns of atrophy with the relapse group but in less

magnitude. Relapse group compared to abstinent group showed patterns of reduced gray matter in the bilateral OFC and right hemisphere's ACC and medial PFC.

Rando and colleagues (49) sought to understand the gray matter changes in forty-five alcohol dependent individuals (ADI), ten females, compared to fifty healthy controls, twenty-two females. ADI had been using alcohol for 18.62 ± 8.65 years. ADI were receiving inpatient treatment and MRI data was collected after a month of abstinence. Follow up data was collected at two weeks, one month and three-month intervals post- rehabilitation. At the two-week interval 11/44 AD had relapsed; one-month interval 19/44 had relapsed; and in three months 30/44 AD had relapsed. AD showed gray matter volume loss in the right hemisphere's lateral PFC cluster (included the dlPFC, and inferolateral PFC), medial frontal cluster (included the ACC, medial and lateral superior frontal part with the supplementary and presupplementary areas, the middle frontal gyrus and posterior cingulate gyrus), and in the parietal and occipital cluster (including the precuneus, cuneus and posterior cingulate) compared to healthy controls. Smaller gray matter volumes in the medial frontal cluster (included the ACC, medial and lateral superior frontal part with the supplementary and presupplementary areas, the middle frontal gyrus and posterior cingulate gyrus), and the parietal-occipital cluster (including the precuneus, cuneus and posterior cingulate) predicted a shorter time of relapse. The medial frontal cluster predicted shorter times of relapse by 44% and the parieto-occipital cluster predicted it by 45%. A negative correlation was observed between volume in the medial frontal cluster and lateral PFC and the number of years of alcohol use and number of days of alcohol use during the 90 days preceding treatment.

Parvaz and colleagues (102) carried out a longitudinal MRI study on abstinent cocaine addicts (CA) at ≥ three-week abstinence and at six months follow up as well as administering neuropsychological tests on the same individuals to monitor executive function improvements. Nineteen CA, six females were recruited and compared to twelve HCs, one female. CAs had used cocaine for an average of 12.74 ± 7.42 years. At baseline, CAs had been abstinent for approximately 5 months on average and at follow up they had been abstinent for approximately 9 months. At baseline healthy controls had greater gray matter volume in the left hemisphere cuneus and right hemisphere precuneus compared to CAs, while CAs had greater gray matter volume in the right hemisphere supplementary motor cortex, left hemisphere superior medial frontal cortex and right hemisphere parietal/occipital cortices. In CAs, gray matter volume at follow up was shown to be improved in the bilateral ventromedial PFC and in the left hemisphere inferior frontal gyrus.

Morales and colleagues (103) compared the gray matter volumes of non-smokers HCs, nicotine smokers (NS) and methamphetamine addicts (MA). MA group were four to seven days abstinent at the initial scan and were rescanned (mean 23.5 ± 1.6 days) of abstinence and matched for NS group. Eighteen HCs, twenty-five NS, and thirty-nine MA were recruited for the study. In the follow up twelve MA and twelve NS were rescanned. MA had been using methamphetamine heavily for 8.4 ± 1.3 years and the follow up longitudinal group had been using methamphetamine for 6.8 ± 2.2 years. Healthy controls had greater gray matter volume than NS and MA in the right hemisphere OFC. HCs had greater gray matter volume than MA in the bilateral precentral gyri, right hemisphere's frontal pole, middle temporal gyrus, precuneus and cingulate gyrus, and the left hemisphere's OFC, superior frontal gyrus, insula and caudate. In the follow up period, MA showed improved gray matter volume in the bilateral superior temporal gyrus, middle temporal gyrus, precuneus, inferior frontal gyrus; right hemisphere's angular gyrus, insula, frontal operculum and ventromedial PFC; and in the left hemisphere's occipital pole. The cerebellum volumes were reduced on follow up compared to the initial scan.

