Research report on

Evidence of Brain Damage in Chronic Ketamine Users – a Brain Imaging Study

Submitted to

Beat Drug Fund Association

Submitted by

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Contents

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The Evangelical Lutheran Church of Hong Kong- Ling Oi Tan Ka Wan Centre;

The Society for the Aid and Rehabilitation of Drug Abusers - Sister Aquinas Memorial Women's Treatment Centre;

The Society for the Aid and Rehabilitation of Drug Abusers - Adult Female Rehabilitation Centre;

Wu Oi Christian Center Tai Mei Tuk Female Training Center.

Abbreviations

ACC: Anterior Cingulate Cortex

ANCOVA: Analysis of Covariance

ANOVA: Analysis of variance

ASI: Addiction Severity Index

ASP: Aspartate

BDI: Beck Depression Inventory

BG: Basal Ganglia

BOLD: Blood Oxygen Level-Dependent

Cho: Choline

Cr: creatine

DLPFC: Dorsolateral Prefrontal Cortex

DMN: Default Mode Network

DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition

FDR: False Discovery Rate

fMRI: functional Magnetic Resonance Imaging

GABA: γ-aminobutyric acid

GIFT: Group ICA of the fMRI Toolbox

Glu: Glutamate

Gln: Glutamine

GPC: Glycerylphosphorylcholine

HADSA: Hospital Anxiety Depression Scale

ICA: Independent Component Analysis

ICs: Independent Components

LCModel: Linear Combination of Model

LTP: Long-term Potentiation

MANCOVA: Multivariate Analysis of Variance

ml: Myoinositol

MNI: Montreal Neurological Institute

MPFC: Medial Prefrontal Cortex

MRI: Magnetic Resonance Imaging

MRS: Magnetic Resonance Spectroscopy

NAA: N-acetylaspartate

NGOs: Non-Governmental Organisations

NMDA: N-Methyl-D-aspartate

OFC: Orbitofrontal Cortex

PAL: Parietal Lobe

PCh: Phosphocholine

PFC: Prefrontal Cortex

PWH: Periventricular White Matter

RAs: Research assistants

ROCF: Rey-Osterrieth Complex Figure

SDS: Severity of Dependence Scale

SCID: Structured Clinical Interview for DSM Disorders

TPL: Temporal Lobe

VF: verbal fluency

VOI: Volumes of Interest

VLPFC: Ventrolateral Prefrontal Cortex

WAIS-III: Wechsler Adult Intelligence Scale, Third Edition

WAIS: Wechsler Adult Intelligence Scale

WCST: Wisconsin Card Sorting Test

WMS-III: Wechsler Memory Scale, Third Edition

Executive summary

The objectives of this study were to ascertain the pattern of grey and white matter volume reduction and regional metabolic and activation abnormalities in chronic ketamine users, and to evaluate the correlations between these brain abnormalities and cognitive impairments in chronic ketamine users in Hong Kong.

One hundred and eighty-one participants were recruited from October 2011 to July 2015. The participants were divided into two groups: ketamine users (124) and healthy controls (57). Amongst the ketamine users, 60 were primarily ketamine users and 64 were poly ketamine users. Psychiatric assessments included self-rated questionnaires and face-to-face interviews. All participants completed a detailed cognitive battery that covered general intelligence, verbal and visual memory, executive functions, motor speed and language. All participants underwent magnetic resonance imaging scan of the brain.

Many participants in the ketamine users group also frequently used cocaine and cannabis. Among the ketamine users, 25% were diagnosed with a mood disorder and 15.3% with an anxiety disorder. The participants in the ketamine users groups, particularly in poly ketamine use group, had worse performance than the healthy controls on tests of general intelligence, verbal, visual and working memory and executive functioning.

In terms of grey matter volumes, the right orbitofrontal cortex, right medial prefrontal cortex, left globus pallidus, left hippocampus, and right nucleus accumbens

were smaller in the ketamine users group. In contrast, the volumes of the left caudate and left thalamus were higher in the ketamine users group. In terms of white matter volumes, the ketamine users group had a lower periventricular white matter volume in the right hemisphere. The grey matter volumes of the right orbitofrontal cortex, right medial prefrontal cortex, and right nucleus accumbens were negatively correlated with the severity of ketamine dependence. The right orbitofrontal cortex, right medial prefrontal cortex, left caudate, left globus pallidus, left hippocampus, right necleus accumbens, left thalamus and right periventricular white matter were also correlated with the performance on the cognitive tests.

In terms of regional metabolism, there were no significant differences in the metabolite ratios between the primarily ketamine users group and the healthy control group; whereas the poly ketamine users group had a higher 'glutamate + glutamine / creatine' ratio in the right basal ganglia than the healthy control group.

A functional connectivity examination of the default mode network revealed significantly decreased connectivity in orbital part of inferior frontal gyrus, anterior cingulate and paracingulate gyri, superior temporal gyrus and vermic lobule VI; and increased connectivity in middle occipital gyrus in ketamine users.

In conclusion, the results provide imaging evidence of brain damage in chronic ketamine users. Chronic ketamine use was associated with reduced grey and white matter volumes in certain regions of the brain. Chronic ketamine use was also associated with altered functional connectivity with the default mode network. Abnormal brain structures and altered functional organisation of the brain network

may underlie the hypersensitivity towards drug related cues but weakened cognitive control in those with ketamine addiction. Longitudinal or prospective studies would help to strengthen the evidence on the reversibility of the structural and functional brain damage caused by ketamine.

行政撮要

本研究的目的旨在 1.) 確定長期氯胺酮使用者灰質和白質容量減少與局部性代謝和激活異常的模式，2.) 評估長期氯胺酮使用者腦部上述結構性、代謝性和功能性異常與認知障礙的相關性。

自 2011 年 10 月至 2015 年 7 月，共 181 名受試者入組。受試者分為 2 組：氯胺酮組和健康對照組，氯胺酮組組有 124 名受試者，而健康對照組則有 57 名受試者。氯胺酮組中包括 60 名主要氯胺酮濫用者和 64 名氯胺酮及多種藥物濫用者。精神狀況評估包括問卷篩查和面談。所有受試者均完成一套詳細的認知測試。該測試涵蓋一般智慧、詞語記憶、視覺記憶、執行功能、動作速度和語言。每名受試者均會接受腦部磁力共振掃描檢查。

氯胺酮組受試者除氯胺酮外主要濫用可卡因和大麻。氯胺酮濫用者中，有 25%患有抑鬱障礙，15.3% 患有焦慮症。兩組氯胺酮濫用組 (尤其氯胺酮及多種藥物濫用者) 與健康對照組在一般智慧、詞語記憶、視覺記憶、執行功能仍存在顯著差異，氯胺酮濫用組得分低於健康對照。

氯胺酮組右側眶額葉、右內側前額葉、左側蒼白球、左側海馬、右側伏隔核的灰質體積小於對照組，而左側尾狀核和左側丘腦的體積則大於對照組。此外，氯胺酮組右側腦室旁白質體積低於對照組。在氯胺酮組中，右側眶額葉、右內側前額葉、右側伏隔核灰質體積與氯胺酮成癮嚴重程度呈負相關關係。右側眶額葉、右內側前額葉、左側尾狀核、左側蒼白球、左側海馬、右側伏隔核、左側丘腦和右側腦室旁白質與認知測試表現呈相關關係。

主要氯胺酮濫用組與對照組相比代謝物濃度沒有顯著差異，而氯胺酮及多種藥物濫用組在右側基底節的“谷氨酸+谷氨醯胺/肌酸”比高於對照組。氯胺酮組靜息態預設模式網路連接在前額眶內部分、前扣帶回和半扣帶回部分、顳上回和 vermic 葉 VI 活性下降，而枕中回活性增強。

總括而言，氯胺酮濫用對大腦的損傷有影像學依據。氯胺酮濫用與特定腦區灰質和白質體積下降有關並且蝕變預設模式網路的功能連線性。異常的腦體積和腦神經網路的功能連接可能導致氯胺酮濫用者的認知功能改變，對濫藥相關線索的敏感性增強而對成癮行為的控制減弱。氯胺酮所致的這些大腦結構及功能變化是否可逆轉還需要縱向或前瞻性的研究來進一步證明。

Background

Introduction to ketamine and ketamine abuse

Ketamine [2-(2-chlorophenyl)-2-(methylamine)-cyclohexanone] was developed in 1962 as an anaesthetic agent to replace phencyclidine, which exerts severe hallucinogenic effects in humans (Sinner & Graf, 2008). Its major effects are pain relief, sedation and amnesia, and it is legally used as an analgesic in cases varying from paediatrics to trauma and cancer (Wolff & Winstock, 2006). It is also used in veterinary practice (Wolff & Winstock, 2006).

Ketamine reacts pharmacologically through non-competitively antagonising NMDA (N-methyl-D-aspartate) receptors (Wolff & Winstock, 2006). NMDA receptor antagonists may prompt the interference transmission of excitatory amino acid glutamate and aspartate, which may underlie ketamine's prevention of the perception of auditory, visual and painful stimuli; responses to the environment; and memory effects (Morgan, Mofeez, Brandner, Bromley, & Curran, 2004a, 2004b). Ketamine enhances the neurotransmission of noradrenaline, serotonin and dopamine systems in a dose-dependent fashion, which causes its psychotomimetic and sympathomimetic effects (Wolff & Winstock, 2006) and addiction potential (Ross & Peselow, 2009). Ketamine can be effectively administered through intranasal, intravenous, subcutaneous, intramuscular and intrathecal routes. In recreational ketamine users, intranasal use is the most common route due to the rapid initiation and long-lasting effect (about 2-3 hours). Ketamine's dissociative effects can be

achieved with a low dose of 50-100 mg, whereas a dose of 5-10 mg/kg is required to achieve anaesthesia (Koesters, Rogers, & Rajasingham, 2002). However, the perception and mood changes indicative of non-medical use are highly diverse based on age, dosage, route of administration and previous experience (Curran & Morgan, 2000). Lower doses of ketamine typically generate a stimulant effect, whereas higher doses produce psychedelic effects and environmental disassociation (Oye, Paulsen, & Maurset, 1992).

Ketamine has been misused in the United States since the 1970s (Wolff & Winstock, 2006), with recreational use reaching its peak around 2000 in the US and gradually decreasing thereafter. In 2002, 2.6% of twelfth graders in US high schools reported witnessing the use of ketamine in the previous year, and this number had dropped to 1.7% by 2009 (National Institute on Durg Abuse, 2010). Trends in Hong Kong differ considerably from those in the US. According to the Narcotics Division's Central Registry of Drug Abuse, ketamine has been recognised as an abused drug in Hong Kong since 2000. Its use increased rapidly and reached a peak in 2008, with a slight decrease in subsequent years. Ketamine is currently the most commonly abused drug in Hong Kong, especially among youths. About 70% of ketamine abusers are under the age of 21, with binge and withdrawal symptoms reported in most recreational users (Critchlow, 2006; Wolff & Winstock, 2006).

Ketamine and cognitive functioning

Ketamine's substantial effects are due to the antagonistic action of NMDA receptors, which is important in inducing long-term potentiation (LTP). LTP is a long-lasting increase in synaptic efficiency induced by brief, high-frequency afferent stimulation, and research has identified it as the underlying mechanism of learning and memory at the neuronal level (Rowland et al., 2005). NMDA receptor antagonists disrupt LTP in the hippocampus, which has been shown to impair information acquisition in animals (Rowland et al., 2005). In human studies, researchers have proven that one-off doses of ketamine lead to temporary memory impairments in healthy volunteers, particulalry in working (N-back test) (Krystal, Perry, et al., 2005; Morgan, Mofeez, et al., 2004a) and episodic memory (Morgan, Mofeez, et al., 2004a; Parwani et al., 2005; Rowland et al., 2005). Ketamine has been found to consistently impair the process of encoding information (Rowland et al., 2005), prose recall (Morgan, Mofeez, et al., 2004a) and Hopkins Verbal Learning Test performance (Krystal, Abi-Saab, et al., 2005), but not the retrieval of information (Rowland et al., 2005). Studies have also noted impairments in the early consolidation of information (Parwani et al., 2005). Studies of executive functioning have found that performance remained intact in the Stroop colour-word test (Parwani et al., 2005), Trail making A/B (Morgan, Mofeez, et al., 2004a) and the fluency test (Morgan, Mofeez, et al., 2004a; Rowland et al., 2005), whereas response inhibition was impaired in the Hayling Test (Morgan, Mofeez, et al., 2004a) and rule learning and shifting in the WCST (Krystal et al., 2000; Krystal et al., 1999). Although acute ketamine use caused no residual effects in healthy volunteers (Morgan, Mofeez, et al., 2004a) and

infrequent ketamine users (Curran & Monaghan, 2001), the same was not true for frequent ketamine users (Curran & Monaghan, 2001).

Most extant studies have explored the acute effects of ketamine, although investigations into the effects of chronic use in humans have been limited for ethical reasons. Studies have found that semantic and episodic memory remained impaired three days after dosage in recreational ketamine users (Curran & Monaghan, 2001; Morgan, Monaghan, & Curran, 2004), but not in healthy volunteers who were given a single dose of ketamine (Morgan, Mofeez, et al., 2004a). In another study, 18 recreational ketamine users were retested 3-4 years after recruitment; those who had decreased their ketamine use showed improvements in semantic memory, but deficits in episodic memory and attention remained (Morgan, Monaghan, et al., 2004). In another recent study by Morgan et al. (2009), frequent ketamine users (more than four times a week) showed sustained impairments in spatial working memory, pattern recognition, the Stockings of Cambridge planning task and category fluency compared to infrequent users, ex-users (abstinent for at least one month), normal controls and poly-drug users (not ketamine). Retrieval from source memory, prose recall (episodic memory), verbal fluency (VF) and performance on the Hayling Test (response initiation and inhibition) were preserved in the frequent ketamine group, with no difference found between ex-, infrequent and non-ketamine users (Morgan, Muetzelfeldt, & Curran, 2009). In addition, a correlation has been found between the amount of ketamine used and pattern recognition and working memory performance in frequent ketamine users (Morgan, Muetzelfeldt, & Curran, 2010). This study

concluded that the chronic effects of ketamine may be reversible, given the similarities between the performances of ex-ketamine users and non-drug users. A local study (Chen, Chan, Chen, & Tang, 2005) was unable to detect cognitive dysfunction among ketamine users. However, there were limitations in the aforementioned studies. First, they featured small sample sizes. Moreover, in Morgan's studies, the ketamine users all co-abused other drugs, such as cocaine or cannabis, which may confound ketamine's effect profile.

Ketamine and brain damage

There is very limited evidence on the brain abnormalities associated with ketamine abuse. In contrast, numerous brain imaging studies have been published on other psychotropics, including amphetamine and cocaine. These studies can be divided into three groups, namely, structural magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS) and functional MRI (fMRI) studies.

MRI studies about substance abuse have revealed reduced grey matter (Berman, O'Neill, Fears, Bartzokis, & London, 2008) and white matter volumes (Schlaepfer et al., 2006) and white matter hyperintensities (Berman et al., 2008). These abnormalities have been identified in a variety of brain regions, including the frontal (Cowan et al., 2003) and prefrontal cortex (Lim et al., 2008), anterior cingulate (Thompson et al., 2004) and hippocampus (Thompson et al., 2004).

MRS does not measure structural damage directly, but rather the level of metabolites in brain tissue, which may serve as a marker of underlying disease activities and indicate regional pathology. For example, N-acetylaspartate (NAA), a metabolite located in the neuron bodies, axons and dendrites, is a marker of neuronal integrity. Choline (Cho), located in the membrane phospholipids, may indicate increased membrane turnover due to neurodegeneration or membrane phosphatidylcholine catabolism. Myoinositol (ml) presents in the glial cells, with a raised level indicating gliosis. Glutamate/glutamine (Glx) are excitatory brain neurotransmitters that are involved in the neurotoxicity of drug abuse (Yamamoto &

Bankson, 2005). Various studies have reported that chronic drug abuse can lead to abnormalities in NAA (Yoon et al., 2010), Cho (Yoon et al., 2010), ml (Sung et al., 2007) and Glx (Sailasuta, Abulseoud, Hernandez, Haghani, & Ross, 2010) levels. The brain regions exhibiting these metabolite abnormalities include the frontal coretx (Yoon et al., 2010), prefrontal cortex (Reneman, Majoie, Schmand, van den Brink, & den Heeten, 2001) and anterior cingulate (R. Salo et al., 2011).

fMRI has emerged as an invaluable method for correlating altered neural substrate activation with the specific cognitive or affective dysfunctions arising from illicit drug use. fMRI is non-invasive and poses no ionising radiation risk, while providing superior spatial resolution to allow the discernment of small brain regions. fMRI reflects brain activation via the blood oxygen level-dependent (BOLD) responses that are indicative of blood flow changes, and its temporal resolution is sufficient for event-related paradigms. fMRI studies conducted on drug abusers have revealed abnormalities in a number of brain regions, including the prefrontal cortex (R. Salo, Ursu, Buonocore, Leamon, & Carter, 2009), dorsolateral prefrontal cortex and anterior cingulate (Aron & Paulus, 2007).

There is a correlation between the extent of these MRI (Aydin, Kircan, Sarwar, Okur, & Balaban, 2009), MRS (R. Salo et al., 2007) and fMRI (Paulus, Lovero, Wittmann, & Leland, 2008) abnormalities and the degree of cognitive deficit in drug abusers.

Objectives

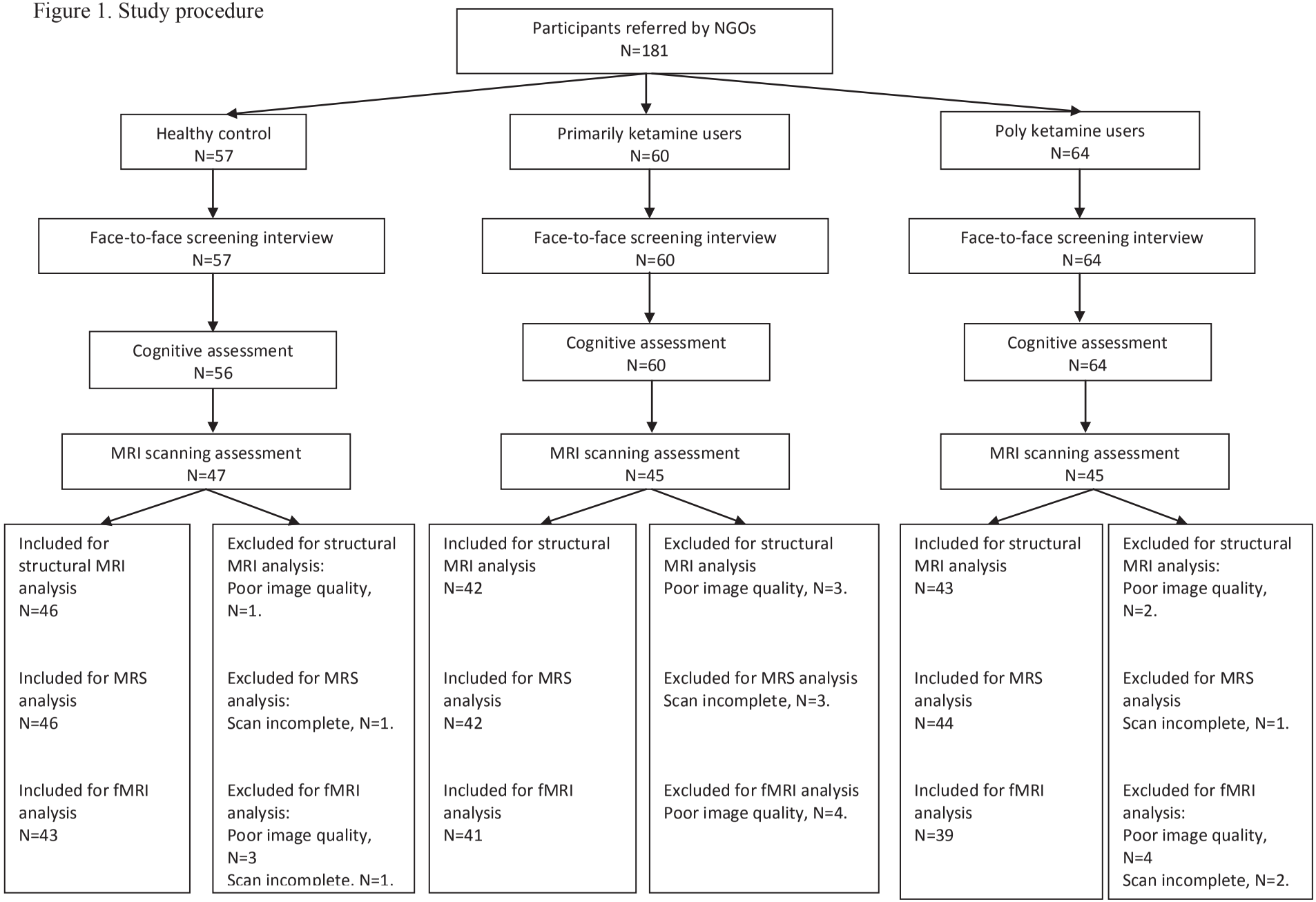
We conducted a cross-sectional study to examine the evidence of brain damage in a group of chronic ketamine users in Hong Kong. The objectives of the proposed study were 1) to ascertain the pattern of grey and white matter volume reduction and regional metabolic and functional abnormalities in chronic ketamine users, and 2) to evaluate the correlations between the aforementioned structural, metabolic and functional abnormalities in the brain and cognitive impairment in chronic ketamine users.

Methods

Design

The participants in this cross-sectional study were recruited to the ketamine abuse group or the healthy control group according to their drug abuse patterns. Cognitive functioning was compared between groups in relation to common confounding factors such as age, gender, education level and psychiatric comorbidities. The procedure for the study is illustrated in Figure 1. Each of the participants was given a $150 coupon as compensation for attending the basic assessment and another $350 coupon for attending the MRI scanning. This study was approved by the Survey and Behavioural Research Ethics Committee of the Chinese University of Hong Kong.

Figure 1. Study procedure



Participants

Participant recruitment sites

The participants were recruited from non-governmental organisations (NGOs) in Hong Kong. Drug abusers were referred by Counselling Centres for Psychotropic Substance Abusers, residential treatment centres and district youth outreach teams, while the normal controls were recruited from community service centres based on the inclusion and exclusion criteria. The NGOs were as follows:

a. Caritas Lok Heep Club;
b. Caritas HUGS Center;
c. Caritas Wong Yiu Nam Center;
d. Chinese Young Men's Christian Association of Hong Kong - Sha Tin Youth Outreaching Social Work Team;
e. Hong Kong Christian Service - Jockey Club Lodge of the Rising Sun;
f. Hong Kong Christian Service - PS33 Tsim Sha Tsui Center;
g. Hong Kong Christian Service - Yuen Long District Youth Outreaching Social Work Team;
h. Hong Kong Lutheran Social Service Cheer Lutheran Center;
i. Hong Kong Sheng Kung Hui Welfare Council Neo-Horizon;
j. Operation Dawn Girl Center;
k. The Evangelical Lutheran Church of Hong Kong - Enlighten Centre;

l. The Evangelical Lutheran Church of Hong Kong-Ling Oi Tan Ka Wan Centre;

m. The Society for the Aid and Rehabilitation of Drug Abusers - Sister Aquinas Memorial Women's Treatment Centre;

n. The Society for the Aid and Rehabilitation of Drug Abusers - Adult Female Rehabilitation Centre;

o. Wu Oi Christian Center Tai Mei Tuk Female Training Center.

Inclusion criteria

Participants were recruited into the study if they met the following inclusion criteria:

a. aged between 18 and 40;

b. right-handed;

c. capable of giving valid consent;

d. receiving service at an NGO;

e. for the primarily ketamine group, use of ketamine at least 24 times over 6 months within the last 2 years and the use of other illicit psychotropic drugs is less than 24 times over 6 months within the last 2 years;

f. for the ketamine poly ketamine group, use of ketamine and, together with other illicit psychotropic drugs such as ecstasy, marijuana or methamphetamine, with frequency at least 24 times over 6 months within the last 2 years;

g. for the healthy youth group – no history of substance abuse (Chen et al., 2005);

h. no history of any neurological disorders, significant medical diseases that required regular medication or severe head injury; and

i. no history of psychotic symptoms.

Data collection

Demographic information

Two research assistants (RAs) approached the participants in the NGOs and performed all of the data collection and cognition function assessments. Demographic information included:

a. age;

b. sex;

c. level of education;

d. marital status;

e. employment status;

f. monthly income;

g. district of residence; and

h. housing type.

Drug use patterns and severity

The Severity of Dependence Scale (SDS) (Gossop et al., 1995), a 5-item self-report scale, was administered to measure the degree of drug dependence in the previous month or the month before abstinence. Each item was scored from 0 to 3 with higher scores indicating increased severity of dependence.

The Addiction Severity Index-Lite Version (ASI-Lite) (Cacciola, Alterman, McLellan, Lin, & Lynch, 2007) is a multi-dimensional index used to measure participants' substance use and health and social problems (McLellan, Cacciola, Alterman, Rikoon, & Carise, 2006). It is a semi-structured scale that covers medical, employment/support, drug and alcohol, legal, family/social and psychiatric issues across the participant's life span. In this study, a composite score was calculated for each area. Each composite score ranged from 0 to 1, with higher scores indicating greater severity of the problems in these areas.

Trained RAs made a diagnosis of lifetime or current drug dependence for each participant according to the criteria for substance dependence in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association, 2000), based on the information recorded during the face-to-face screening interview.

Psychiatric comorbidities

The 21-item version of the Beck Depression Inventory (BDI) (Shek, 1990) was used to screen for depressive disorder. The BDI was applied in a previous study of ecstasy users in Hong Kong (Chen et al., 2005), in which total BDI scores ranged from 0 to 63. The sensitivity and specificity of the scale are 100% and 82%, respectively (D. T. Lee, Yip, Chiu, Leung, & Chung, 2001).

The anxiety subscale of the Hospital Anxiety Depression Scale (HADSA) (Leung, Ho, Kan, Hung, & Chen, 1993) was used in this study to screen for anxiety disorders. The HADSA has 7 items, each graded from 0 to 3. Scores are summed to produce a total score, and higher scores indicate greater severity of symptoms.

Mood disorder, anxiety disorder and psychosis screening questions derived from the Chinese version of the Structured Clinical Interview for DSM-IV (So et al., 2003) were administered to screen for possible mood, anxiety and psychosis disorders. Two RAs screened the same 20 participants with a kappa of 1.0, indicating that the two RAs were highly consistent in their assessment of whether a participant displayed possible psychiatric symptoms.

Cognition function evaluation

The cognitive battery was composed of the following domains and tests:

a. General intelligence: the 3-subtest short form of the WAIS–III (Chan, Chen, & Chan, 2005).
b. Executive function: the Stroop Test (Stroop, 1935), modified VF test (Chiu et al., 1997) and the WCST (Heaton, Chelune, Talley, Kay, & Curtiss, 1993).
c. Attention and working memory: Digit Span Forward and Digit Span Backward (D. Wechsler, 1997a).

d. Verbal memory: WMS–III logical memory immediate recall, delayed recall and recognition (Hua et al., 2005; D. Wechsler, 1997b); and wordlist immediate recall, delayed recall and recognition (Hua et al., 2005; D. Wechsler, 1997b);

e. visual memory: the Rey-Osterrieth Complex Figure (Osterrieth, 1944; E. M. Taylor, 1959).

f. Language: Modified Boston Naming Test (D. Wechsler, 1997a).

General intelligence was examined using the 3-subtest short form of the Wechsler Adult Intelligence Test–III. Although the full WAIS-III with 13 subscales is used worldwide for the evaluation of intelligence, its application is time-consuming. Therefore, the 3-subtest short form was developed (Axelrod, Ryan, & Ward, 2001; Blyler, Gold, Iannone, & Buchanan, 2000). This version includes the information, arithmetic and digit-symbol coding subtests. In the information subtest, participant will be asked a series of questions about general knowledge. The test contains 28 items, participant will be scored 1 for each correct response, test disoncintues after 6 consecutive incorrect responses. For the arithmetic subtest, participant is presented with a series of arithmetic problems to be solved mentally, without the use of pcncil or paper, and responds orally within a time limit. There are 20 items in this substest, the test terminates after 4 consecutive incorrect responses. For the last 2 items (items 19 and 20), 2 points will be given for each correct resonse provided within 10 seconds or 1 point for a correct response provided within the time limit but not in 10 seconds or less. For digit-symbol coding subtest, participant will be instructed to copy symbols

that are paired with numbers. Using a key, the participant draws each symbol under its corresponding number. Score is determined by the number of correct symbol drawn within the 120-second time limit. A study on a local normal population (Chan et al., 2005) reported scores ranging from 0-28, 0-22 and 0-133 for the respective subtests, with higher scores indicating better performance.

A simple version of the Stroop Test (T. M. Lee & Chan, 2000) was adopted to measure executive functioning in this study. This test consists of 72 items and is divided into 3 types of stimuli: color dot naming (part D), neuter colored words (part W), and incongruently colored words (part C). Each condition consists of 24 items and participants are required to read the given color of the dots in part D, the color of the unrelated words in part W, and the printed color of the incongruently colored words in part C. The number of errors and reaction times are then recorded for each condition. Participants tend to take a longer time and make more errors in part C due to the activation of the inhibitory process or interference effect. The additional time spent in part C is considered time spent on inhibiting word reading or solving interference (Ludwig, Borella, Tettamanti, & de Ribaupierre, 2010), whereas the increased error rates are seen as an index of temporally maintaining the task goal (Kane & Engle, 2003). After standardising the scores, the number of errors in part C and the differences in reaction times between parts C and D provide the Stroop score for executive function.

In the modified VF test, the participant was given one minute to generate as many animal names as possible, 30 seconds to generate as many fruit names as

possible and 30 seconds to generate as many vegetable names as possible. The number of words, perseverative errors (repeated words) and intrusive errors (non-category words) in each category were then counted. The total number of correct responses and the total number of perseverative and intrusive errors were used as the index scores for the modified VF test.

The WCST is composed of 4 stimulus cards and 64 response cards. The response cards differ in three dimensions: colour (red, green, yellow or blue), pattern (triangle, star, cross or circle) and number (one, two, three or four). The participants were asked to work out a sorting principle for matching each response card to the four stimulus cards (one red triangle, two green stars, three yellow crosses or four blue circles) according to the feedback given by the examiner (correct or incorrect). Once the participant had made 10 consecutive correct matches to the sorting principle, the sorting principle was changed without warning and the participant had to work out a new sorting principle. The test was terminated when the participant had: (1) successfully maintained 6 correct sorting principles (colour, pattern, number, colour, pattern, number) or (2) made 128 attempts. To evaluate the participant's abstract reasoning ability and ability to shift cognitive strategies, each response was recorded as either correct, a perseverative response, a perseverative error or a non-perseverative error for subsequent scoring. The number of categories completed, the total number of attempts and the number of perseverative errors were selected as the index scores.

The Digit Span test is a standardised measure that assesses attention and working memory. It consists of two modes - digit forward and digit backward - that

are administered separately. In the forward mode, the participant was instructed to listen to digit strings of 2 to 9 digits presented by the examiner and immediately repeat them back in the same order. In the backward mode, the digit strings contained up to 8 digits and the participant was instructed to repeat each string in reverse order. Each session started with a 2-digit item and the test terminated when the participant failed to repeat the digit string correctly after two attempts, or once all of the items had been successfully completed. Subscores and a total score were generated for this test. The backward mode requires more working memory effort than the digit forward mode, thus it is more sensitive in detecting deficiencies (Davis, Donald, & Zhu, 2003). The subscores of the digit backward mode were selected as the index of working memory and ranged from 0 to 14 with higher scores reflecting superior performance.

Verbal memory capacity was measured using the word list and logical memory subtests of the Wechsler Memory Scale - Third edition (WMS-III). Both subtests include three elements: immediate recall, delayed recall and recognition. In the word list subtest, the participant was presented with two separate word lists (A and B), each of which contained 12 unrelated words. The examiner read List A aloud and the participant was asked to perform a free recall of the words. The participants were given four consecutive attempts to recall List A, then the total number of words recalled correctly across all four attempts was calculated. The examiner then read List B aloud once and the participant was asked to recall the words in any order. Finally, the examiner asked the participant to recall the words from List A again, without

rereading the list. Three subscores were generated: the learning slope (the difference between the participant's first and fourth attempts to recall List A), the difference between the first attempts for Lists A and B and the difference between the fourth attempt at and delayed recall of List A. After a 30-minute interval, a list of 24 words was read aloud and the participant was asked to recognise the words from List A by responding 'yes' if he/she recalled the word from the list and 'no' if not. The number of words correctly recognised was recorded and all of the measurements were transformed into standard scores.

In the logical memory subtests, the participant was asked to listen to and then retell two stories (Stories A and B). Story A was read by the examiner and the participant was asked to immediately recall as much of the story as possible. Story B was then read aloud and the participant had to recall it immediately. This story was read and immediately recalled twice. After 30 minutes, the participant was again asked to recall both stories. Finally, the examiner posed 15 questions about the content of each story. The elements within the retelling were divided into story (content-related) and thematic (theme-related) units. The test was scored by calculating the total number of story units in the immediate recall of Stories A and B, the total number of story units in the delayed recall of Stories A and B and the number of questions about the story answered correctly. The story unit retention score was calculated using the following formula: (immediate recall for story A ± second immediate recall of story B) / (delayed recall of story A ± delayed recall of story B. Again, all measurements were transformed into standard scores.

Visual construction and visual memory were tested using the Rey-Osterrieth Complex Figure (ROCF), which comprises four conditions: copy, immediate recall, delayed recall and recognition. The participant was instructed to copy a drawing of a complex figure, which was removed from sight once the copy had been completed. The participant was asked to redraw the figure 3 minutes (immediate recall) and then 30 minutes (delayed recall) later, without looking at the original drawing. The accuracy and placement of the elements in the figure were counted according to the 36-point scoring system (E. M. Taylor, 1959). After completing the delayed recall, the participant was shown 24 geometric items and asked to identify which had been present in the complex figure. The number of items correctly recognised (sum of true positive and false negative items) was used as the index score.

Language ability was evaluated using the Modified Boston Naming Test, in which the participants named 15 pictures of common objects. The total number of correctly named objects was taken as the index score for this test.

The cognitive tests and maximum scores are listed in Table 1.

Table 1. Cognitive battery

	Tests	Maximum score
General intelligence	Wechsler Adult Intelligence Scale – III	
	Digit Symbol-Coding	133
	Arithmetic	22
	Information	28
Executive function	Stroop	
	Reaction Time (seconds)	
	Colour Dots	---
	Chinese Characters	---

	Colour Words	---
	Number of Errors	
	Colour Dots	0
	Chinese Characters	0
	Colour Words	0
	Modified Verbal Fluency Test	
	Animals	---
	Fruits	---
	Vegetables	---
	Wisconsin Card Sorting Test	
	Number of Attempts Administered	---
Working memory	Digit Span	
	Forward	16
	Backward	14
	Total	20
Verbal memory	Wechsler Memory Scale – III Logical Memory	
	Logical Memory I	
	Total Immediate Recall	50
	Logical Memory II	
	Delayed Recall	50
	Recognition	30
	Percent Retention	---
	Wechsler Memory Scale – III Word List Memory	
	Word List I	
	First Recall	12
	Total Recall	48
	Learning slope	-/+12
	Word List II	
	Total Recall	12
	Total Recognition	24
	Percent Retention	---
Visual memory	Rey-Osterrieth Complex Figure	
	Copy	36
	Immediate Recall	36
	Delayed Recall	36
	Recognition Total Correct	24
Language	Modified Boston Naming Test	15

Radiological examination

All of the imaging procedures were carried out at the MRI centre located in the basement of the Prince of Wales Hospital's cancer centre. All of the participants provided a urine sample on the day of scan. All of the scans were performed with a 3T scanner (Philips Achieva 3.0T, X Series, Quasar Dual MRI System) and experienced neuroradiologists, who were blind to the participants' drug use status, assessed the MRIs.

Structural MRI

Conventional T1 weighted, T2 weighted, Fluid Attenuation Inversion Recovery (FLAIR) and T2* weighted gradient echo sequences were acquired for the morphological assessment of the participants to detect any ketamine-related insults to the brain.

Whole-brain volume acquisition was obtained using a T1-weighted FLASH (Fast low angle shot) sequence. The regions of interest were the frontal lobe and its subregions (prefrontal cortex, orbitofrontal cortex, dorsolateral prefrontal cortex and anterior cingulate), basal ganglia and hippocampus. The volumetry analysis of brain regions was performed using an automatic image analysis pipeline written in C++ programming language and based on the Insight Segmentation and Registration

Toolkit (http://www.itk.org). All of the brain region volumes were adjusted by the intracranial volume using analysis of covariance (ANCOVA).

MR Spectroscopy (MRS)

MRS data were acquired on the same machine, equipped with a standard eight-channel head coil. Head motion was restricted by comfortable padding placed around the participant's head. Standard scout MR images were acquired first, followed by axial T2-weighted MR images to guide the positioning of the MRS volumes of interest (VOI), measuring $3 \times 3 \times 3$ cm^3. The regions of interest were the bilateral anterior cingulate and right basal ganglia. The bilateral visual cortex was selected as a control region. We opted for a conventional MRS method rather than spectral editing methods as we were also interested in obtaining reliable NAA measures from the same VOI. The MRS data were acquired using the PRESS (point resolved spectroscopy) sequence with a repetition time of TR=2000 ms, an echo time of TE=40 ms and an average of 128 acquisitions. Second-order shimming was used to obtain optimal shimming in the selected VOI, with an automated water-suppression procedure. Metabolites were quantified using the Linear Combination of Model (LCModel), a fully automated and user-independent spectral evaluation tool, well suited to quantify complex signals such as glutamate. The advantage of the LCModel is that it uses information from the whole chemical shift range of a spectrum to fit the

amplitudes of the model spectra. The metabolites of interest were NAA, Cho, mI, Glx and creatinine.