Changes to gray matter have been shown to mediate the risk of relapse (49,101–103). Previous studies found that relapse could be predicted by reduced gray matter volume in the OFC, ACC, medial PFC, lateral and medial superior frontal cortex (included presupplementary and supplementary areas) middle frontal and PCC, and parietooccipital cluster (included precuneus, cuneus and posterior cingulate) (49,101). Furthermore, abstinent patient showed improved gray matter volume in the frontal regions

(ventromedial PFC, inferior frontal gyrus, frontal operculum, OFC, superior frontal) temporal, regions (parietal (precuneus and angular), caudate, insula, cingulate, occipital pole (102). Rando and colleagues (49) were able to predict that gray matter atrophy in the medial frontal cluster would increase probability of relapse by 44% and gray matter atrophy of the parieto-occipital cluster would predict atrophy by 45%, which suggests the importance of understanding neuroanatomical changes that influence relapse.

1.2. The link between SUD and psychiatric disorders

There has been a well-documented co-occurrence of SUDs and psychiatric disorders (104). McGovern and colleagues (104) found that from a population of addicts seeking treatment, the most co-occurring psychiatric disorders were: mood disorders, anxiety disorders, post-traumatic stress disorder, severe mental issues, antisocial personality disorder and borderline personality disorder. Additional studies have emphasized the importance of treating SUDs in conjunction with underlying psychiatric disorders (105) and a need for research to expand on SUDs and co-occurring psychiatric disorders (106).

Research has shown that dopamine dysregulation may play a role in several affective disorders (107–109). In major depression (a common mood disorder), dopaminergic innervation is found reduced in regions that mediate mood and motivation (i.e. prefrontal regions and the cingulate cortex), and the hyperactivity of the prefrontal regions is a key target for treatment in treating depression symptoms (107,108).

Interestingly, the aberrant dopaminergic innervation in regions of target such as the NAcc, PFC and cingulate gyri that underlie the pathophysiology of affective disorders is also observed in substance use pathology. This may explain the high rates of psychiatric comorbidity and addiction. Using depression as an example, meta-analysis study of gray matter changes in major depressive disorder found that gray matter was reduced in: the bilateral insulas extending into the posterior part of the inferior frontal gyrus and anterior superior temporal gyrus, and a large region of the ventromedial PFC predominantly inferior to the ACC, in the posterior cingulate cortex and dorsal ACC, in the lateral PFCs, in the left inferior parietal gyrus and right fusiform and in the left caudate, left hippocampus and left parahippocampus (110). Increased gray matter volume was found in the bilateral superior occipital gyri extending into the cuneus and smaller elevations in the right angular gyrus and right postcentral gyrus (110). Changes in the frontal and cingulate regions were hypothesized to be a key factor in mood dysregulation seen in major depression patients (110).

2. Study aims and objectives

2.1. Specific aim and objective 2- Association between neuroimaging data and treatment outcome.

We expected to find frontal cortical thickness significantly correlated to positive treatment outcome (94). Using the follow up data collected nine months after rehabilitation of nyaope patients to divide them into abstinent- and relapse patients, we ran regional analysis on cortical thickness.

2.2. Specific aim and objective 3- Association between nyaope addiction and co-morbid psychiatric disorders.

Although patients were screened for a previous history of a psychiatric disorder and those presenting with symptoms of psychiatric comorbidity on the MSE were excluded, during subsequent assessment, the MINI detected the presence of current depressive episodes and antisocial personality disorder in our cohort. We expected that additional gray matter atrophy would be detected in such cases (110–113). Therefore, we divided NDI into those with detected psychiatric co-morbidities and those without and ran regional analyses to compare cortical thickness.

3. Methods and Data analysis

The methods and data analysis techniques are described in chapter one sections 3 and 4.

4. Results

4.1. Follow up results on abstinence

At the three-month follow up appointment, 11/28 of NDI had relapsed, 8/28 had remained abstinent, and 9/28 were lost to follow-up. At the nine-month follow-up appointment, the number of relapsed patients had increased to 17/28, 3/28 of NDI remained abstinent, and 8/28 were lost to follow-up (*Table 3*).