Functional MRI (fMRI)

Blood-oxygen-level-dependent (BOLD) imaging was performed. With patients lying supine on the MRI scanning table, the head was held by padding and a chin strap to prevent motion. Structural and functional images were acquired using an eight-channel receive-only head coil in a transverse orientation from the same section of the brain. A whole brain anatomical data set was acquired with a T1-weighted sequence (TR/TE: 7.6/3.5 ms, field of view: 230 mm, 250 contiguous slices, 0.6 mm thickness, reconstruction matrix: 224 x 224) for co-registration of functional to anatomical images. The functional scans were then performed using echo shifting with a train of observation (PRESTO) sequence (TR: 28ms, TE: 12ms, flip angle 7, slab thickness: 125 mm, field of view: 230 mm, data matrix: 80 x 51 x 25). Sixty dynamic scans were acquired in each period with a nominal in-plane resolution of 2.8 x 2.8 mm and a temporal resolution of 2.7 sec/scan.

Functional connectivity analysis

Independent component analysis was conducted using the Informix algorithm in the Group ICA of the fMRI Toolbox (GIFT) software (Medical Image Analysis

Lab, University of New Mexico, Albuquerque, New Mexico; http://icatb.sourceforge.net/). The data obtained from each patient were decomposed into 30 spatially separated components using GIFT software. The number of independent components (ICs) was determined according to the minimum description length criteria (Li, Adali, & Calhoun, 2007) and the resulting averaged 30 ICs. The averaged IC was then used for independent component analysis (ICA) separation for each participant. Principal component analysis was used to reduce the number of dimensions in each data set. Then, a single ICA was performed on each participant's data, followed by back-reconstruction of single-subject time courses and spatial maps from the raw data. The time course for each IC corresponds to the waveform of a specific pattern of coherent brain activity, and the intensity of this pattern of activity across the voxels is expressed in the associated spatial map (Mantini, Perrucci, Del Gratta, Romani, & Corbetta, 2007). To display the voxels related to a particular IC, the intensity values in each map are converted to z values (Greicius, Srivastava, Reiss, & Menon, 2004). After ICA separation, a template of the default mode network (DMN) is used to select the best fitting component for each participant. The standard DMN template is based on the meta-analytic modeling provided by Angela R. Laird, Ph.D. (Research Imaging Institute, University of Texas Health Science Center, San Antonio, Texas) (Laird et al., 2009). Processing of the fMRI data was conducted with the Statistical Parametric Mapping (SPM8) software from the Wellcome Department of Cognitive Neurology in London, implemented in Matlab (Mathworks Inc., Sherborn MA, USA).

Statistical methods

Data analyses were performed using IBM SPSS 20.0. The continuous variables are described as mean ± SD and categorical variables are described as n (%). Descriptive statistics were computed for the participant characteristics, and the demographic and clinical data of the all ketamine-user and healthy control groups were compared using χ^2 and t tests. ANCOVA was used to analyse potential confounding influences, including age, sex and education. The fMRI data were analysed via SPM8. Finally, we also examined the correlations between the structural, metabolic and functional MRI parameters and cognitive task performance in chronic ketamine users. The significance level was set at 0.05.

In order to examine the differences between the primarily ketamine and poly-ketamine groups, the demographic and clinical data of the two ketamine-user groups and healthy control group were compared using χ^2 and analysis of variance (ANOVA). MRI and MRS data of all three groups were analysed by ANOVA. The significance level was set at 0.05. MANCOVA was used to analyse potential confounding influences, including age, sex and education.

Results

Demographics

The demographic characteristics of the study sample are summarised in Table 2a. One hundred and twenty four ketamine users and fifty-seven healthy controls were recruited for the study. Ketamine users were older than healthy control (25.8 ± 5.0 versus 23.9 ± 4.5, p = 0.015). There were significantly more male in ketamine users than that in healthy control (58.9% versus 42.1%, p = 0.036). No significant differences were found in terms of marital status or religious preference. However, the ketamine users had significantly lower levels of education (9.4 ± 1.9 versus 13.6 ± 2.4, p < 0.001) and monthly incomes (5386 ± 8590 versus 8755 ± 7555, p < 0.001) compared to the healthy controls, and fewer ketamine users were employed (48.4% versus 94.6%, p < 0.001). 44.4% of the ketamine users were recruited from non-residential community service centres (Table 2a).

Table 2a. Descriptive statistics of demographic characteristics between all ketamine users and healthy control groups.

	All Ketamine Users (N = 124)	Healthy Control (N = 57)	p
Age	25.8 ± 5.0	23.9 ± 4.5	0.015 [a]
Sex (male), n (%)	73 (58.9%)	24 (42.1%)	0.036 [b]
Education (year)	9.4 ± 1.9	13.6 ± 2.4	<0.001 [a]
Marital status (single)	114 (91.9%)	50 (90.9%)	0.778 [c]
Monthly income (HK$)	5386 ± 8590	8755 ± 7555	<0.001 [d]
Employed/homemaker/student	60 (48.4%)	53 (94.6%)	<0.001 [b]

Have religious preference	51 (41.1%)	19 (33.9%)	0.359 [b]
Resource of referral			
Non-residential	55 (44.4%)	57 (100.0%)	<0.001 [b]
Residential	69 (55.6%)	0 (0)	

[a] t test; [b] chi-square test; [c] Fisher`s exact test; [d] Mann-Whitney U test. α was set at 0.05 for all p values.

Amongst the ketamine users, 60 were primarily ketamine users and 64 were poly ketamine users. Age was significantly different between groups, with 26.0 ± 4.8 years for primarily ketamine users, 25.6 ± 5.2 years for poly ketamine users, and 23.9±4.5 years for healthy controls (p = 0.048). There was significantly more male in primarily ketamine users than healthy control (61.7% versus 42.1%, p = 0.001). Both ketamine users groups had significantly lower levels of education (primarily ketamine, 9.2 ± 2.2; poly ketamine, 9.6 ± 1.5), compared to the healthy controls (13.6 ± 2.4). Besides, both primarily and poly ketamine users had significantly lower monthly income than healthy controls (5410 ± 6184 versus 8754 ± 7555, p = 0.011; 5359 ± 10660 versus 8754 ± 7555, p < 0.001). Also, there were significantly fewer primarily (53.3%) and poly (43.8%) ketamine users employed than healthy control group (94.6%) (Table 2b).

Table 2b. Descriptive statistics of demographic characteristics among three groups

	Primarily Ketamine Users (N = 60)	Poly Ketamine Users (N = 64)	Healthy Control (N = 57)	p	p^1	p^2	p^3
Age	26.0 ± 4.8	25.6 ± 5.2	23.9 ± 4.5	0.048 [a]	0.063 [a]	0.160 [a]	1.000 [a]
Sex (male), n (%)	37 (61.7)	36 (56.3)	24 (42.1)	0.092 [b]	0.001 [b]	0.120 [b]	0.540 [b]
Education (year)	9.2 ± 2.2	9.6 ± 1.5	13.6 ± 2.4	<0.001 [a]	<0.001 [a]	<0.001 [a]	0.877 [a]
Marital status (single) , n (%)	57 (95.0)	57 (89.1)	50 (90.9)	0.478 [b]	0.477 [c]	0.739 [b]	0.326 [c]
Monthly income (HKD$)	5410 ± 6184	5359 ± 10660	8754 ± 7555	0.001 [d]	0.011 [e]	<0.001 [e]	0.379 [e]
Occupation							
Employed/homemaker/student, n (%)	32 (53.3)	28 (43.8)	53 (94.6)	<0.001 [b]	<0.001 [b]	<0.001 [b]	0.286 [b]
Resource of referral							
Non-residential	36 (60.0)	19 (29.7)	57 (100.0)	<0.001 [b]	<0.001 [b]	<0.001 [b]	0.001 [b]
Residential	24 (40.0)	45 (70.3)	0 (0)				

[a] ANOVA; [b] Chi-square test; [c] Fisher`s exact test; [d] Kruskal-Wallis test; [e] Mann-Whitney U test.

p^1: primarily ketamine users versus healthy control; p^2: poly ketaminer users versus health control; p^3: primarily ketamine users versus poly ketamine users. p^{1-3}: post hoc comparison with Bonferroni correction. α was set at 0.017 except age and education with α of 0.05.

HKD: Hong Kong dollar.

Pattern of ketamine use

According to the 124 ketamine users' self-reported drug use patterns, 87.7% were diagnosed with lifetime dependence and 46.7% with lifetime abuse. Current ketamine dependence was diagnosed in 56.2% of ketamine users, and 30.8% were classified as current ketamine abusers (Table 3a).

The mean Severity of Dependence Scale (SDS) score was 8.42. Ketamine users had a significantly higher percentage of alcohlo use (82.1% versus 60.7%, $p = 0.002$), longer duration of alcohol use (7.7 ± 6.0 versus 3.6 ± 4.0, $p < 0.001$) and younger age of first alcohol use (15.8 ± 3.43 versus $17.7 \pm 2.$, $p = 0.003$) than the healthy control group. In addition, ketamine users had significantly worse scores for medical, employment, drugs, legasl, family and psychiatric conditions as measured by the Addiction Severity Index (ASI) – Lite Version (Table 3a).

Table 3a. Pattern of substances use between ketamine use group and healthy control

	All Ketamine Users (N = 124)	Healthy Control (N = 57)	p
Age of first ketamine use	16.5 ± 3.1	-	-
Duration of ketamine use (months)	88.0 ± 46.8	-	-
Days of ketamine use in the past one year (mean / median)	243.6 ± 107.0 / 267.5	-	-
Days of ketamine use in previous month	5.0 ± 9.6	-	-
Abstinent from ketamine more than 1 month	66 (54.1)	-	-
Lifetime diagnosis of ketamine dependence, n (%)	107 (87.7)	-	-

	All Ketamine Users (N = 124)	Healthy Control (N = 57)	p
Lifetime diagnosis of ketamine abuse, n (%)	7 (46.7)	-	-
Current diagnosis of ketamine dependence, n (%)	50 (56.2)	-	-
Current diagnosis of ketamine abuse, n (%)	12 (30.8)	-	-
SDS	8.4 ± 3.2	-	-
Any use of alcohol	101 (82.1)	34 (60.7)	0.002 [a]
Age of first alcohol use	15.8 ± 3.4	17.7 ± 2.4	0.003 [b]
Duration of alcohol use (years)	7.7 ± 6.0	3.6 ± 4.0	<0.001 [c]
Days of alcohol use in previous month	3.2 ± 6.9	0.9 ± 1.4	0.681 [c]
ASI Composite Score – Medical	0.30 ± 0.36	0.17 ± 0.23	0.026 [c]
ASI Composite Score – Employment	0.77 ± 0.26	0.70 ± 0.22	0.003 [c]
ASI Composite Score – Alcohol	0.06 ± 0.11	0.01 ± 0.02	0.175 [c]
ASI Composite Score – Drugs	0.11 ± 0.08	0	<0.001 [c]
ASI Composite Score – Legal	0.03 ± 0.10	0	0.005 [c]
ASI Composite Score - Family	0.14 ± 0.21	0.02 ± 0.06	<0.001 [c]
ASI Composite Score - Psychiatry	0.20 ± 0.16	0.03 ± 0.08	<0.001 [c]

[a] chi square test; [b] t test; [c] Mann-Whitney U test. α was set at 0.05 for all p values.
ASI=The Addiction Severity Index-Lite Version; SDS=Severity of Dependence Scale.

There was a trend that the poly ketamine users had an earlier age of first exposure to ketamine, longer duration of ketamine use but lower use of ketamine in preivous one month than the primarily ketamine users (Table 3b). The lifetime use of alcohol was more common in poly ketamine group (92.1%) than the primarily ketamine (73.3%) and healthy control group (60.7%). However, both primarily and poly ketamine users had significant longer duration of alcohol use than the healthy controls (3.1 ± 6.8, 3.3 ± 7.0 versus 0.9 ± 1.4, $p < 0.001$). Both the primarily (6.18 ±

5.12 years) and poly (9.23 ± 6.43 years) ketamine groups had longer duration of alcohol use than healthy controls (3.61 ± 3.99 years). Besides, both ketamine users groups had significantly worse scores for drugs, family and psychiatric conditions than healthy controls, as measured by the Addiction Severity Index (ASI) – Lite Version. Primarily ketamine users also had significantly worse medical scores than healthy control, while poly ketamine users had significantly worse employment and legal scores than healthy control (Table 3b).

Table 3b. Pattern of ketamine and alcohol use among three study groups.

	Primarily Ketamine Users (N = 60)	Poly Ketamine Users (N = 64)	Healthy Control (N = 57)	P	p^1	p^2	p^3
Age of first ketamine use	17.0 ± 3.1	16.0 ± 3.0	-	0.050 [a]	-	-	-
Duration of ketamine use (months)	79.9 ± 44.3	95.7 ± 48.2	-	0.063 [a]	-	-	-
Days of ketamine use in the past one year	245.3 ± 112.0	242.0 ± 104.0	-	0.864 [a]	-	-	-
Days of ketamine use in previous month (days)	9.3 ± 11.5	5.6 ± 10.0	-	0.059 [a]	-	-	-
Abstinent from ketamine more than 1 month	28 (46.7)	38 (61.3)	-	0.105 [b]	-	-	-
Lifetime diagnosis of ketamine dependence, n (%)	50 (84.7)	57 (90.5)	-	0.335 [b]	-	-	-
Lifetime diagnosis of ketamine abuse, n (%)	4 (44.4)	3 (50.0)	-	1.000 [b]	-	-	-
Current diagnosis of ketamine dependence, n (%)	23 (56.1)	27 (56.3)	-	0.988 [b]	-	-	-
Current diagnosis of ketamine abuse, n (%)	9 (50.0)	3 (14.3)	-	0.016 [b]	-	-	-
SDS	8.29 ± 3.10	8.54 ± 3.29	-	0.666 [a]	-	-	-
Any use of alcohol	44 (73.3)	58 (92.1)	34 (60.7)	<0.001 [b]	0.148 [b]	<0.001 [b]	0.013 [b]

Age of first alcohol use	16.6 ± 3.3	15.1 ± 3.4	17.7 ± 2.4	0.001 [c]	0.381 [c]	0.001 [c]	0.065 [c]
Duration of alcohol use (years)	6.2 ± 5.1	9.2 ± 6.4	3.6 ± 4.0	<0.001 [d]	0.005 [e]	<0.001 [e]	0.008 [e]
Days of alcohol use in previous month	3.1 ± 6.8	3.3 ± 7.0	0.9 ± 1.4	0.914 [d]	0.688 [e]	0.753 [e]	0.914 [e]
ASI Composite Score – Medical	0.38 ± 0.39	0.23 ± 0.32	0.17 ± 0.23	0.011 [d]	0.004 [e]	0.302 [e]	0.047 [e]
ASI Composite Score– Employment	0.72 ± 0.28	0.82 ± 0.24	0.70 ± 0.22	0.001 [d]	0.165 [e]	<0.001 [e]	0.020 [e]
ASI Composite Score – Alcohol	0.05 ± 0.09	0.07 ± 0.13	0.01 ± 0.02	0.308 [d]	0.391 [e]	0.133 [e]	0.465 [e]
ASI Composite Score – Drugs	0.11 ± 0.08	0.10 ± 0.08	0	<0.001 [d]	<0.001 [e]	<0.001 [e]	0.432 [e]
ASI Composite Score – Legal	0.03 ± 0.10	0.04 ± 0.10	0	0.005 [d]	0.028 [e]	0.001 [e]	0.182 [e]
ASI Composite Score - Family	0.15 ± 0.22	0.13 ± 0.19	0.02 ± 0.06	<0.001 [d]	<0.001 [e]	<0.001 [e]	0.565 [e]
ASI Composite Score - Psychiatry	0.19 ± 0.14	0.21 ± 0.18	0.03 ± 0.08	<0.001 [d]	<0.001 [e]	<0.001 [e]	0.407 [e]

ASI:The Addiction Severity Index-Lite Version; SDS: Severity of Dependence Scale.

[a] t test, $\alpha = 0.05$; [b]Chi-Square test, $\alpha = 0.05$; [c] ANOVA , $\alpha = 0.05$; [d] Kruskal-Wallis test, $\alpha = 0.05$; [e] Mann-Whitney U test.

p^1: primarily ketamine users versus healthy control; p^2: poly ketamine users versus healthy control; p^3: primarily ketamine users versus poly ketamine users. p^{1-3}: post hoc comparison with Bonferroni correction. α was set at 0.017 except "age of first alcohol use" with α of 0.05.

Patterns of other drugs use

The patterns of other drug use for ketamine users were shown in Table 4. According to the 124 ketamine users' self-reported drug use patterns, the lifetime use of cocaine, cannabis, hypnotics, ecstasy, methamphetamine and cough medicine, were 80.6%, 65.9%, 58.5%, 57.9%, 46.8% and 9.0%, respectively. The mean duration of other drug use ranged from 26.8 to 48.0 months, and the mean number of days of drug use was 0-0.8 per month or 0-46.4 per year. The most heavily misused drug in last year other than ketamine was cocaine (46.4 days), followed by cough medicine (20.9 days), methamphetamine (15.6 days), hypnotics (6.6 days) and cannabis (2.2 days) (Table 4).

Lifetime diagnoses of cocaine dependence were more common in poly ketamine group than in primarily ketamine group (47.6% versus 18.6%, $p = 0.001$). Poly ketamine users also had significant more days of cocaine consumption in the past year (80.8 ± 88.9 versus 3.6 ± 6.6, $p < 0.001$) and past month (2.1 ± 5.6 versus 0.2 ± 0.6, $p = 0.043$) as well as a longer duration of cocaine use (48.9 ± 35.2 versus 25.0 ± 23.7, $p < 0.001$) than primarily ketamine users.