Table 3: Relapse rates and psychiatric comorbidity in NDI

Relapsed vs abstinent NDI			
Follow up period	Relapsed	Abstinent	Unknown
3 months	11	8	9
9 months	17	3	8
Psychiatric comorbidities in NDI			
	NDI (n=28)	Percentage (%)	
Depressive episode	15	53.57	
Antisocial personality disorder	12	42.86	
Depressive episode and antisocial personality disorder	8	28.57	

4.2 Differences in neuroanatomy between NDI who relapsed and NDI who remained abstinent at follow-up

No strong effects were found with the potential to relapse. A post-hoc analysis on abstinent vs relapsed NDI showed cortical atrophy in relapsed NDI in the right caudal anterior cingulate, the right posterior cingulate and in the right caudate nucleus ($p<0.05$). These effects, however, were marginal and did not reach FDR correction. Duration of drug use was also not found to be particularly related to the pattern of atrophy. Duration of drug use was marginally associated with cortical atrophy in the left superior temporal sulcus, bilateral caudal-anterior cingulate, left fusiform, bilateral middle temporal, left superior parietal, left superior temporal, right inferior parietal, right pars opercularis, and right posterior cingulate ($p<0.05$), but these effects did not reach significance following FDR correction.

4.3 Psychiatric comorbidities

Although the NDI had no previous psychiatric history and no comorbidity was apparent in the MSE, subsequent MINI evaluation detected the presence of current depressive episodes- 15/28, and antisocial personality disorder- 12/28.

4.4 Psychiatric comorbidity and cortical thickness

Psychiatric comorbidity was not associated with any specific cortical atrophy. NDI with a current depressive episode (n=15) and antisocial personality disorder (n=12) underwent separate analyses (two-sided t-test). Depressive episodes were marginally associated with atrophy in the right transverse temporal sulcus and antisocial personality disorder was marginally associated with atrophy in the right isthmus cingulate ($p<0.05$). These effects, however, did not survive FDR, suggesting that no significant differences in cortical thickness were found between the groups with psychiatric features and those without.

5. Discussion

5.1 Risk of relapse neuroanatomy

Our results showed that relapse patients had marginally reduced cortical thickness in the right hemisphere's caudal ACC, PCC and caudate nucleus. The results did not survive FDR testing, although further studies with larger sample sizes may uncover stronger effects and thus, the marginal effects reported here are worth exploring.

Previous neuroimaging studies found that there was a reduction in gray matter of the ACC, PCC, and striatum (formed partially by the caudate) in patients who relapsed back into their substance use disorder (49,101). There

is scant literature on the correlation between gray matter thickness and risk of relapse in heroin, possibly attributable to the high relapse rates (69), something we also encountered as a limitation. Nevertheless, these results suggest a possible role of the ACC, PCC and caudate in mediating risk of relapse in nyaope use disorder.

5.1.1 Anterior cingulate cortex

The ACC has extensive connections with the PFC, parietal, temporal poles, entorhinal areas and with motor regions (supplementary motor area, pre-motor and motor cortices) (114). It is thought to be organized in a rostro-caudal axis and has its main functions- affective and cognitive processing arranged along this axis respectively (114,115). The rostral ACC is positively connected to the amygdala, hippocampus, ventromedial PFC, and PCC and through these connections mediates affective processing (115). The caudal ACC is positively connected to frontoparietal, sensorimotor processing regions (115) and is part of the frontoparietal attention network (115). The intermediate ACC is positively connected to the lateral PFC and mediates higher cognitive functions (working memory, cognitive control), and may function to integrate affective and sensorimotor information to produce a crucial function of the ACC- conflict detection and error monitoring (115).

The ACC is involved in the processing a range of cognitive functions that include autonomic regulation, pain perception, affective and higher cognitive functions (114,115). Specifically, in cognitive control of behavior, the ACC has been identified as being important in detecting conflicts when presented with tasks and error monitoring (114–116). For example, the ACC is active during tasks that require the suppression of a pre-potent response, generation of a novel response, tasks that cause divided attention or when arbitrarily selecting a response from a large possible range of responses (114).

The functions of error detection and conflict monitoring have been implicated in substance use disorders. It is suggested that the inability of the ACC to successfully monitor conflict and error, in conjunction with an altered OFC is important in producing a continued overvaluation of the substance in spite of detrimental outcomes for the addict (117). In our sample, individuals with greater ACC integrity may be able to remain abstinent because their cognitive function of conflict monitoring and error detection in tasks may be intact and are thus able to successfully inhibit actions that are detrimental i.e. relapse (28). Leland and colleagues (118) found that the ACC was activated by drug predictive cues in a group of substance use disorder patients and interestingly, the greater the activation of the ACC in response to the cues, the better the subjects performed on an inhibitory task. Therefore, the greater ACC volume in our abstinent patients may contribute to greater activity in response to drug related cues and improved inhibition of the impulse to relapse. Furthermore, Goldstein and colleagues (119) found that the greater the activation of the caudal ACC in response to drug related cues, the greater the negative valence attributed to that drug cue. This indicates that an intact ACC may offset relapse in part by contributing to the conscious awareness of the detrimental effects of the substance in the presence of drug related cues.

It is worth noting that executive top-down inhibition of behavior is reliant on the ACC, dlPFC, inferior frontal cortex and OFC (28), and in our overall study we found gray matter atrophy in the dlPFC and OFC. The ACC (along with the OFC) regulate dopamine cell activity and are necessary for the increased motivational value of

drugs in patients as they, hence they have been shown to be triggered by conditioned cues for rewards and this culminates in craving (24). The ACC thus seems to produce craving in patients and paradoxically, is also responsible for inhibiting the impulses produced by craving. Both these functions are important in relapse i.e., craving initiates the desire to use the drug while the inhibitory circuit prevents actions initiated towards taking the drug, thus an intact ACC may be a pivotal constituent in a patient remaining abstinent.

5.1.2 Posterior cingulate cortex

The PCC is part of and is extensively connected to the posteromedial cortex (retrosplenial cortex and medial parietal cortex), the paralimbic and limbic areas, medial temporal lobe, para-hippocampal cortex and hippocampal formation, ventromedial PFC, ACC, frontal poles, PFC, thalamus and striatum (120). As part of the posteromedial cortex, the PCC is an important hub in the default mode network (120).

The functions of the PCC are not well understood and are subject of ongoing investigations. However, it is thought to play important roles in the production of arousal and awareness, internally directed thought, controlling the balance between internal and external attention and the detection of environmental changes (120). In the context of SUDs, internally directed thought, internal vs external attention and environmental change detection may play important roles in keeping the individual abstinent. During internally directed thought, a function mediated by the DMN, an individual may be engaged in processes such as planning for the future, something that may be crucial in remaining abstinent. The PCC is able to control the balance or shift from internal to external attention because of its position in the hippocampocentric subdivision of the paralimbic zone, that provides a link between the hippocampal formation and higher cognitive centers (120). Its ventral portion is a part of the DMN while its dorsal portion is part of the fronto-parietal control network and is thought to be important in enabling the switch from 'mind wandering' to external attention in-order to carry out tasks. This may be important in helping the abstinent patient maintain focus on other tasks outside of their internal states e.g. anhedonia and amotivation (96), which sometimes contribute to relapse . Indeed many clinical disorders involving lesions to the PCC result in an inability to maintain attentional focus (120). Lastly the PCC has also been shown to play an important role in signaling changes in the environment in order to control behavior, i.e. produce a vigilant state (120). Some of its neurons have been shown to be responsive to behaviorally salient information in the environment (120). Furthermore, its activity has been shown to increase when focusing on targets that are of high motivational value and appears to integrate the history of rewards obtained, consequences of behavior over time and provide a signal for strategy change if the reward is suboptimal (120).