Table 4. Patterns of drug use in ketamine users

	All Ketamine Users (N = 124)	Primarily Ketamine Users (N = 60)	Poly Ketamine Users (N = 64)	p
Any use of Cocaine (Yes)	100 (80.6)	45 (75.0)	55 (85.9)	<0.001 [a]
Age of first use	19.4 ± 3.7	20.0 ± 4.1	19.0 ± 3.3	0.157 [b]

	All Ketamine Users (N = 124)	Primarily Ketamine Users (N = 60)	Poly Ketamine Users (N = 64)	p
Duration of use (months)	39.3 ± 33.1	25.0 ± 23.7	48.9 ± 35.2	0.001[d]
Days of use in past year	46.4 ± 76.5	3.6 ± 6.6	80.8 ± 88.9	<0.001[d]
Days of use in past month	0.8 ± 5.3	0.2 ± 0.6	2.1 ± 5.6	0.043 [d]
Lifetime diagnosis of dependence	41 (33.6)	11 (18.6)	30 (47.6)	0.001 [a]
Lifetime diagnosis of abuse	15 (12.3)	9 (15.3)	6 (9.5)	0.335 [a]
Current diagnosis of dependence	6 (4.9)	4 (6.8)	2 (3.2)	0.428 [c]
Current diagnosis of abuse	6 (4.9)	4 (6.8)	2 (3.2)	0.428 [c]
Any use of Cannabis (Yes)	81 (65.9)	37 (61.7)	44 (69.8)	0.339 [a]
Age of first use	16.8 ± 3.2	17.2 ± 3.2	16.5 ± 3.1	0.402 [b]
Duration of use (months)	48.0 ± 58.3	38.3 ± 37.6	53.1 ± 66.9	0.833 [d]
Days of use in past year	2.2 ± 9.6	0.2 ± 0.5	9.8 ± 25.7	0.961 [d]
Days of use in past month	0.1 ± 0.8	0.1 ± 0.4	0.2 ± 1.1	0.974 [d]
Lifetime diagnosis of dependence	6 (4.9)	1 (1.7)	5 (7.9)	0.209 [c]
Lifetime diagnosis of abuse	8 (6.6)	3 (5.1)	5 (7.9)	0.718 [c]
Current diagnosis of dependence	2 (1.6)	0 (0.0)	2 (3.2)	0.496 [c]
Current diagnosis of abuse	1 (0.8)	1 (1.7)	0 (0.0)	0.484 [c]
Any use of Hypnotics (Yes)	72 (58.5)	32 (53.3)	40 (63.5)	0.253 [a]
Age of first use	18.2 ± 3.8	18.5 ± 3.4	17.9 ± 4.1	0.489 [b]
Duration of use (months)	45.8 ± 59.5	31.0 ± 40.7	58.7 ± 70.3	0.230 [d]
Days of use in past year	6.6 ± 44.1	2.0 ± 6.6	10.8 ± 60.9	0.224 [d]
Days of use in past month	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-
Lifetime diagnosis of dependence	14 (11.5)	5 (8.5)	9 (14.3)	0.314 [a]
Lifetime diagnosis of abuse	12 (9.8)	5 (8.5)	7 (11.1)	0.625 [a]
Current diagnosis of dependence	2 (1.6)	1 (1.7)	1 (1.6)	1.000 [c]
Current diagnosis of abuse	1 (0.8)	1 (1.7)	0 (0.0)	0.484 [c]
Any use of Ecstasy (Yes)	70 (57.9)	35 (60.3)	35 (55.6)	0.284 [a]
Age of first use	16.6 ± 2.9	16.6 ± 2.9	16.5 ± 3.0	0.894 [b]
Duration of use (months)	26.8 ± 29.9	26.9 ± 28.1	26.7 ± 32.2	0.806 [d]
Days of use in past year	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-
Days of use in past month	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-

	All Ketamine Users (N = 124)	Primarily Ketamine Users (N = 60)	Poly Ketamine Users (N = 64)	p
Lifetime diagnosis of dependence	4 (3.3)	2 (3.4)	2 (3.2)	1.000 [c]
Lifetime diagnosis of abuse	10 (8.2)	5 (8.5)	5 (7.9)	0.914 [c]
Current diagnosis of dependence	1 (0.8)	1 (1.7)	0 (0.0)	0.484 [c]
Current diagnosis of abuse	1 (0.8)	1 (1.7)	0 (0.0)	0.484 [c]
Any use of Methamphetamine (Yes)	58 (46.8)	27(45.0)	31 (48.4)	0.701 [a]
Age of first use	19.4 ± 5.1	18.8 ± 4.8	19.8 ± 5.3	0.472 [b]
Duration of use (months)	34.6 ± 48.9	31.9 ± 43.8	36.6 ± 53.6	0.506 [d]
Days of use in past year	15.6 ± 42.9	4.9 ± 15.6	24.6 ± 55.3	0.456 [d]
Days of use in past month	0.2 ± 1.4	0.1 ± 0.4	0.3 ± 1.9	0.948 [d]
Lifetime diagnosis of dependence	12 (9.8)	5 (8.5)	7 (11.1)	0.625 [a]
Lifetime diagnosis of abuse	9 (7.4)	4 (6.8)	5 (7.9)	1.000 [c]
Current diagnosis of dependence	3 (2.5)	0 (0.0)	3 (4.8)	0.245 [c]
Current diagnosis of abuse	3 (2.5)	2 (3.4)	1 (1.6)	0.610 [c]
Any use of Cough medicine (Yes)	11 (9.0)	6 (10.3)	5 (7.8)	0.626 [a]
Age of first use	16.2 ± 2.7	16.8 ± 3.3	15.3 ± 1.3	0.387 [b]
Duration of use (months)	40.7 ± 60.0	4.0 ± 5.4	114.0 ± 42.4	0.133 [d]
Days of use in past year	20.9 ± 69.7	11.8 ± 33.1	25.9 ± 83.8	0.792 [d]
Days of use in past month	0.2 ± 1.4	0.2 ± 0.9	0.2 ± 1.8	0.535 [d]
Lifetime diagnosis of dependence	3 (2.5)	1 (1.7)	2 (3.2)	1.000 [c]
Lifetime diagnosis of abuse	0 (0.0)	0 (0.0)	0 (0.0)	-
Current diagnosis of dependence	3 (2.5)	1 (1.7)	2 (3.2)	1.000 [c]
Current diagnosis of abuse	0 (0.0)	0 (0.0)	0 (0.0)	-

[a] Chi-Square test; [b] t test; [c] Fisher`s exact test; [d] Mann-Whitney test. α was set at 0.05 for all p values.

Comorbid psychiatric problems

The ketamine users had significantly higher Beck Depression Inventory (BDI) (13.7 ± 10.3 versus 3.8 ± 4.3, $p < 0.001$) and Anxiety subscale of the Hospital Anxiety Depression Scale (HADSA) (6.1 ± 4.2 versus 2.78 ± 2.38, $p < 0.001$) scores than healthy controls. The prevalence of mood disorders and anxiety disorders in ketamine users were 25.0% and 15.3%, respectively. Depressive disorders were the most commonly diagnosed mood disorders, while generalised anxiety disorder and panic disorder were the most common anxiety disorder in ketamine users (Table 5a).

Table 5a. Psychiatric problems across primarily ketamine users and healthy control groups.

	All Ketamine Users (N = 124)	Healthy Control (N = 57)	p
BDI score	13.7 ± 10.3	3.8 ± 4.3	<0.001 [a]
HADSA score	6.1 ± 4.2	2.8 ± 2.4	<0.001 [a]
Previous visit in a psychiatric outpatient setting	29 (23.4)	0	<0.001 [b]
Previous visit in a psychiatric inpatient setting	11 (8.9)	0	0.018 [c]
Psychiatric screening with SCID			
Current psychiatric diagnosis, n (%)	30 (24.2)	0	<0.001 [b]
Current or past mood disorders, n (%)	31 (25.0)	0	<0.001 [b]
Current depressive disorders, n (%)	7 (5.6)	0	0.100 [c]
Previous depressive disorders, n (%)	18 (14.5)	0	0.002 [b]
Current dysthymia disorders, n (%)	15 (12.1)	0	0.003 [c]
Current anxiety disorders, n (%)	19 (15.3)	0	0.002 [b]
Current generalised anxiety disorder, n (%)	9 (7.3)	0	0.059 [c]
Current panic disorder, n (%)	9 (7.3)	0	0.059 [c]
Current social phobia disorder, n (%)	6(4.8)	0	0.179 [c]

BDI=Beck Depression Inventory; HADSA=Anxiety subscale of the Hospital Anxiety Depression Scale; SCID=Structured Clinical Interview for DSM Disorders.
[a] Mann-Whitney test; [b] chi-square test; [c] Fisher`s exact test. α was set at 0.05 for all p values.

Subjects in the primarily and poly ketamine users groups had significantly higher BDI (12.5 ± 9.8 versus 3.75 ± 4.29, $p < 0.001$; 14.7 ± 10.7 versus 3.75 ± 4.29, $p < 0.001$) and HADSA (5.3 ± 3.8 versus 2.78 ± 2.38, $p < 0.001$; 6.9 ± 4.3 versus 2.78 ± 2.38, $p < 0.001$) scores than healthy controls. Also, both primarily and poly ketamine users had significantly more previous visits in a psychiatric outpatient setting than healthy controls (25% and 21.9% versus 0, $p < 0.001$). Lifetime psychiatric treatment and ccurrent psychiatric diagnoses were significantly more common in the primarily ketamine users group and poly ketamine users group than healthy controls group. The prevalence of current mood disorders were 15.0% in primarily ketamine users group and 6.3% in poly ketamine users group, and that of current anxiety disorders was 6.7% and 7.8% in primarily and poly ketamine users groups, respectively. No significant differences were found in terms of previous visits in psychiatric outpatient & inpatient settings and the BDI & HADSA scores between the two ketamine users groups (Table 5b).

Table 5b. Psychiatric problems among the three groups.

Variables	Primarily Ketamine users (N = 60)	Poly Ketamine Users (N = 64)	Healthy Control (N = 57)	p	p^1	p^2	p^3
BDI score	12.5 ± 9.8	14.7 ± 10.7	3.8 ± 4.3	<0.001 [a]	<0.001 [b]	<0.001 [b]	0.271 [b]
HADSA score	5.3 ± 3.8	6.9 ± 4.3	2.8 ± 2.4	<0.001 [a]	<0.001 [b]	<0.001 [b]	0.022 [b]
Previous visit in a psychiatric outpatient setting	15 (25)	14 (21.9)	0	<0.001 [a]	<0.001 [b]	<0.001 [b]	0.445 [b]
Previous visit in a psychiatric inpatient setting	5 (8.3)	6 (9.5)	0	0.069 [a]	0.028 [b]	0.018 [b]	0.850 [b]
Psychiatric screening with SCID							
Life-time history of psychiatric treatment, n (%)	16 (27.1)	8 (18.2)	0	<0.001 [c]	<0.001 [c]	0.001 [d]	0.289 [c]
Current psychiatric diagnosis, n (%)	13 (21.7)	26 (40.6)	0	<0.001 [d]	<0.001 [d]	<0.001 [d]	0.023 [c]
Current or past mood disorder, n (%)	9 (15.0)	4 (6.3)	0	0.002 [c]	0.003 [d]	0.121 [d]	0.112[b]
Current depressive disorders, n (%)	2 (3.3)	5 (7.8)	0	0.081[c]	0.496 [d]	0.059 [d]	0.441[c]
Past depressive disorders, n (%)	10 (16.7)	8 (12.5)	0	0.007 [c]	0.001 [d]	0.007 [d]	0.510 [c]
Dysthymia disorders, n (%)	8 (13.3)	7 (10.9)	0	0.021 [c]	0.006 [d]	0.014 [d]	0.683 [c]
Current anxiety disorders, n (%)	4 (6.7)	4 (6.3)	0	0.044 [c]	0.119 [d]	0.121 [d]	1.000 [d]
Current generalized anxiety disorder, n (%)	2 (3.3)	7 (10.9)	0	0.007 [c]	0.496 [c]	0.014 [d]	0.166 [d]
Current panic disorder, n (%)	4 (6.7)	5 (7.8)	0	0.029 [c]	0.119 [c]	0.059 [d]	1.000 [d]
Current social phobia, n (%)	0	6 (9.4)	0	0.002 [c]	-	0.029 [d]	0.028 [d]

BDI: Beck Depression Inventory; HADSA: The anxiety subscale of the Hospital Anxiety Depression scale; SCID=Structured Clinical Interview for DSM Disorders. [a] Kruskal-Wallis test; [b] Mann-Whitney U test. [c] Chi square test; [d] Fisher's Exact test.
p^1: primarily ketamine users versus healthy control; p^2: poly ketamine users versus healthy control; p^3: primarily ketamine users versus poly ketamine users. α was set at 0.05 for p and 0.017 for p^{1-3}.

Cognitive functioning

There were differences between the ketamine users group and healthy control group in general intelligence (Wechsler Adult Intelligence scale, 'WAIS' III Digit Symbol-Coding, arithmetic and information), attention (digit span-backward, digit span-total), verbal memory (WAIS III logical memory and word list), visual memory (Rey-Osterrieth Complex Figure, ROCF), executive functioning (Wisconsin Card Sorting Test 'WCST', Stroop and Verbal Fluency), and language scores.

After adjusting for age, sex, education and BDI score, ketamine users still had significantly lower scores or worse performance than healthy controls on WAIS III arithmetic and information; logical memory for immediate recall, delayed recall and recognition; ROCF for immediate recall and delayed recall; total errors of Stroop test and language scores (Table 6a).

Table 6a. Performance on cognitive tests for all ketamine users and healthy control.

	All Ketamine Users (N = 124)	Healthy Control (N = 57)	p^a	p^b
WAIS III Digit Symbol Coding	83.1 ± 16.4	96.1 ± 11.3	<0.001	0.797
WAIS III Arithmetic	12.95 ± 3.6	17.6 ± 2.6	<0.001	<0.001
WAIS III Information	11.4 ± 4.5	17.9 ± 3.9	<0.001	<0.001
WAIS III Digit Span (Forward)	15.3 ± 1.3	15.6 ± 1.0	0.079	0.522
WAIS III Digit Span (Backward)	8.4 ± 3.1	10.6 ± 3.0	<0.001	0.138
WAIS III Digit Span total	23.6 ± 3.7	26.1 ± 3.5	<0.001	0.145
WMS III Logical Memory: immediate recall	18.6 ± 8.0	29.0 ± 7.1	<0.001	0.001
WMS III Logical Memory: delayed recall	15.8 ± 8.4	26.0 ± 7.1	<0.001	0.001

	All Ketamine Users (N = 124)	Healthy Control (N = 57)	p^a	p^b
WMS III Logical Memory: recognition	21.4 ± 4.3	25.8 ± 2.3	<0.001	<0.001
WMS III Logical Memory: percent retention	76.5 ± 23.8	87.3 ± 13.5	<0.001	0.100
WMS III word list: immediate recall	29.7 ± 6.3	35.5 ± 5.4	<0.001	0.209
WMS III word list: delayed recall	7.4 ± 2.6	9.2 ± 2.4	<0.001	0.196
WMS III word list: recognition	22.9 ± 1.5	23.7 ± 0.7	<0.001	0.309
WMS III word list: percent retention	78.3 ± 22.2	88.9 ± 16.8	0.001	0.306
ROCF: copy	33.0 ± 3.0	34.3 ± 2.4	0.002	0.863
ROCF: immediate recall	20.1 ± 7.1	25.4 ± 6.7	<0.001	0.006
ROCF: delayed recall	20.6 ± 6.5	25.4 ± 6.9	<0.001	0.008
ROCF: recognition total	20.0 ± 2.5	21.2 ± 1.6	<0.001	0.102
WCST: total attempts	98.0 ± 21.9	85.1 ± 15.9	<0.001	0.100
WCST: categories completed	5.4 ± 1.3	5.9 ± 0.4	<0.001	0.691
WCST: preservative errors	13.4 ± 10.4	8.2 ± 5.3	<0.001	0.431
Stroop Test: interference (seconds)	10.6 ± 6.4	8.3 ± 5.7	0.027	0.179
Stroop Test: total reaction time (seconds)	52.2 ± 12.5	46.6 ± 10.1	0.002	0.842
Stroop Test: total errors	2.2 ± 2.4	1.1 ± 1.7	0.003	0.017
Verbal Fluency: total correct responses	42.4 ± 7.8	48.2 ± 8.4	<0.001	0.218
Language	14.5 ± 1.2	14.9 ± 0.3	<0.001	0.025

ROCF=the Rey-Osterrieth Complex Figure; WAIS III= Wechsler Adult Intelligence scale - Third Edition; WCST: Wisconsin Card Sorting Test; WMS III= Wechsler Memory Scale - Third Edition.

p^a: t-test; p^b: ANCOVA, adjusted for age, sex, level of education and BDI score; α was set at 0.05 for all p values.

Both the primarily and poly ketamine users had significantly lower scores than healthy controls on WAIS III arithmetic / information, WAIS III Logical Memory immediate recall / delayed recall / recognition. In addition, the primarily ketamine users had significantly lower scores than healthy controls ($p = 0.031$) and poly ketamine users ($p = 0.025$) on WAIS III Logical Memory percent retention. The poly ketamine users had higher scores than healthy controls on Stroop Test total errors ($p=0.019$); lower scores than primarily ketamine users on ROCF copy ($p = 0.032$) and lower scores than healthy controls on ROCF immediate ($p = 0.006$) and delayed ($p = 0.006$) recall (Table 6b).

Table 6b Performance on cognitive tests for the three groups.

	Primarily Ketamine users (N = 60)	Poly Ketamine users (N = 64)	Healthy Control (N = 57)	P^a	P^b	p^1	p^2	p^3
WAIS III Digit Symbol Coding	84.1 ± 17.6	82.2 ± 15.2	96.1 ± 11.3	<0.001	0.572	-	-	-
WAIS III Arithmetic	12.9 ± 3.4	12.8 ± 3.9	17.6 ± 2.6	<0.001	0.001	0.004	0.001	1.000
WAIS III Information	11.5 ± 4.7	11.2 ± 4.4	17.9 ± 3.9	<0.001	<0.001	<0.001	<0.001	1.000
WAIS III Digit Span (Forward)	15.4 ± 1.1	15.1 ± 1.4	15.6 ± 1.0	0.122	0.223	-	-	-
WAIS III Digit Span (Backward)	8.5 ± 3.1	8.2 ± 3.1	10.6 ± 3.0	<0.001	0.270	-	-	-
WAIS III Digit Span total	23.9 ± 3.6	23.4 ± 3.8	26.1 ± 3.5	<0.001	0.194	-	-	-
WMS III Logical Memory: immediate recall	17.7 ± 9.0	19.4 ± 7.0	29.0 ± 7.1	<0.001	0.002	0.001	0.009	0.885
WMS III Logical Memory: delayed recall	14.1 ± 9.0	17.3 ± 7.5	26.0 ± 7.1	<0.001	0.001	<0.001	0.028	0.139
WMS III Logical Memory: recognition	20.8 ± 4.8	21.9 ± 3.7	25.8 ± 2.3	<0.001	0.001	<0.001	0.007	0.424
WMS III Logical Memory: percent retention	71.2 ± 27.4	81.5 ± 18.7	87.3 ± 13.5	<0.001	0.008	0.031	1.000	0.025
WMS III word list: immediate recall	29.3 ± 6.8	30.1 ± 6.0	35.5 ± 5.4	<0.001	0.104	-	-	-
WMS III word list: delayed	7.2 ± 2.5	7.6 ± 2.7	9.2 ± 2.3	<0.001	0.226	-	-	-

	Primarily Ketamine users (N = 60)	Poly Ketamine users (N = 64)	Healthy Control (N = 57)	P^a	P^b	p^1	p^2	p^3
recall								
WMS III word list: recognition	22.8 ± 1.5	22.9 ± 1.4	23.7 ± 0.7	0.001	0.405	-	-	-
WMS III word list: percent retention	74.3 ± 20.2	82.1 ± 23.5	88.9 ± 16.8	0.001	0.056	-	-	-
ROCF: copy	33.6 ± 2.8	32.3 ± 3.0	34.3 ± 2.4	0.001	0.037	1.000	1.000	0.032
ROCF: immediate recall	21.3 ± 6.9	19.1 ± 7.2	25.4 ± 6.7	<0.001	0.006	0.160	0.006	0.327
ROCF: delayed recall	21.73 ± 6.0	19.5 ± 6.7	25.4 ± 6.9	<0.001	0.006	0.233	0.006	0.213
ROCF: Recognition Total	20.1 ± 2.6	19.8 ± 2.4	21.2 ± 1.5	0.003	0.234	-	-	-
WCST: total attempts	97.3 ± 21.6	98.7 ± 22.3	85.1 ± 15.9	0.001	0.220	-	-	-
WCST: category completed	5.5 ± 1.2	5.4 ± 1.4	5.9 ± 0.4	0.013	0.725	-	-	-
WCST: preservative errors	14.3 ± 11.8	12.5 ± 8.9	8.2 ± 5.3	0.002	0.451	-	-	-
Stroop Test: interference (seconds)	10.6 ± 7.1	10.6 ± 5.8	8.3 ± 5.7	0.088	0.404	-	-	-
Stroop Test: total reaction time (seconds)	52.7 ± 12.9	51.7 ± 12.2	46.6 ± 10.1	0.013	0.881	-	-	-
Stroop Test: total errors	1.9 ± 2.3	2.4 ± 2.5	1.1 ± 1.7	0.004	0.019	0.303	0.019	0.392
Verbal Fluency: total correct responses	40.9 ± 7.2	43.9 ± 8.0	48.2 ± 8.4	<0.001	0.075	-	-	-
Language	14.6 ± 0.9	14.5 ± 1.4	14.9 ± 0.3	0.032	0.069	-	-	-

ROCF: the Rey-Osterrieth Complex Figure; WAIS III: Wechsler Adult Intelligence scale - Third Edition; WCST: Wisconsin Card Sorting test.
p^a: ANOVA; $p^{b,1-3}$: ANCOVA, adjusted for age, sex, level of education, and BDI total score with Bonferroni correction method.

p^1: primarily ketamine users versus healthy control; p^2: poly ketamine users versus healthy control; p^3: primarily ketamine users versus poly ketamine users. α was set at 0.05 for all p values.

Structural MRI

In terms of grey matter volumes, the ketamine group had decreased volumes in the right orbitofrontal cortex (OFC) (10.42 ± 1.60 versus 11.46 ± 1.58, p = 0.005), right medial prefrontal cortex (MPFC) (9.26 ± 1.33 versus 10.20 ± 1.35, p = 0.001), left globus pallidus (1.24 ± 0.23 versus 1.40 ± 0.24, p = 0.002), left hippocampus (4.48 ± 0.44 versus 4.78 ± 0.39, p < 0.001), and right nucleus accumbens (0.36 ± 0.08 vs 0.41 ± 0.09, p = 0.019). On the other hand, the ketamine group had increased volumes in left caudate (2.70 ± 0.47 versus 2.56 ± 0.44, p = 0.029) and left thalamus (9.19 ± 0.95 vs 8.94 ± 0.97, p = 0.045) (Table 7a). In terms of white matter volumes, the ketamine group had a lower periventricular white matter volume in the right hemisphere (18.89 ± 4.35 versus 20.8 ± 4.89, p = 0.024) (Table 7a).

Table 7a. Grey matter and white matter volumes across groups.

	All Ketamine Users (N = 85)	Healthy Control (N = 46)	p^a	p^b
Total white matter	591.38 ± 99.13	603.76 ± 103.01	0.502	0.612
Total grey matter	551.17 ± 79.81	586.76 ± 71.08	0.013	0.094
Frontal lobe (left)	71.20 ± 11.76	73.64 ± 10.04	0.236	0.944
Frontal lobe (right)	70.73 ± 11.69	75.19 ± 10.95	0.035	0.300
PFC (left)	48.74 ± 7.70	49.89 ± 7.02	0.401	0.563
PFC (right)	49.05 ± 8.17	51.92 ± 7.44	0.050	0.405
OFC (left)	10.94 ± 1.53	11.61 ± 1.58	0.020	0.137
OFC (right)	10.42 ± 1.60	11.46 ± 1.58	0.001	0.005
DLPFC (left)	15.55 ± 2.96	15.55 ± 2.66	0.996	0.223
DLPFC (right)	15.51 ± 3.06	16.21 ± 3.03	0.212	0.943
VLPFC (left)	9.24 ± 1.76	9.12 ± 1.73	0.703	0.083
VLPFC (right)	9.31 ± 1.93	9.51 ± 1.73	0.554	0.837

	All Ketamine Users (N = 85)	Healthy Control (N = 46)	p^a	p^b
MPFC (left)	9.09 ± 1.39	9.73 ± 1.35	0.012	0.110
MPFC (right)	9.26 ± 1.33	10.20 ± 1.35	<0.001	0.001
ACC (left)	4.17 ± 0.96	4.55 ± 0.99	0.035	0.144
ACC (right)	4.30 ± 0.82	4.45 ± 0.89	0.346	0.816
Temporal lobe (left)	44.27 ± 8.30	46.67 ± 8.29	0.115	0.416
Temporal lobe (right)	43.46 ± 7.35	46.66 ± 7.89	0.022	0.177
Parietal lobe (left)	44.82 ± 8.02	48.35 ± 7.72	0.016	0.121
Parietal lobe (right)	45.35 ± 7.88	48.17 ± 7.57	0.050	0.240
Occipital lobe (left)	17.36 ± 3.69	17.88 ± 3.52	0.437	0.885
Occipital lobe (right)	16.54 ± 4.05	17.32 ± 4.00	0.291	0.631
Basal ganglia (left)	8.32 ± 1.10	8.41 ± 1.03	0.643	0.490
Basal ganglia (right)	7.75 ± 1.03	8.01 ± 1.08	0.173	0.544
Caudate (left)	2.70 ± 0.47	2.56 ± 0.44	0.114	0.029
Caudate (right)	2.67 ± 0.55	2.74 ± 0.49	0.494	0.926
Putamen (left)	4.39 ± 0.72	4.45 ± 0.63	0.619	0.562
Putamen (right)	3.93 ± 0.53	4.10 ± 0.56	0.079	0.209
Globus pallidus (left)	1.24 ± 0.23	1.40 ± 0.24	<0.001	0.002
Globus pallidus (right)	1.15 ± 0.18	1.17 ± 0.20	0.542	0.894
Nucleus accumbens (left)	0.42 ± 0.09	0.45 ± 0.10	0.092	0.278
Nucleus accumbens (right)	0.36 ± 0.08	0.41 ± 0.09	0.004	0.019
Hippocampus (left)	4.48 ± 0.44	4.78 ± 0.39	<0.001	<0.001
Hippocampus (right)	4.38 ± 1.10	4.43 ± 0.5	0.755	0.963
Thalamus (left)	9.19 ± 0.95	8.94 ± 0.97	0.147	0.045
Thalamus (right)	7.71 ± 0.67	7.69 ± 0.67	0.850	0.251
Periventricular white matter (left)	19.66 ± 4.90	21.06 ± 4.63	0.113	0.075
Periventricular white matter (right)	18.89 ± 4.35	20.80 ± 4.89	0.024	0.024
Deep white matter (left)	239.54 ± 28.51	238.47 ± 30.90	0.843	0.232
Deep white matter (right)	249.67 ± 55.92	242.18 ± 31.16	0.403	0.215

Note: all data are presented in cm^3 (mean±standard deviation).
ACC=anterior cingulate cortex; DLPFC=dorsolateral prefrontal cortex; MPFC= medial prefrontal cortex; OFC=orbitofrontal cortex; PFC=prefrontal cortex; VLPFC= ventrolateral prefrontal cortex.
p^a: t-test unless otherwise specified; p^b: adjusted for age, sex and intracranial volume by ANCOVA. α was set at 0.05 for all p values.

After adjusting for age, sex and intracranial volume, the primarily ketamine users had decreased grey volumes in right MPFC ($p = 0.022$) and left hippocampus ($p = 0.005$) than the healthy control, while the poly ketamine users had decreased volumes in right OFC ($p = 0.012$), right MPFC ($p = 0.004$), left globus pallidus ($p = 0.001$), right nucleus accumbens ($p = 0.012$) and left hippocampus ($p = 0.005$) than the healthy control. On the contrary, the poly ketamine users had increased grey volumes in the left caudate ($p = 0.006$) and left thalamus ($p = 0.016$) than the healthy control. The primarily ketamine users had lower grey matter volume in the left caudate ($p = 0.048$) and lower deep white matter volume ($p = 0.043$) than the poly ketamine users (table 7b).

Table 7b. Grey matter and white matter volumes across groups.

	Primarily Ketamine Users (N = 42)	Poly Ketamine Uers (N = 43)	Healthy Control (N = 46)	p^a	p^b	p^1	p^2	p^3
Total white matter	603.36 ± 98.76	579.67 ± 99.25	603.76 ± 103.01	0.444	0.672	-	-	-
Total grey matter	558.37 ± 74.82	544.14 ± 84.69	586.76 ± 71.08	0.031	0.228	-	-	-
Frontal lobe (left)	71.63 ± 1.37	72.67 ± 1.37	72.02 ± 1.35	0.496	0.862	-	-	-
Frontal lobe (right)	71.41 ± 1.40	72.02 ± 1.39	73.51 ± 1.38	0.106	0.558	-	-	-
PFC (left)	49.17 ± 0.90	49.63 ± 0.89	48.75 ± 0.88	0.684	0.792	-	-	-
PFC (right)	49.97 ± 0.95	49.53 ± 0.95	50.74 ± 0.94	0.109	0.671	-	-	-
OFC (left)	10.91 ± 0.17	11.23 ± 0.17	11.38 ± 0.17	0.062	0.132	-	-	-
OFC (right)	10.67 ± 0.19	10.43 ± 0.19	11.24 ± 0.19	0.001	0.013	0.125	0.012	1.000
DLPFC (left)	15.73 ± 0.38	15.79 ± 0.38	15.19 ± 0.37	0.942	0.474	-	-	-
DLPFC (right)	15.69 ± 0.38	15.82 ± 0.38	15.79 ± 0.37	0.439	0.968	-	-	-
VLPFC (left)	9.43 ± 0.22	9.31 ± 0.22	8.90 ± 0.22	0.674	0.208	-	-	-
VLPFC (right)	9.59 ± 0.25	9.23 ± 0.25	9.35 ± 0.25	0.395	0.577	-	-	-
MPFC (left)	9.19 ± 0.17	9.22 ± 0.17	9.54 ± 0.17	0.041	0.278	-	-	-
MPFC (right)	9.42 ± 0.16	9.30 ± 0.16	10.03 ± 0.16	0.001	0.003	0.022	0.004	1.000
ACC (left)	4.30 ± 0.13	4.15 ± 0.13	4.46 ± 0.13	0.056	0.256	-	-	-
ACC (right)	4.39 ± 0.12	4.30 ± 0.12	4.38 ± 0.11	0.414	0.814	-	-	-
Temporal lobe (left)	44.33 ± 1.09	45.20 ± 1.08	45.86 ± 1.07	0.291	0.610	-	-	-
Temporal lobe (right)	43.30 ± 0.91	44.85 ± 0.91	45.61 ± 0.90	0.067	0.195	-	-	-
Parietal lobe (left)	44.83 ± 0.98	46.03 ± 0.98	47.32 ± 0.97	0.055	0.206	-	-	-
Parietal lobe (right)	45.57 ± 0.97	46.22 ± 0.96	47.30 ± 0.95	0.147	0.449	-	-	-
Occipital lobe (left)	17.18 ± 0.48	17.90 ± 0.47	17.62 ± 0.47	0.644	0.557	-	-	-

	Primarily Ketamine Users (N = 42)	Poly Ketamine Uers (N = 43)	Healthy Control (N = 46)	p^a	p^b	p^1	p^2	p^3
Occipital lobe (right)	16.32 ± 0.54	17.13 ± 0.54	17.04 ± 0.53	0.489	0.506	-	-	-
Basal ganglia (left)	8.35 ± 0.14	8.45 ± 0.14	8.28 ± 0.13	0.899	0.669	-	-	-
Basal ganglia (right)	7.86 ± 0.14	7.75 ± 0.14	7.91 ± 0.14	0.260	0.711	-	-	-
Caudate (left)	2.60 ± 0.07	2.83 ± 0.07	2.53 ± 0.07	0.038	0.005	1.000	0.006	0.048
Caudate (right)	2.74 ± 0.08	2.66 ± 0.08	2.69 ± 0.07	0.464	0.759	-	-	-
Putamen (left)	4.45 ± 0.09	4.43 ± 0.08	4.38 ± 0.08	0.763	0.835	-	-	-
Putamen (right)	3.98 ± 0.07	3.93 ± 0.07	4.06 ± 0.07	0.147	0.393	-	-	-
Globus pallidus (left)	1.30 ± 0.03	1.20 ± 0.03	1.38 ± 0.03	0.000	0.001	0.246	0.001	0.098
Globus pallidus (right)	1.14 ± 0.03	1.17 ± 0.03	1.16 ± 0.03	0.799	0.847	-	-	-
Nucleus accumbens (left)	0.43 ± 0.01	0.41 ± 0.01	0.44 ± 0.01	0.092	0.309	-	-	-
Nucleus accumbens (right)	0.38 ± 0.01	0.35 ± 0.01	0.40 ± 0.01	0.002	0.015	0.642	0.012	0.262
Hippocampus (left)	4.50 ± 0.06	4.50 ± 0.06	4.76 ± 0.06	0.001	0.001	0.005	0.005	1.000
Hippocampus (right)	4.45 ± 0.14	4.36 ± 0.14	4.41 ± 0.14	0.741	0.898	-	-	-
Thalamus (left)	9.03 ± 0.12	9.39 ± 0.12	8.90 ± 0.12	0.159	0.014	1.000	0.016	0.104
Thalamus (right)	7.70 ± 0.09	7.80 ± 0.09	7.62 ± 0.09	0.959	0.352	-	-	-
Periventricular white matter (left)	19.42 ± 0.68	19.84 ± 0.68	21.14 ± 0.67	0.286	0.188	-	-	-
Periventricular white matter (right)	18.80 ± 0.63	19.09 ± 0.63	20.72 ± 0.62	0.078	0.075	-	-	-
Deep white matter (left)	235.83 ± 2.85	245.79 ± 2.85	236.39 ± 2.81	0.680	0.024	1.000	0.069	0.043
Deep white matter (right)	249.54 ± 6.59	252.17 ± 6.58	240.66 ± 6.50	0.693	0.446	-	-	-

Note: all data were presented in cm3 (mean ± standard error) as the estimated marginal means.

ACC=anterior cingulate cortex; DLPFC=dorsolateral prefrontal cortex; MPFC= medial prefrontal cortex; OFC=orbitofrontal cortex; PFC=prefrontal cortex; VLPFC= ventrolateral prefrontal cortex.

P^a: ANOVA; $p^{b,1-3}$: ANCOVA, adjusted for age, sex and intracranial volume with Bonferroni correction method.

p^1: primarily ketamine users versus healthy control; p^2: poly ketamine users versus healthy control; p^3: primarily ketamine users versus poly ketamine users;
α was set at 0.05 for all p values.

The regions showing significant differences between the ketamine groups and healthy controls were correlated with clinical factors and cognitive functions. In the all ketamine users group, the grey matter volumes of the right OFC, right MPFC and right nucleus accumbens were negatively correlated with the severity of ketamine dependence in terms of SDS scores. In the primarily ketamine users group, the grey matter volumes of the right OFC, right MPFC, left globus pallidus and right nucleus accumbens volume were negatively correlated with the severity of ketamine dependence in terms of SDS scores. In the poly ketamine users group, only the grey matter volumes of the right OFC were negatively correlated with the severity of ketamine dependence in terms of SDS scores (Table 8).

Table 8. Correlations between brain volumes and SDS scores (N=85).

	All Ketamine Users (N = 85)		Primarily Ketamine users (N = 42)		Poly Ketamine users (N = 43)	
	Pearson correlation coefficient	p	Pearson correlation coefficient	p	Pearson correlation coefficient	p
OFC (right)	-0.342	0.002	-0.354	0.029	-0.329	0.033
MPFC (right)	-0.335	0.002	-0.382	0.018	-	NS
Globus pallidus (left)	-	NS	-0.333	0.041	-	NS
Caudate (left)	-	NS	-	NS	-	NS
Nucleus accumbens (right)	-0.265	0.017	-0.465	0.003	-	NS
Hippocampus (left)	-	NS	-	NS	-	NS
Thalamus (left)	-	NS	-	NS	-	NS
Periventricular white matter (right)	-	NS	-	NS	-	NS

MPFC=medial prefrontal cortex; NS=non-significant; OFC=orbitofrontal cortex.
α was set at 0.05 for all p values.

In all ketamine users group, the right OFC volume was negatively correlated with total attempts in WCST; the right MPFC volume was negatively correlated with interference score and total reaction time of Stroop test; the left caudate volume was positively correlated with recognition score of WMS III Logical Memory; the left globus pallidus volume was positively correlated with forward WAIS III Digit Span score and negatively correlated with total correct response of verbal fluency; the right nucleus accumbens volume was positively correlated with WMS III digit symbol coding and ROCF copy scores; the left hippocampus volume was positively correlated with both forward WAIS III digit span and WMS III information scores; the left thalamus volume was negatively correlated with correct recognition of ROCF score; the right periventricular white matter volume was negatively correlated with immediate recall score of WMS III Logical Memory (Table 9a).

Table 9a. Correlations between brain volumes and performance on cognitive tests in all ketamine users (N = 85).