PCC neurons were found to be active in tasks whereby there is gathering of information about alternative options (121).The PCC may be a part of a network involved in monitoring the outcomes of each decision and integrating them with other higher level strategy planning networks (121,122). The computation of reward and reward predictions occurs in the striatum, OFC and medial PFC, and is then integrated with information about recent reward outcome, choice history maintained in the PCC and is finally relayed to the ACC where it is used to select for appropriate actions (e.g. inhibition of behavior) (121,123). Hence in an abstinent patient, a healthy

PCC may confer the patient the ability to monitor behavioral outcomes and improve the ability to inhibit the impulse to take a substance through its connections with the ACC.

5.1.3 Caudate

The caudate nucleus forms the dorsal striatum with the putamen. The dorsal striatum is important in developing habitual compulsive behavior in substance use disorders (16,17,24,28). The dorsal striatum has been shown to be responsible in producing the subjective feeling of craving, studies have shown that drug-associated cues caused an increase in dopamine in the dorsal striatum and this is correlated to craving (124,125). The development of habitual behavior occurs due to a change in the dopamine spiraling circuit that results in the gradual overstimulation of the dorsal striatum (*see chapter 1*), producing the phenotype of habitual drug seeking behavior (16). In addition to this, the dorsal striatum is under the influence of PFC centers such as the ACC that inhibit the increased activity in the region thus suppressing the automated response to drug seeking (28). Therefore, the dorsal striatum is involved in not only craving (from dopaminergic activity) but also automated drug seeking that is suppressed by the ACC, hence a healthy dorsal striatum would be more responsive to signals from the ACC, inhibiting drug-seeking behavior.

5.2 Co-morbid psychiatric disorders

Although the patients recruited for this study were not receiving treatment for a previously diagnosed comorbid psychiatric disorder and did not show signs of apparent comorbid psychiatric disorders at the MSE, the MINI detected comorbidities such as depression and antisocial personality disorder. No significant differences in cortical thickness were found in these groups in comparison to the NDI. Marginal effects were found in the right transverse temporal sulcus and the right isthmus cingulate, however, these effects did not survive correction for multiple comparisons. Our results, thus, suggest that the effects of nyaope use have stronger effects on brain morphology than do underlying comorbidities but this should be interpreted lightly as the sample sizes of these subgroups were small.

We found 15/28 NDI had experienced a current or recent major depressive episode (MDE). A MDE according to the DSM V is a period of at least two weeks in which there is either a depressed mood or a loss of interest or pleasure in nearly all activities (15). The individual must also experience at least four other symptoms that may include changes in appetite or weight, sleep, psychomotor, decreased energy, feelings of worthlessness or guilt, difficulty thinking, concentrating or making decisions or recurrent thoughts or ideations of suicide (15). The symptoms must persist for a day, nearly daily for at least 2 consecutive weeks, and must be accompanied by social or occupational impairments (15). According to the DSM V (15), MDE commonly co-occurs with substance use disorders which is consistent with our results. Although we were unable to isolate any significant regions of atrophy in nyaope and MDE co-morbidity, we found marginal atrophy in the right transverse

temporal sulcus. The transverse temporal sulcus is involved in language processing (126). It has also been reported to be atrophied in people who were diagnosed to MDE that was linked to childhood trauma (127). Its cortical thickness thinning was related to the extent of childhood maltreatment (127). This finding warrants further examination by recruiting a greater NDI cohort presenting with MDE for greater statistical power.