	OFC R	MPFC R	Caudate L	Globus Pallidus L	Nucleus accumbens R	Hippocampus L	Thalamus L	PWM R
WAIS III Digit Symbol Coding	-	-	-	-	0.296	-	-	-
WAIS III Arithmetic	-	-	-	-	-	-	-	-
WAIS III Information	-	-	-	-	-	0.254	-	-
WAIS III Digit Span (Forward) [a]	-	-	-	0.257	-	0.266	-	-
WAIS III Digit Span (Backward)	-	-	-	-	-	-	-	-
WAIS III Digit Span total	-	-	-	-	-	-	-	-
WMS III Logical Memory: immediate recall	-	-	-	-	-	-	-	-0.258
WMS III Logical Memory: delayed recall	-	-	-	-	-	-	-	-
WMS III Logical Memory: recognition [a]	-	-	0.221	-	-	-	-	-
WMS III Logical Memory: percent retention	-	-	-	-	-	-	-	-
WMS III Word List: immediate recall	-	-	-	-	-	-	-	-
WMS III Word List: delayed recall	-	-	-	-	-	-	-	-
WMS III Word List: recognition [a]	-	-	-	-	-	-	-	-
WMS III Word List: percent retention	-	-	-	-	-	-	-	-
ROCF: copy	-	-	-	-	0.249	-	-	-

	OFC R	MPFC R	Caudate L	Globus Pallidus L	Nucleus accumbens R	Hippocampus L	Thalamus L	PWM R
ROCF: immediate recall	-	-	-	-	-	-	-	-
ROCF: delayed recall	-	-	-	-	-	-	-	-
ROCF: correct recognition	-	-	-	-	-	-	-0.374	-
WCST: total attempts	-0.222	-	-	-	-	-	-	-
WCST: category completed [a]	-	-	-	-	-	-	-	-
WCST: preservative errors	-	-	-	-	-	-	-	-
Stroop Test: interference	-	-0.272	-	-	-	-	-	-
Stroop Test: total reaction time (seconds)	-	-0.270	-	-	-	-	-	-
Stroop Test: total errors	-	-	-	-	-	-	-	-
Verbal Fluency: total correct responses	-	-	-	-0.238	-	-	-	-
Modified Boston Naming Test [a]	-	-	-	-	-	-	-	-

OFC=orbitofrontal cortex; PWM= periventricular white matter; MPFC=medial prefrontal cortex; WM= white matter; L =left; R=right.
ROCF= Rey-Osterrieth Complex Figure; WAIS III=Wechsler Adult Intelligence scale - Third Edition; WCST=Wisconsin Card Sorting test; WMS III=Wechsler Memory scale - Third Edition.
Only the significant results are shown ($p < 0.05$) unless otherwise specified. α was set at 0.05 for all p values.
The Pearson correlation coefficient unless otherwise specified, adjusted for intracranial volume by partial correlation.
[a]Spearman's rho, adjusted for intracranial volume by volume/ intracranial volume.

In addition, in the primarily ketamine users group, the right MPFC volume was negatively correlated with interference score of Stroop test; the left hippocampus volume was positively correlated with WAIS III information and percent retention scores of WMS III Logical Memory (Table 9b).

Table 9b. Correlations between brain volumes and performance on cognitive tests in primarily ketamine users (N = 42).

	MPFC R	Hippocampus L
WAIS III Digit Symbol Coding	-	-
WAIS III Arithmetic	-	-
WAIS III Information	-	0.339
WAIS III Digit Span (Forward) [a]	-	-
WAIS III Digit Span (Backward)	-	-
WAIS III Digit Span total	-	-
WMS III Logical Memory: immediate recall	-	-
WMS III Logical Memory: delayed recall	-	-
WMS III Logical Memory: recognition [a]	-	-
WMS III Logical Memory: percent retention	-	0.384
WMS III Word List: immediate recall	-	-
WMS III Word List: delayed recall	-	-
WMS III Word List: recognition [a]	-	-
WMS III Word List: percent retention	-	-
ROCF: copy	-	-
ROCF: immediate recall	-	-
ROCF: delayed recall	-	-
ROCF: correct recognition	-	-
WCST: total attempts	-	-
WCST: category completed [a]	-	-
WCST: preservative errors	-	-
Stroop Test: interference	-0.331	-
Stroop Test: total reaction time (seconds)	-	-
Stroop Test: total errors	-	-
Verbal Fluency: total correct responses	-	-
Modified Boston Naming Test [a]	-	-

OFC=orbitofrontal cortex; PWM= periventricular white matter; MPFC=medial prefrontal cortex; WM= white matter; L =left; R=right.

ROCF= Rey-Osterrieth Complex Figure; WAIS III=Wechsler Adult Intelligence scale - Third Edition; WCST=Wisconsin Card Sorting test; WMS III=Wechsler Memory scale - Third Edition.

Only the significant results are shown ($p < 0.05$) unless otherwise specified. α was set at 0.05 for all p values.

The Pearson correlation coefficient unless otherwise specified, adjusted for intracranial volume by partial correlation.

[a]Spearman's rho, adjusted for intracranial volume by volume/ intracranial volume.

In the poly ketamine users group, the right OFC volume was negatively correlated with recognition score of WMS III Word List; the left caudate volume was negatively correlated with the score of Modified Boston Naming Test; the left globus pallidus volume was negatively correlated with forward digit span score of WAIS III, and positively correlated with copy score of ROCF; the right accumbens volume was positively correlated with total digit span of WAIS III and percent retention scores of WMS III Word List; the left hippocampus volume was negatively correlated with immediate recall score of WMS III Logical Memory; and the left thalamus volume was negatively correlated with delayed recall of WMS III Word List and correct recognition scores of ROCF (Table 9c).

Table 9c. Correlations between brain volumes and performance on cognitive tests in poly ketamine users (N = 43).

	OFC R	MPFC R	Caudate L	Globus Pallidus L	Nucleus accumbens R	Hippocampus L	Thalamus L
WAIS III Digit Symbol Coding	-	-	-	-	-	-	-
WAIS III Arithmetic	-	-	-	-	-	-	-
WAIS III Information	-	-	-	-	-	-	-
WAIS III Digit Span (Forward) [a]	-	-	-	-0.460	-	-	-
WAIS III Digit Span (Backward)	-	-	-	-	-	-	-
WAIS III Digit Span total	-	-	-	-	0.315	-	-
WMS III Logical Memory: immediate recall	-	-	-	-	-	-0.313	-
WMS III Logical Memory: delayed recall	-	-	-	-	-	-	-
WMS III Logical Memory: recognition [a]	-	-	-	-	-	-	-
WMS III Logical Memory: percent retention	-	-	-	-	-	-	-
WMS III Word List: immediate recall	-	-	-	-	-	-	-
WMS III Word List: delayed recall	-	-	-	-	-	-	-0.317
WMS III Word List: recognition [a]	-0.325	-	-	-	-	-	-
WMS III Word List: percent retention	-	-	-	-	0.326	-	-
ROCF: copy	-	-	-	0.355	-	-	-
ROCF: immediate recall	-	-	-	-	-	-	-
ROCF: delayed recall	-	-	-	-	-	-	-
ROCF: correct recognition	-	-	-	-	-	-	-0.514
WCST: total attempts	-	-	-	-	-	-	-
WCST: category completed [a]	-	-	-	-	-	-	-

	OFC R	MPFC R	Caudate L	Globus Pallidus L	Nucleus accumbens R	Hippocampus L	Thalamus L
WCST: preservative errors	-	-	-	-	-	-	-
Stroop Test: interference	-	-	-	-	-	-	-
Stroop Test: total reaction time (seconds)	-	-	-	-	-	-	-
Stroop Test: total errors	-	-	-	-	-	-	-
Verbal Fluency: total correct responses	-	-	-	-	-	-	-
Modified Boston Naming Test [a]	-	-	-0.450	-	-	-	-

OFC=orbitofrontal cortex; PWM= periventricular white matter; MPFC=medial prefrontal cortex; WM= white matter; L =left; R=right.
ROCF= Rey-Osterrieth Complex Figure; WAIS III=Wechsler Adult Intelligence scale - Third Edition; WCST=Wisconsin Card Sorting test; WMS III=Wechsler Memory scale - Third Edition.
Only the significant results are shown ($p < 0.05$) unless otherwise specified. α was set at 0.05 for all p values.
The Pearson correlation coefficient unless otherwise specified, adjusted for intracranial volume by partial correlation.
[a]Spearman's rho, adjusted for intracranial volume by volume/ intracranial volume.

MR Spectroscopy (MRS)

Compared with healthy control group, the ketamine users group had a lower ASP / Cr (aspartate / creatine) ratio (0.20 ± 0.06 versus 0.21 ± 0.06, p = 0.019) in the bilateral visual cortex. There were no significant differences in the metabolite ratios in the anterior cingulate and basal ganglia regions between the all ketamine users group and healthy control group (Table 10a).

Table 10a. Metabolite ratios of three brain regions across groups.

	All Ketamine Users (N = 86)	Healthy Control (N = 46)	p^a	p^b
Right Basal Ganglia				
ASP/Cr	0.11 ± 0.07	0.11 ± 0.08	0.945	0.780
GABA/Cr	0.22 ± 0.07	0.21 ± 0.08	0.349	0.385
Glu/Cr	0.90 ± 0.12	0.91 ± 0.13	0.673	0.895
Glu+Gln/Cr	1.10 ± 0.24	1.04 ± 0.20	0.198	0.185
MI/Cr	0.41 ± 0.11	0.39 ± 0.07	0.387	0.469
NAA/Cr	1.19 ± 0.16	1.21 ± 0.16	0.685	0.671
GPC/Cr	0.24 ± 0.05	0.25 ± 0.04	0.383	0.178
GPCPch/Cr	0.25 ± 0.03	0.25 ± 0.03	0.269	0.670
Bilateral Anterior Cingulate				
ASP/Cr	0.16 ± 0.07	0.17 ± 0.08	0.524	0.535
GABA/Cr	0.28 ± 0.07	0.26 ± 0.08	0.272	0.097
Glu/Cr	1.30 ± 0.15	1.33 ± 0.13	0.273	0.774
Glu+Gln/Cr	1.51 ± 0.20	1.53 ± 0.21	0.423	0.840
MI/Cr	0.76 ± 0.11	0.74 ± 0.07	0.209	0.261
NAA/Cr	1.09 ± 0.12	1.12 ± 0.12	0.335	0.475
GPC/Cr	0.26 ± 0.04	0.26 ± 0.05	0.775	0.635
GPCPch/Cr	0.27 ± 0.03	0.28 ± 0.03	0.592	0.320
Bilateral Visual Cortex				
ASP/Cr	0.20 ± 0.06	0.21 ± 0.06	0.074	0.019
GABA/Cr	0.15 ± 0.06	0.14 ± 0.05	0.350	0.111
Glu/Cr	0.96 ± 0.12	0.94 ± 0.12	0.351	0.433
Glu+Gln/Cr	1.33 ± 0.25	1.29 ± 0.23	0.365	0.998
MI/Cr	0.57 ± 0.08	0.57 ± 0.06	0.970	0.658
NAA/Cr	1.12 ± 0.12	1.12 ± 0.11	0.730	0.430
GPC/Cr	0.15 ± 0.02	0.15 ± 0.02	0.700	0.804
GPCPch/Cr	0.16 ± 0.02	0.16 ± 0.02	0.201	0.328

p^a : t test; p^b: ANCOVA, adjusted for age and sex. α was set at 0.05 for all p values. Asp = aspartate; Cr = creatine; GABA = γ-aminobutyric acid; Glu = glutamate; MI = myo-Inositol; NAA = N-acetyl compounds; Gln = glutamine; GPC = Glycerylphosphorylcholine; PCh = phosphocholine.

After adjusting for age and sex, there were significant differences between groups in the Glu + Gln / Cr ratio (p = 0.012) in right basal ganglia, MI / Cr ratio (p = 0.023) in bilateral anterior cingulate and GPCPch / Cr ratio (p = 0.033) in bilateral visual cortex. The primarily ketamine users group had a lower Glu+Gln/Cr ratio (p = 0.023) in the right basal ganglia, lower MI / Cr ratio (p = 0.037) in the bilateral anterior cingulate, higher GPCPch/Cr ratio (p = 0.047) in the bilateral visual cortex than the poly ketamine users. The poly ketamine users group also had a higher Glu+Gln/Cr ratio (p = 0.041) in the right basal ganglia than healthy control (Table 10b).

Table 10b. Metabolite ratios of the primarily ketamine users and poly ketamine users.

	Primarily Ketamine users (N = 42)	Poly Ketamine users (N = 44)	Healthy Control group (N = 46)	p^a	p^b	p^1	p^2	p^3
Right Basal Ganglia								
ASP/Cr	0.11 ± 0.08	0.11 ± 0.07	0.11 ± 0.08	0.940	0.929	-	-	-
GABA/Cr	0.21 ± 0.07	0.23 ± 0.06	0.21 ± 0.08	0.320	0.355	-	-	-
Glu/Cr	0.88 ± 0.14	0.92 ± 0.11	0.91 ± 0.13	0.258	0.274	-	-	-
Glu+Gln/Cr	1.04 ± 0.26	1.15 ± 0.20	1.04 ± 0.20	0.025	0.012	1.000	0.041	0.023
MI/Cr	0.40 ± 0.11	0.41 ± 0.10	0.39 ± 0.07	0.731	0.752	-	-	-
NAA/Cr	1.20 ± 0.18	1.19 ± 0.14	1.21 ± 0.16	0.914	0.909	-	-	-
GPC/Cr	0.24 ± 0.05	0.23 ± 0.05	0.25 ± 0.04	0.290	0.202	-	-	-
GPCPch/Cr	0.26 ± 0.03	0.25 ± 0.02	0.25 ± 0.03	0.037	0.079	-	-	-
Bilateral Anterior Cingulate								
ASP/Cr	0.15 ± 0.06	0.17 ± 0.08	0.17 ± 0.08	0.276	0.237	-	-	-
GABA/Cr	0.27 ± 0.06	0.29 ± 0.08	0.26 ± 0.08	0.122	0.060	-	-	-
Glu/Cr	1.29 ± 0.13	1.31 ± 0.18	1.33 ± 0.13	0.332	0.537	-	-	-
Glu+Gln/Cr	1.48 ± 0.19	1.54 ± 0.21	1.53 ± 0.21	0.261	0.187	-	-	-
MI/Cr	0.74 ± 0.08	0.79 ± 0.12	0.74 ± 0.07	0.024	0.023	1.000	0.083	0.037
NAA/Cr	1.08 ± 0.11	1.11 ± 0.13	1.11 ± 0.12	0.299	0.342	-	-	-

GPC/Cr	0.26 ± 0.04	0.26 ± 0.04	0.26 ± 0.05	0.816	0.849	-	-	-
GPCPch/Cr	0.27 ± 0.03	0.27 ± 0.03	0.27 ± 0.03	0.862	0.609	-	-	-
Bilateral Visual Cortex								
ASP/Cr	0.19 ± 0.06	0.20 ± 0.06	0.21 ± 0.06	0.189	0.055	-	-	-
GABA/Cr	0.15 ± 0.07	0.15 ± 0.05	0.14 ± 0.05	0.589	0.273	-	-	-
Glu/Cr	0.99 ± 0.15	0.94 ± 0.09	0.94 ± 0.12	0.092	0.133	-	-	-
Glu+Gln/Cr	1.34 ± 0.31	1.31 ± 0.19	1.28 ± 0.23	0.600	0.987	-	-	-
MI/Cr	0.57 ± 0.09	0.57 ± 0.07	0.57 ± 0.06	0.995	0.905	-	-	-
NAA/Cr	1.11 ± 0.12	1.14 ± 0.11	1.12 ± 0.11	0.348	0.338	-	-	-
GPC/Cr	0.16 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.173	0.159	-	-	-
GPCPch/Cr	0.17 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.017	0.033	0.104	1.000	0.047

P^{a}: ANOVA; $p^{b,1-3}$: ANCOVA, adjusted for age and sex with Bonferroni correction method. α was set at 0.05 for all p values.

p^{1}: primarily ketamine users versus healthy control; p^{2}: poly ketamine users versus healthy control; p^{3}: primarily ketamine users versus poly ketamine users.

Asp = aspartate; Cr = creatine; GABA = γ-aminobutyric acid; Glu = glutamate; MI = myo-Inositol; NAA = N-acetyl compounds; Gln = glutamine; GPC = Glycerylphosphorylcholine; PCh = phosphocholine.

Resting functional MRI (fMRI)

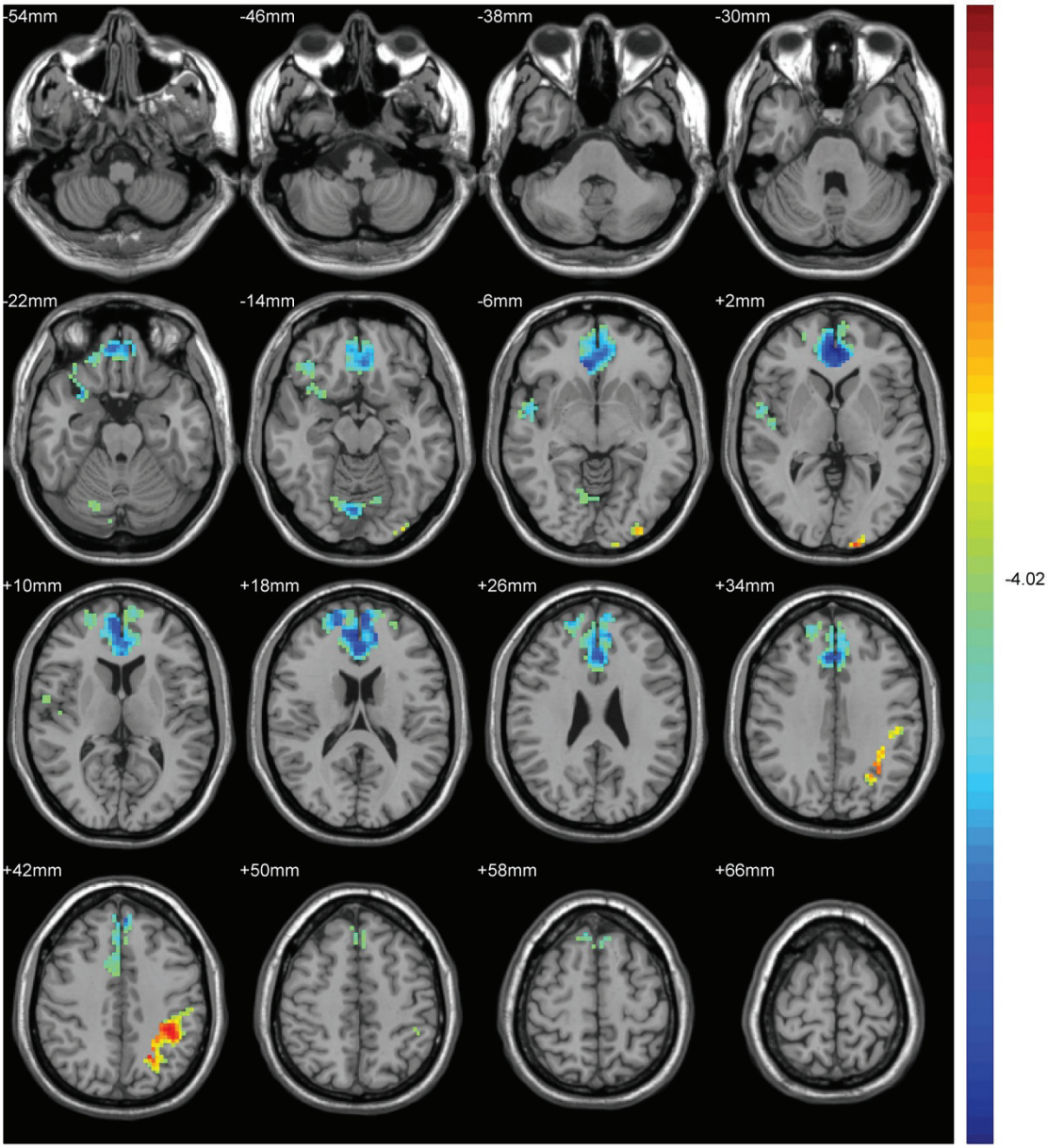
In comparison with healthy controls, all ketamine users group displayed significantly decreased connectivity in orbital part of right inferior frontal gyrus, left anterior cingulate and paracingulate gyri, right superior temporal gyrus and bilateral vermic lobule VI ($p < 0.01$). The all ketamine users group also displayed significantly increased connectivity in left middle occipital gyrus ($p < 0.01$) (Table 11a & Figure 2).

Table 11a. Regions with significantly different connectivity in the default mode network in all ketamine users (n=80) and healthy control (n=43).

Regions	Cluster size (voxels)	Peak MNI coordinate			Brodmann's area	Peak intensity	FDR corrected p value
		x	y	z			
Decreased connectivity in all Ketamine users							
Right inferior frontal gyrus, orbital part	73	30	30	-18	47	-6.5301	0.01
Left anterior cingulate and paracingulate gyri	1751	0	45	3	10	-8.2659	0.01
Right superior temporal gyrus	90	57	0	0	48	-6.2002	0.01
Bilateral vermic lobule VI	112	6	-78	-15	18	-6.4864	0.01
Increased connectivity in all Ketamine users							
Left middle occipital gyrus	55	-15	-105	3	17	5.7013	0.01
Left middle occipital gyrus	297	-24	-60	39	7	6.8325	0.01

Adjusted for age and sex by ANCOVA.
The minimum cluster size was 50 voxels.
FDR=false discovery rate; MNI=Montreal Neurological Institute.

Figure 2. DMN resting connectivity of all ketamine users compared to healthy control. Blue and red indicate connectivity decreases and increases, respectively, in various regions of the brain. Threshold of $p < 0.01$ (false discovery rate corrected), voxel size >/= 50. The coloured bar represents the t value of each voxel. Red–yellow indicates increased connectivity, deep–light blue represents decreased connectivity.



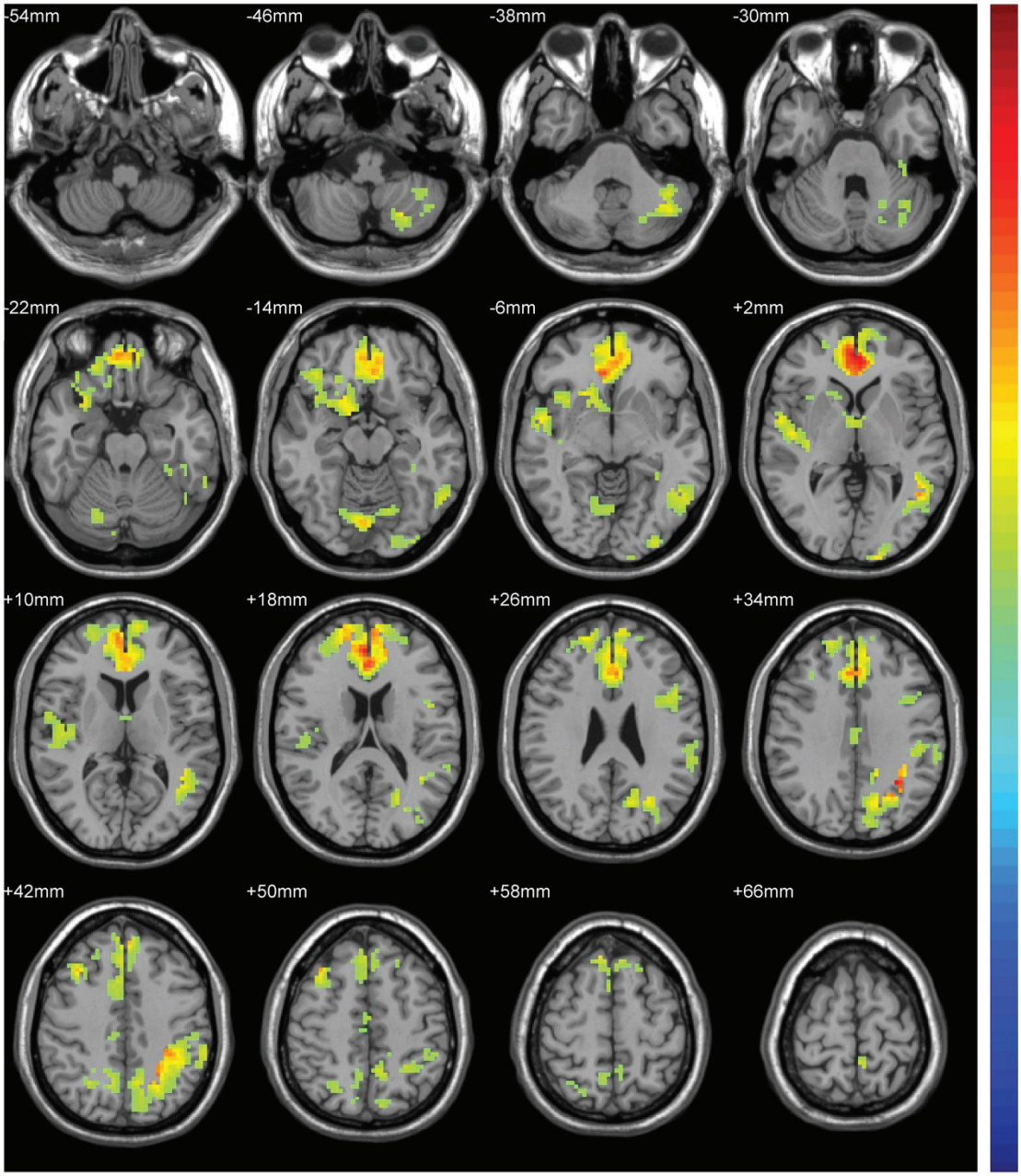
In the comparison among the primarily ketamine users, the poly ketamine users and healthy control, it displayed significantly different connectivity in bilateral crus I, left inferior temporal gyrus, left anterior cingulate and paracingulate gyri, bilateral vermic lobule VI, left middle occipital gyrus, right superior temporal gyrus, left precentral gyrus, right median cingulate and paracingulate gyri, right middle frontal gyrus and right precuneus ($p < 0.01$) (Table 11b & Figure 3).

Table 11b. Regions with significantly different connectivity in the default mode network in primarily ketamine users (n=41), poly ketamine users (n=39) and healthy control (n=43).

Regions	Cluster size (voxels)	Peak MNI coordinate			Brodmann's area	Peak intensity	FDR corrected p value
		x	y	z			
Different connectivity in 3 groups` comparison							
Right middle frontal gyrus	103	36	24	48		30.1404	0.01
Left anterior cingulate and paracingulate gyri	2502	-3	45	3		36.1921	0.01
Right median cingulate and paracingulate gyri	59	9	-24	45		12.1565	0.01
Left inferior temporal gyrus	99	-48	-39	-27		19.3584	0.01
Right superior temporal gyrus	304	57	0	0		22.6352	0.01
Left precentral gyrus	95	-45	0	21		15.1525	0.01
Left middle occipital gyrus	1294	-27	-54	36		45.4358	0.01
Left middle occipital gyrus	96	-15	-105	3		16.5177	0.01
Right precuneus	157	9	-60	39		13.5077	0.01
Bilateral vermic lobule VI	161	3	-78	-15		21.3354	0.01
Bilateral crus I	283	-42	-63	-36		18.7962	0.01

Adjusted for age and sex by ANCOVA.
The minimum cluster size was 50 voxels.
FDR=false discovery rate; MNI=Montreal Neurological Institute.

Figure 3. DMN resting connectivity of the primarily ketamine users, the poly ketamine users and healthy control. Blue and red indicate connectivity decreases and increases, respectively, in various regions of the brain. Threshold of $p < 0.01$ (false discovery rate corrected), voxel size >/= 50. The coloured bar represents the t value of each voxel. Red–yellow indicates increased connectivity, deep–light blue represents decreased connectivity.



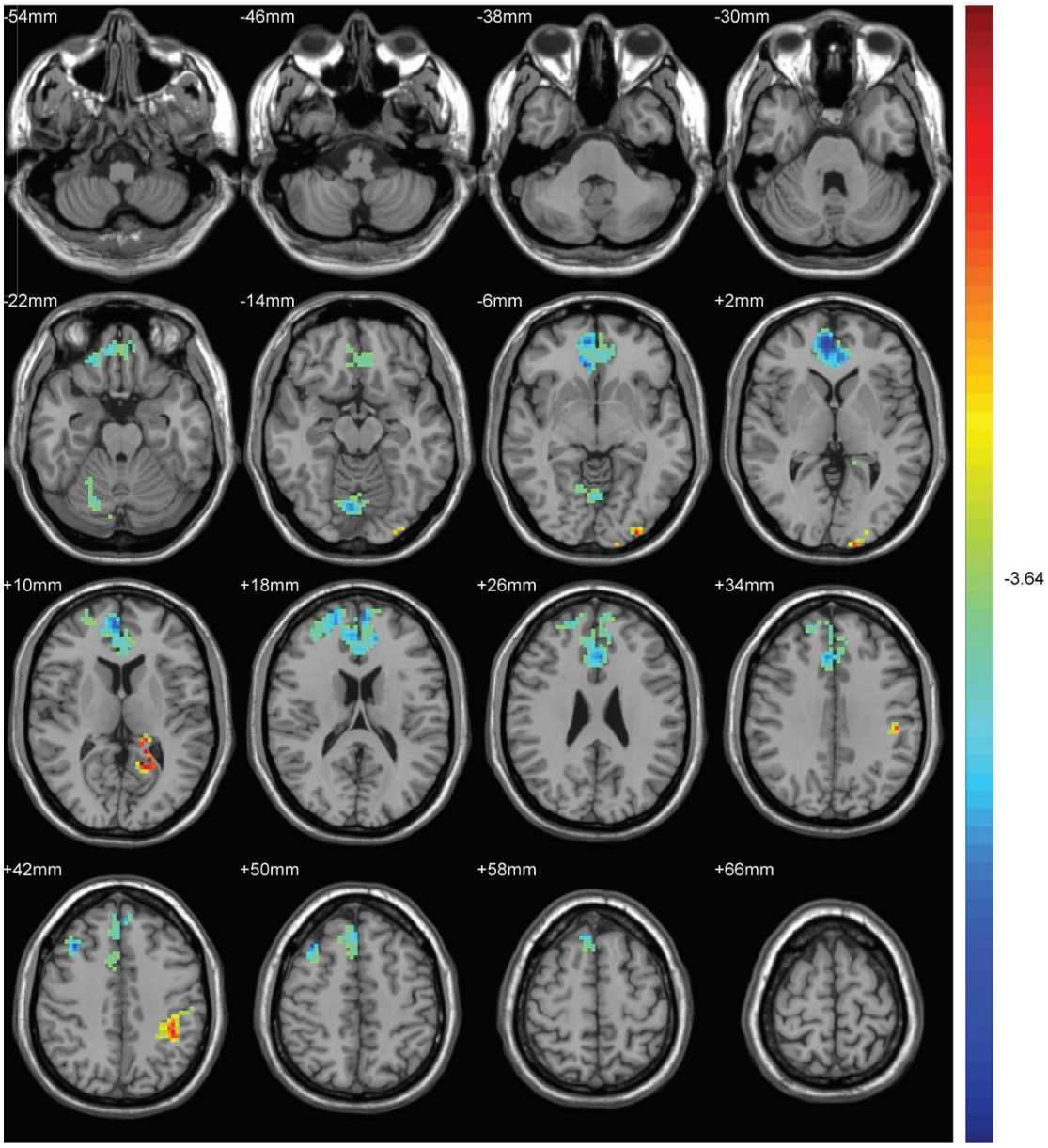
In comparison with healthy controls, the primarily ketamine users displayed significantly decreased connectivity in orbital part of right middle frontal gyrus, right middle frontal gyrus and bilateral vermic lobule VI ($p < 0.01$). The primarily ketamine users group also displayed significantly increased connectivity in left supramarginal gyrus, left inferior occipital gyrus, and left calcarine fissure and surrounding cortex ($p < 0.01$) (Table 11c & Figure 4).

Table 11c. Regions with significantly different connectivity in the default mode network in primarily ketamine users (n=41) versus control participants (n=43).

Regions	Cluster size (voxels)	Peak MNI coordinate			Brodmann's area	Peak intensity	FDR corrected p value
		x	y	z			
Decreased connectivity in primarily Ketamine Users							
Right middle frontal gyrus, orbital part	1261	6	51	0	10	-7.9028	0.01
Right middle frontal gyrus	81	36	24	48	9	-7.5807	0.01
Bilateral vermic lobule VI	137	6	-75	-15	17	-5.6648	0.01
Increased connectivity in primarily Ketamine Users							
Left supramarginal gyrus	100	-48	-27	36	2	5.0113	0.01
Left inferior occipital gyrus	50	-33	-96	-9	18	5.1800	0.01
Left calcarine fissure and surrounding cortex	52	-15	-54	9	17	5.1757	0.01

Adjusted for age and sex by ANCOVA.
The minimum cluster size was 50 voxels.
FDR=false discovery rate; MNI=Montreal Neurological Institute.

Figure 4. DMN resting connectivity of the primarily ketamine users compared to healthy controls. Blue and red indicate connectivity decreases and increases, respectively, in various regions of the brain. Threshold of $p < 0.01$ (false discovery rate corrected), voxel size >/= 50. The coloured bar represents the t value of each voxel. Red–yellow indicates increased connectivity, deep–light blue represents decreased connectivity.



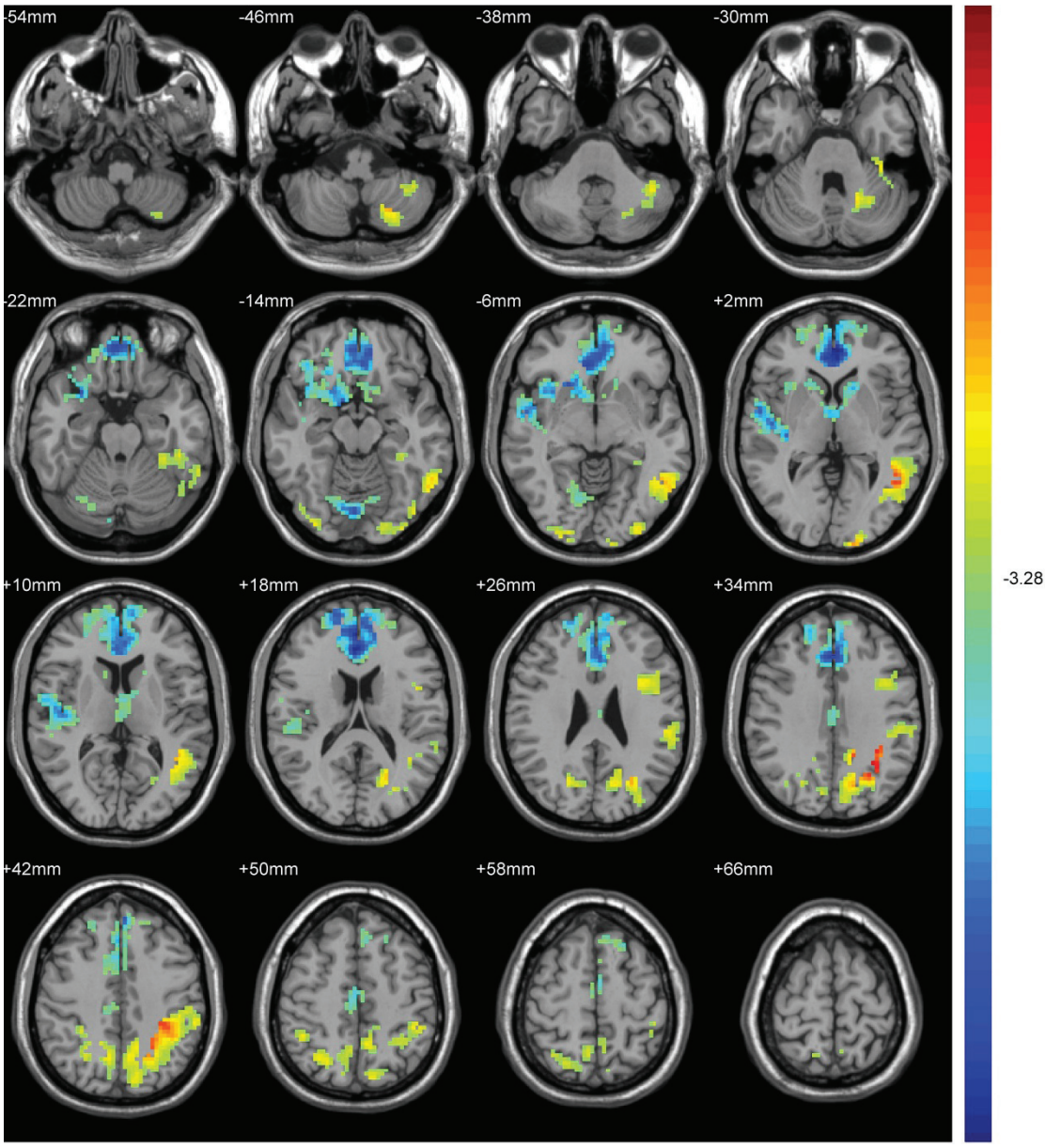
When compared with healthy controls, the poly ketamine users showed decreased connectivity in left anterior cingulate and paracingulate gyri, right median cingulate and paracingulate gyri and bilateral vermic lobule VI ($p < 0.01$). The poly ketamine users group also showed significantly increased connectivity in left precentral gyrus, left middle temporal gyrus, left hemispheric lobule VIII , right inferior occipital gyrus, and left middle occipital gyrus ($p < 0.01$) (Table 11d & Figure 5).

Table 11d. Regions with significantly different connectivity in the default mode network in poly ketamine users (n=39) and healthy controls (n=43).

Regions	Cluster size (voxels)	Peak MNI coordinate			Brodmann's area	Peak intensity	FDR corrected p value
		x	y	z			
Decreased connectivity in Poly Ketamine users							
Left anterior cingulate and paracingulate gyri	3080	-3	45	3	10	-7.6953	0.01
Right median cingulate and paracingulate gyri	134	6	-21	48	23	-5.1245	0.01
Bilateral vermic lobule VI	170	3	-81	-15	17	-6.3561	0.01
Increased connectivity in Poly Ketamine users							
Left precentral gyrus	118	-45	0	21	48	5.1596	0.01
Left middle temporal gyrus	738	-48	-57	0	37	7.3721	0.01
Right inferior occipital gyrus	52	33	-93	-12	18	4.9187	0.01
Left middle occipital gyrus	1499	-27	-54	36	7	8.9079	0.01
Left hemispheric lobule VIII	246	-21	-66	-48	--	5.8728	0.01

Adjusted for age and sex by ANCOVA.
The minimum cluster size was 50 voxels.
FDR=false discovery rate; MNI=Montreal Neurological Institute.