Nyaope use disorder, as a consequence of its neuroanatomical signature may also produce symptoms that collectively manifest as MDE co-morbidity i.e., MDE in the patients is a consequence of nyaope use disorder. The DSM V recognizes that substances of abuse can be 'associated with depression like phenomenon'(15). Thus, in our patients, MDE may be a consequence of the substance as opposed to a uniquely altered neurobiology resulting in a major depressive episode. It is also possible that the major depression in our cohort may be represented by changes to neural circuitry due to substance abuse as they had no history of MDEs.

Twelve out of the 28 NDI in our cohort were also diagnosed with antisocial personality disorder (ASPD). ASPD according to the DSM V is primarily the pervasive pattern of disregard for, and violation of the rights of others that begin at childhood/adolescences and continues into adulthood (15). Often the individual is deceitful for personal gain, impulsive, irritable and aggressive, reckless disregard for the safety of self or others, is excessively irresponsible and displays a general lack of remorse (15). The prevalence of ASPD is reportedly very high in substance use disorder patients (15). There is often an overlap of ASPD behavior and substance use disorders such as neglect for responsibilities and the use of crime in order to sustain the habit (15). A metanalysis of brain changes in ASPD reveals that compared with controls, gray matter volume in subjects with antisocial behavior is reduced in the right lentiform nucleus, left insula and left frontopolar cortex, and is increased in the right fusiform gyrus, right inferior parietal lobule, right superior parietal lobule, right cingulate gyrus and the right postcentral gyrus (128). These regions do not overlap with our findings of marginal effects in the right isthmus cingulate or with our main findings but further research with larger cohorts may uncover whether antisocial traits lead to a predisposition to initiate and continue drug use or if there is a common neurobiological disease pattern.

6. Conclusion

We found that greater volumes of the ACC, PCC and caudate nucleus conferred greater chances of remaining abstinent. Our results did not survive FDR testing suggesting a small influence. This may be due to our small population of individuals who were able to remain abstinent. Future studies may benefit from recruiting abstinent patients to compare with relapse patients. Gray matter atrophy was found in regions associated with craving (ACC and dorsal striatum), error detection and strategy review (ACC and PCC) and inhibitory control (ACC). This suggests that in abstinent patients, there is improved modulation of craving and suppression of drug seeking behavior, and these two aspects may be key in preventing relapse in patients. We found that MDE and ASPD were the most prevalent psychiatric co-morbidities in our cohort which is supported by the DSM V

linking MDE and ASPD to SUDs. Due to the fact that no significant neuroanatomical changes were noted in the MDE and antisocial groups, and the marginal effects had no overlap with our main findings, we can deduce that nyaope use has a stronger effect on cortical atrophy compared to underlying psychiatric features in this cohort. This is likely due to the fact that our cohort had no previous history and had no obvious psychiatric comorbidity present at during the MSE. Further longitudinal research with larger cohorts will uncover whether underlying psychiatric features drive drug initiation and maintenance or if years of drug use could precipitate depressive and antisocial features and, if so, to what extent.

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Appendices

Ethics clearance certificate

R14/49 Dr Tanya Calvey et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170302

NAME: (Principal Investigator)	Dr Tanya Calvey et al
DEPARTMENT:	School of Anatomical Sciences Parklane Clinic Chris Hani Baragwanath Academic Hospital University of the Witwatersrand Empilweni Substance Abuse Rehabilitation Centre
PROJECT TITLE:	Structural and Connectivity Characterization of the Brains of Nyaope Users
DATE CONSIDERED:	31/03/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	19/05/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review March and will therefore be due in the month of March each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

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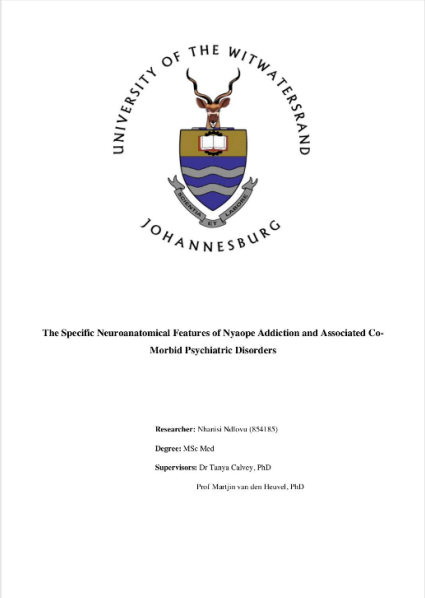
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UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