Figure 5. DMN resting connectivity of the poly ketamine users compared to normal controls. Blue and red indicate connectivity decreases and increases, respectively, in various regions of the brain. Threshold of $p < 0.01$ (false discovery rate corrected), voxel size >/= 50. The coloured bar represents the t value of each voxel. Red–yellow indicates increased connectivity, deep–light blue represents decreased connectivity.



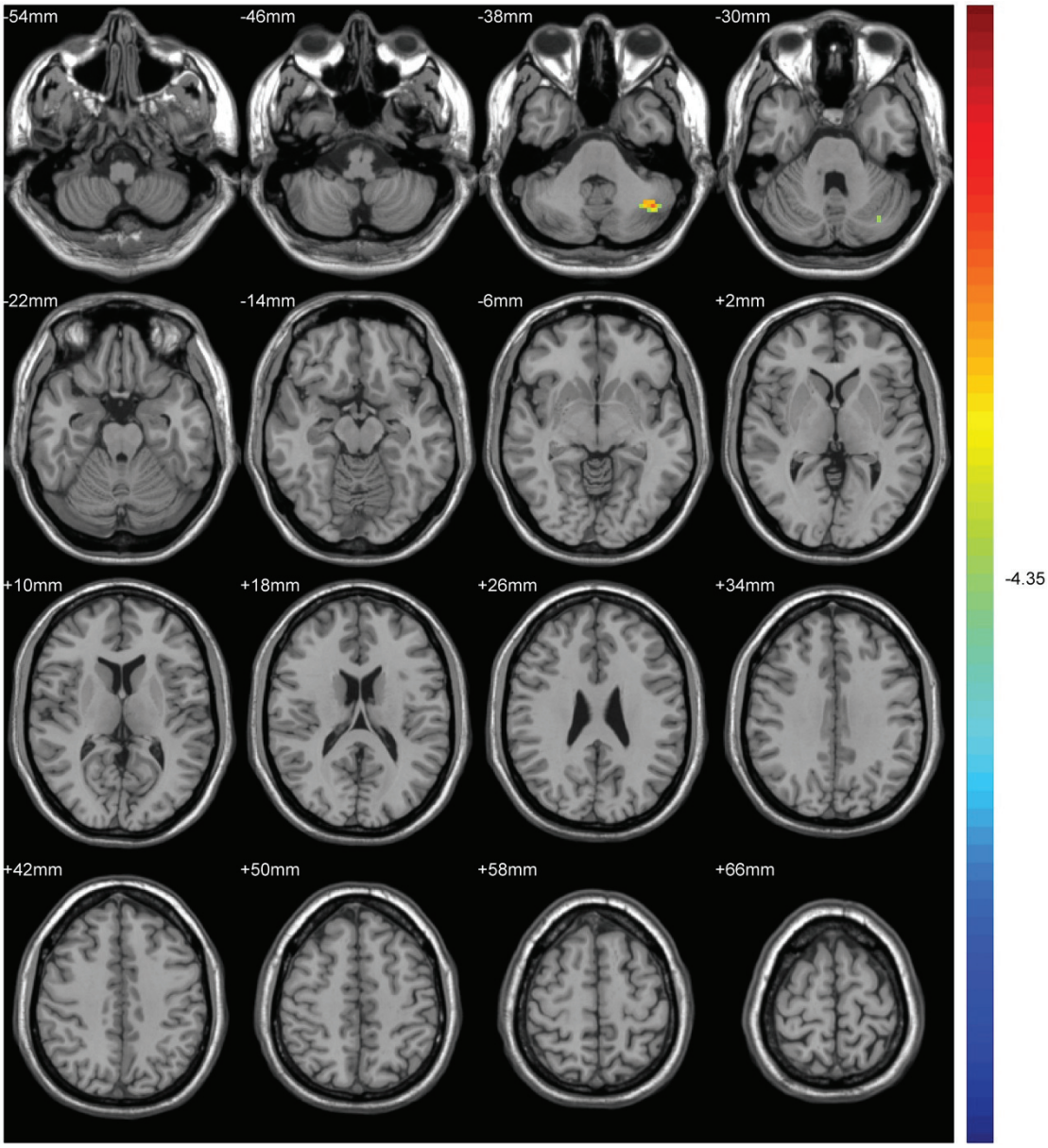
The primarily ketamine users and the poly ketamine users differed only in the connectivity in left crus I ($p < 0.01$) (Table 11e & Figure 6).

Table 11e. Regions with significantly different connectivity in the default mode network in primarily ketamine users (n=41) and poly ketamine users (n=39).

Regions	Cluster size (voxels)	Peak MNI coordinate			Brodmann's area	Peak intensity	FDR corrected p value
		x	y	z			
Increased connectivity in Poly Ketamine users							
Left crus I	67	-42	-63	-36	--	5.8528	0.01

Adjusted for age and sex by ANCOVA.
The minimum cluster size was 50 voxels.
FDR=false discovery rate; MNI=Montreal Neurological Institute.

Figure 6. DMN resting connectivity of the primarily ketamine users compared to the poly ketamine users. Blue and red indicate connectivity decreases and increases, respectively, in various regions of the brain. Threshold of $p < 0.01$ (false discovery rate corrected), voxel size >/= 50. The coloured bar represents the t value of each voxel. Red–yellow indicates increased connectivity, deep–light blue represents decreased connectivity.



Discussion

Demographics and drug use patterns

The ketamine users participated in this study were predominantly single male young adults, and being less educated, unable to find (or maintain) a job and had lower income. These demographic characteristics are in line with the recent local literatures (Liang et al., 2013; Narcotics Division, 2013).

Most drug abusers started to use ketamine when they were aged 16-20 and had an average duration of ketamine use as many as 88 months. The mean SDS score for ketamine users was 8.4, which indicates a severe level of addiction (Cuenca-Royo et al., 2012). Actually, the majority ketamine users had lifetime dependence in this study.

Many of the ketamine users also used other substances, especially alcohol, cocaine and cannabis. An association between ketamine use and the use of other psychotropic drugs has been reported previously (Degenhardt & Dunn, 2008). The poly drug use pattern in ketamine users is also consistent with the previous studies (Morgan et al., 2009; Morgan et al., 2010).

Effects of ketamine on psychological health

The all ketamine users group had higher BDI and HADSA scores than the healthy control group, consistent with a previous report (Liang et al., 2013). This suggests that ketamine users suffer from more depressive and anxiety symptoms than non-users. In previous studies, ketamine users also had a higher level of depressive symptoms both at the baseline assessment (Morgan, Curran, & Independent Scientific Committee on, 2012) and at the 1-year follow-up assessment (Morgan et al., 2010). The anxiety-depression subscale of the Brief Psychiatric Rating Scale was also used to measure the severity of anxiety and depression in a previous study (Zhang et al., 2014), which found that ketamine users had significantly more severe anxiety and depression symptoms than those who used amphetamine-type stimulants (ATS) and ATS + ketamine.

The association between ketamine and depression has been well reported (Morgan et al., 2009; Morgan et al., 2010). The present results are partially in line with those of a previous local study (Chen et al., 2005). The rate of current psychiatric disorder in this study (24.2%) was close to that of 26.3% reported locally by Chen et al. (2005), but the prevalence of mood disorders and anxiety disorders was higher. These results are congruent with abundant evidence that mood disorders are common comorbidities in substance use disorder (Chen et al., 2005; Compton, 2007; Kessler et al., 1996; A. Tang, Cheung, Liang, Ungvari, & Tang, 2011; A. Tang, Liang, Ungvari, & Tang, 2011). The association between depression and illicit substance use is most commonly explained either by a causal relationship or shared etiologic factors

(genetic predisposition, prenatal environment and disruptive family environment) underlying both disorders (Swendsen & Merikangas, 2000). The association between anxiety disorders and psychoactive drug use may be explained by the underlying mechanism of specific anxiety disorders (Buckner, Mallott, Schmidt, & Taylor, 2006; Kushner, Abrams, Thuras, Thuras, & Hanson, 2000). For instance, the tension-reduction expectation in alcohol addicts with panic disorder increased their risk of self-medicating to reduce anxiety (Kushner et al., 2000). Another study suggested that patients with social phobia were more prone to peer pressure (Buckner et al., 2006), and peer influence has been shown to be a risk factor for ketamine use (K. H. Lee et al., 2012) and cannabis use disorder (Buckner et al., 2006).

Effect of ketamine on cognitive functioning

The ketamine users had significant impairments in cognitive functions, including verbal, visual and working memory, and executive functioning. The impaired verbal and visual memory in ketamine users is also consistent with the previous studies in our team (Liang et al., 2013; W. K. Tang, Liang, Lau, Tang, & Ungvari, 2013), which provided further evidence of impairments in working memory and executive functions (Curran & Monaghan, 2001; Morgan, Monaghan, et al., 2004; Morgan et al., 2009; W. K. Tang et al., 2013).

Effects of ketamine on brain structure

Compared with the healthy control group, ketamine users had altered regional brain volumes. The ketamine users had reduced grey matter volumes in right OFC, right MPFC, left globus pallidus, left hippocampus, right nucleus accumbens, and reduced right periventricular white matter volume. The volumes of right OFC, right MPFC and right nucleus accumbens volumes were negatively correlated with the severity of ketamine dependence, suggesting a dose effect of ketamine use and brain volume reduction. On the other hand, ketamine users had increased grey matter volumes in left caudate and left thalamus than healthy control. None of the these regional brain volume changes has been reported among ketamine users.

No previous studies have specifically examined OFC and MPFC volumes in ketamine users. In a voxel-based morphometry study, Liao et al. (2011) reported reduced volumes in the left superior and right middle frontal gyrus in a group of 41 ketamine users. Similarly, atrophy of the frontal regions, namely the prefrontal cortex and basal prefrontal gyrus, was reported in a case series of 21 ketamine users (Wang, Zheng, Xu, Lam, & Yew, 2013).

In addition to frontal atrophy, the ketamine group also had reduced left hippocampal volumes. No previous studies have examined the hippocampal volume in ketamine users, although Wang et al. (2013) reported atrophy of the parahippocampal gyrus in a case series of ketamine users. Hippocampal atrophy has been reported in other forms of chemical addiction, such as in cocaine users (Alia-

Klein et al., 2011). An animal study found that daily methamphetamine use altered hippocampal volume (Mandyam et al., 2008). In an animal model of alcohol dependence, adult neurogenesis was inhibited during dependence, with a pronounced increase in the formation of new hippocampal neurons after weeks of abstinence (Nixon & Crews, 2004). Finally, in a study of 79 participants with substance use disorder, hippocampal volume reduction was related to childhood maltreatment (Van Dam, Rando, Potenza, Tuit, & Sinha, 2014).

The ketamine users had reduced grey volume in right nucleus accumbens. In a study of heroin dependent users, reduced volume of the left nucleus accumbens was observed (Seifert et al., 2015). It has been suggested that nucleus accmbens is part of the reward circuitry involved in drug-seeking behaviors (Carlezon & Thomas, 2009).

In contrast to the atrophy in the frontal and limbic regions, the left caudate and left thalamus volumes were higher in the ketamine group than healthy control. No previous studies have reported basal ganglia volume changes in ketamine users. However, in a study of 50 cocaine-dependent subjects, cocaine users showed a significant increase of grey matter volumes in caudate nucleus (Ersche et al., 2011). Similarly, enlarged striatal structures have been reported in methamphetamine users (Chang et al., 2005). There is evidence to suggest that striatal enlargement is an imaging marker of cocaine dependence, which may reflect reduced dopamine neurotransmission and could indeed be a predisposing factor rather than a consequence of cocaine use (Ersche et al., 2011). In the contrary, Reid et al reported abstinent opioid addicts had reduced thalamic grey volume, and this brain structure

change seemed to be affected by chronic heroin use (Reid et al., 2008). Other clues may also be implicated in the neurobiology of addiction due to its widespread afferent dopamine connections (Garcia-Cabezas, Rico, Sanchez-Gonzalez, & Cavada, 2007).

The ketamine users group also had reduced periventricular white matter in the right hemisphere. Reduction of white matter volume in ketamine users has not been reported previously. There were negative associations between the grey and white matter volumes and the severity of ketamine dependence. Negative associations between brain volumes and the duration of ketamine use and estimated total lifetime ketamine consumption were reported by Liao et al (2011). It has been suggested that ketamine use has a cumulative effect; the longer the ketamine use, the lower the grey matter volume (Liao et al., 2011). Thus, early intervention might be pivotal for the treatment of ketamine addiction.

Wang et al. (2013) reported white matter lesions in ketamine users, and suggested that chronic ketamine use may lead to axonal damage and white matter degeneration. Recent studies have found reduced white matter integrity in the right hemisphere (Roberts, Curran, Friston, & Morgan, 2014) and the bilateral frontal and left temporoparietal cortices in ketamine users (Liao et al., 2010).

In the present study, the grey and white matter volume loss was related to the severity of ketamine dependence. The duration of use and total lifetime consumption of ketamine have been shown to be correlated with volume loss (Liao et al., 2011). Similarly, negative correlations have been found between the durations of drug use and grey matter volumes in heroin, cocaine and toluene abusers, respectively (Aydin

et al., 2009; Ersche et al., 2011; Yuan et al., 2010). Finally, a study by Matichil et al.(2005) reported a negative correlation between white matter concentration and the duration of cannabis use .

In this study sample, the left caudate, right nucleus accumbens and left hippocampal volumes were positively correlated with certain measures of attention, memory and executive functions. Although an association between grey and white matter loss and cognitive impairment has not been reported previously in ketamine users, such an association has been observed among abusers of other substances. In amphetamine users, correlations have been found between hippocampal volume reduction and impaired word recall (Thompson et al., 2004); striatum volume reduction and impaired attention, delayed verbal memory and cognitive performance (Chang et al., 2005; Chang et al., 2004); and between frontal cortex volume reduction and decreased WCST performance (Kim et al., 2006). Similarly, grey matter volumes were correlated with cognitive functions in toluene abusers (Aydin et al., 2009).

Effects of ketamine on brain metabolism

In the present study, the only difference in metabolite ratios between all ketamine users and healthy control was found in the visual cortex, which is regarded as a control region (Nordahl et al., 2002). The poly ketamine users groups had a higher 'glutamate + glutamine / creatine' ratio in the right basal ganglia than the primarily ketamine and healthy control group. Decreased levels of Glutamate have been found in the rostral anterior cingulate in cocaine-dependent patients (Yang et al., 2009), alcohol-dependent patients (Thoma et al., 2011) and opiate addiction (Yucel et al., 2007). On the contrary, increased Glu level is found in the putamen in cocaine treated squirrel monkeys (Liu et al., 2011), and acute ketamine administration results in increased glutamate release in the anterior cingulate in healthy men (Rowland et al., 2005). Further studies on other regions of the brain, such as the thalamus (Stone et al., 2012), and other metabolites, particularly gamma-aminobutyric acid (Stone et al., 2012), are warranted.

Effects of ketamine on brain function

To our knowledge, this is the first study to examine the long-term effects of ketamine exposure on the functional connectivity of the DMN. Our findings indicate that chronic ketamine use is associated with alterations in the functional connectivity in certain brain areas, especially in the frontal and temporal cortices. These findings are consistent with an association between long-term ketamine use and altered brain function. Liao et al.(2012) reported an association between ketamine dependence and alterations in the functional connectivity of the anterior cingulate cortex and precentral prefrontal cortex. The connectivity alterations were similar to those observed in task-related fMRI studies of acute ketamine administration in healthy volunteers (G. D. Honey et al., 2008; R. A. Honey et al., 2004). This similarity suggests that acute changes induced by the drug may translate into more enduring functional brain organisation features following chronic exposure.

Abnormal DMN functional connectivity has been found in cocaine (X. Ding & Lee, 2013), nicotine (Sutherland, McHugh, Pariyadath, & Stein, 2012) and heroin addiction (Ma et al., 2011). Compared with healthy controls, heroin users showed increased functional connectivity in the right hippocampus and decreased functional connectivity in the right dorsal anterior cingulate cortex and left caudate in the DMN (Ma et al., 2011). Similarly, decreased functional connectivity in the left precentral gyrus, hippocampus and left middle frontal gyrus has been reported in cocaine addicts (X. Ding & Lee, 2013).

Within the DMN network, we observed altered connectivity in the superior and middle frontal gyrus, anterior and median cingulate, middle temporal gyrus, inferior and middle occipital gyrus, precentral gyrus, vermic lobule VI and VIII. Many of these regions have been implicated in other forms of addiction. For instance, the right ventral striatum demonstrated increased connectivity to the right superior and middle frontal gyrus and cerebellum in pathological gambling patients (Koehler et al., 2013), whereas nicotine withdrawal was associated with increased connectivity in the superior frontal gyrus (Huang et al., 2014). Pathological gambling patients also demonstrated decreased connectivity from the right middle frontal gyrus to other prefrontal areas (Koehler et al., 2013). In cannabis-dependent adult volunteers, craving induced by visual cues was correlated with brain activation in the superior temporal pole (Charboneau et al., 2013). Subjects with Internet gambling addiction exhibited decreased resting connectivity in the right inferior temporal gyrus and cerebellum, and right precuneus connectivity was positively correlated with the severity of addiction (W. N. Ding et al., 2013). In an fMRI Go/No-Go task study, current and former cocaine users exhibited over-activation of the angular gyrus (Castelluccio, Meda, Muska, Stevens, & Pearlson, 2014). The duration of heroin use was correlated with altered bilateral connectivity in the cerebellum in heroin-dependent individuals (Yuan et al., 2010).

These findings suggest that abnormal functional organisation of the DMN in ketamine abusers may explain the abnormally increased memory processing but diminished cognitive control related to attention and self-monitoring, which may

underlie the hypersensitivity towards drug-related cues but weakened strength of cognitive control in ketamine addiction (Ma et al., 2011).

Limitations

First, this was a cross-sectional study and the possibility of pre-existing differences in cognitive functioning or psychiatric symptoms cannot be ruled out. Similarly, the reversibility of cognitive and structural changes in the brain is also uncertain. Prospective studies of participants at high risk of ketamine use or participants who become abstinent from ketamine would shed additional light on the abovementioned issue. Second, although the extent of alternate drug use in addition to ketamine was relatively low, we cannot rule out the effects of these compounds on the findings. Third, there were differences between the ketamine users and healthy controls in terms of age, sex and education level that were only partially solved by statistical adjustments.

Conclusions

In conclusion, this study provides imaging evidence of brain damage in chronic ketamine users. Chronic ketamine use was associated with reductions in both grey and white matter in certain regions of the brain. These volume reductions were related to the severity of ketamine dependence. Chronic ketamine use was also associated with altered functional connectivity in the DMN. Abnormal functional organisation of the network in ketamine abusers may underlie the hypersensitivity towards drug related cues but weakened cognitive control in ketamine addiction. Longitudinal or prospective studies are needed to strengthen the evidence on the reversibility of the structural and functional brain damage caused by ketamine.

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