The Specific Neuroanatomical Features of Nyaope Addiction and Associated Co-Morbid Psychiatric Disorders

Researcher: Nhanisi Ndlovu (854185)

Degree: MSc Med

Supervisors: Dr Tanya Calvey, PhD

Prof Martjin van den Heuvel, PhD

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C: Biographical questionnaire of healthy controls

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Faculty of Health Sciences
School of Anatomical Sciences
7 York Road
Parktown
Johannesburg
2193

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

BIOGRAPHICAL QUESTIONNAIRE

Please take the time to answer these questions thoroughly and honestly

Thank you

Participant number: ……

Sex: ❒ Male
❒ Female
❒ Other

Date of birth: ……...

Age: ……...

Country of birth: ……...

Home language: ……...

Handedness: ❒ Left
❒ Right
❒ Ambidextrous

Would you like a summary of the findings when the research is completed? ❒ Yes
❒ No

1. **Have you ever been diagnosed with a chronic or acute psychiatric disorder?**

❒ No (skip this section) ❒ Yes (continue with section)

Please give details of the diagnosis:

……
……
……
……
……

What is the history of the condition? (e.g., when you were first diagnosed, how long you have had/had the problem, etc)

……
……
……
……
……

2. Have you ever been diagnosed with an acute or chronic heart condition?

❒ No (skip this section) ❒ Yes (continue with section)

Please describe the diagnosis:

……
……
……
……
……

What is the history of the condition? (e.g., when you were first diagnosed, how long you have had/had the problem, etc)

……
……

……

…....……...

……..

3. Do you currently smoke?

❒ No (skip this section) ❒ Yes (continue with section)

Number of cigarettes a day:

❒ Nil

❒ 1-10 per day

❒ 11-20 per day

❒ 20 +

How long have you been smoking for?

……

……

……

…....……...

……..

4. Alcohol Consumption

How often do you drink alcohol and how much do you consume?

❒ Don’t drink

❒ Drink occasionally (less than once a week)

❒ Drink only on weekends – moderate use

❒ Drink only on weekends – uncontrolled

❒ Drink every day in moderate amounts

❒ Drink every day and sometimes get drunk

❒ Drink to intoxication (excess) every day

❒ Other

When was your last drink? How much did you drink?

……

……

……

……

……

5. Head Injury

Have you ever suffered a head injury?

❒ Yes
❒ No
❒ Unsure

If you HAVE had a head injury, did you lose consciousness?

❒ Yes
❒ No
❒ Unsure

If you HAVE had a head injury, were you unconscious for longer than 5 minutes?

❒ Yes
❒ No
❒ Unsure

If you HAVE had a head injury, did you have to go to hospital?

❒ Yes
❒ No
❒ Unsure

6. Are you currently taking any <u>prescribed</u> medication(s)?

❒ No (skip this section) ❒ Yes (continue with section)

Medication name	Medication purpose	Last date taken	Dosage	How long have you been taking them?

7. Do you take any <u>non-prescription</u> medication(s)?

❒ No (skip this section) ❒ Yes (continue with section)

Medication name	Medication purpose	Last date taken	Dosage	How long have you been taking them?

8. Do you use any <u>recreational</u> drugs?

❒ No (skip this section) ❒ Yes (continue with section)

Medication name	Medication purpose	Last date taken	Dosage	How long have you been taking them?
Medication name	Medication purpose	Last date taken	Dosage	How long have you been taking them?

9. Do you suffer from claustrophobia?

❒ No ❒ Yes

10. To your knowledge, are you HIV positive?

❒ No ❒ Yes