Research report on

Change in cognitive function and biomarkers of neurotoxicity following abstinence of ketamine: a prospective longitudinal study

Submitted to

Beat Drug Fund Association

Submitted by

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Abbreviations

AD: Alzheimer's disease

ASI-Lite: Addiction Severity Index-Lite Version

BDI: Beck Depression Inventory

BDNF: Brain derived neurotropic factor

DACARS: Drug Addicts Counselling and Rehabilitation Services

δ: delta

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

GDNF: glial cell line-derived neurotrophic factor

HADSA: anxiety subscale of the Hospital Anxiety Depression Scale

κ: kappa

LTP: long term potentiation

NGF: nerve growth factor

NGOs: non-governmental organizations

NMDA: N-methyl D-aspartate

MCI: Mild Cognitive Impairment

μ: mu

p75NTR: p75 neurotrophin receptors

ROCF: Rey-Osterrieth Complex Figure

SDS: Severity of Dependence Scale

TrkB: tropomyosin receptor kinase B receptors

WCST: Wisconsin Card Sorting Test

WMS-III: Wechsler Memory Scale - Third edition

Introduction

1. Overview of recreational ketamine use

1.1 Recreational use of ketamine and its prevalence

1.1.1 Pharmacological effect

Ketamine was first developed in 1962 as an anaesthetic to replace phencyclidine. It is a non-competitive antagonist of the N-methyl D-aspartate (NMDA) receptor, a glutamate receptor that is a major excitatory neurotransmitter in the brain (Antagonism, 1996). The NMDA receptor plays an important role in synaptic plasticity, which is central to learning and memory (Morgan & Curran, 2012). Ketamine also has less interaction with mu (μ), delta (δ) and kappa (κ) opioid receptors, which activate dopamine release as an analgesic (Kegeles *et al.*, 2000). In addition, ketamine can increase endogenous acetylcholine concentrations by interacting with muscarinic acetylcholine receptors (Hustveit *et al.*, 1995). Ketamine can be used as an analgesic, anaesthetic and anti-depressant due to its pharmacological effect (Antagonism, 1996, Morgan & Curran, 2012).

1.1.2 Recreational use of ketamine

In addition to the above medical uses, ketamine's effect also appeals to recreational drug users. Non-medical use of ketamine began in the United States in the early 1970s (Huff & Roth, 2015), and increased until end of the century (Jansen, 1993). In Hong Kong, there was a rapid rise in recreational ketamine use as part of the dance culture at the end of the 1990s (Joe-Laidler & Hunt, 2008). At a low dose, ketamine can produce a dissociative state (Giannini *et al.*, 2000). At a high dose, users may experience 'K-hole', a state of extreme dissociation with visual and auditory hallucinations (Stewart, 2001). Ketamine is usually inhaled in a powder form, and is occasionally administered orally or by intra-muscular or intravenous injection (Dillon *et al.*, 2003, Morgan & Curran, 2012). In Hong Kong, 94% of ketamine abusers took the drug by sniffing (Narcotics Division, 2015). Many ketamine users in Hong Kong are also poly drug users (Winstock *et al.*, 2012), especially cocaine and methamphetamine (Narcotics Division, 2015).

1.1.3 Prevalence of recreational ketamine use

In a report by the United Nations Office on Drug Control, 58 countries and territories reported recreational ketamine use, including Australia, Brazil, the United Kingdom, Untied States and Hong Kong (United Nations Office on Drug Control, 2016). Lifetime prevalence of ketamine use was 1.5% among 12th grade students in the United States, 0.05–1.08% among university students in South America, 2.6% among 16–24-year olds in the United Kingdom in 2014 and 1.7% among those aged 14 and above in Australia in 2013 (United Nations Office on Drug Control, 2016). In Hong Kong, 0.03% of people aged 11 and above used ketamine in the previous year according to the Central Registry of Drug Abuse in 2014 (United Nations Office on Drug Control, 2016). In 2014, a quarter of all reported drug abusers in Hong Kong were ketamine users, ranking second in all types of drug abuse (Narcotics Division, 2015).

1.2 Harmful outcomes of ketamine use

1.2.1 Acute physical problems

Ketamine abusers present in the emergency department with acute clinical physical problems. Acute ketamine intoxication is the most common reason for emergency admission (Morgan & Curran, 2012). The typical syndrome involves confusion, dizziness, impaired consciousness or a reported transient period of loss of consciousness (Chan, 2012). In a retrospective study of 233 cases in Hong Kong, the most common symptoms of ketamine recreational use were impaired consciousness (45%), abdominal pain (21%), lower urinary tract symptoms (12%) and dizziness (12%) (Ng *et al.*, 2010). Ketamine has a wide therapeutic range, which makes death from overdose difficult (Kalsi *et al.*, 2011). Only 4 of the 23 deaths in which ketamine was identified at post-mortem were attributed to ketamine poisoning alone in the United Kingdom between 1993 and 2006 (Schifano *et al.*, 2008).

In addition to poisoning, ketamine also increases acute cardiac risks (Morgan & Curran, 2012). Ketamine stimulates the cardiovascular system, causing increased heart rate, cardiac output and blood pressure (Morgan & Curran, 2012). In a study of 188 acute ketamine abuse cases, 27% of abusers presented with cardiovascular features such as hypertension and tachycardia (heart rate >100 per minute) (Chan, 2012).

Ketamine also increases the risk of injury after falling into the ‘K-hole’, which is associated with agitation, aggression, paranoid and dissociative-type symptoms (Muetzelfeldt *et al.*, 2008). Users put

themselves at significant risk of injury through jumping from heights, road traffic accidents, drowning and hypothermia (Jansen, 2000).

1.2.2 Chronic physical and psychological problems

Physical damage induced by chronic ketamine use includes ulcerative cystitis, kidney dysfunction and 'K-cramps' (Morgan & Curran, 2012). Ketamine-induced ulcerative cystitis was first documented in 2007 and the symptoms include increased frequency and urgency of urination, dysuria, urge incontinence and occasionally painful haematuria (blood in urine), with marked thickening of the bladder wall, small bladder capacity and severe inflammation (Shahani *et al.*, 2007). The symptoms are alleviated after cessation of ketamine (Shahani *et al.*, 2007). Another physical problem is kidney dysfunction in the form of hydronephrosis. In a study of 59 ketamine abusers, 51% had hydronephrosis (Chu *et al.*, 2008). Furthermore, 'K-cramps', described as intense abdominal pain, are not rare. A third of 90 heavy ketamine users reported 'K-cramps' (Muetzelfeldt *et al.*, 2008) and a study in Hong Kong found that 18% of 233 ketamine abusers had abdominal tenderness (Ng *et al.*, 2010).

Ketamine induces psychological problems including depression and psychosis (Morgan & Curran, 2012). Long-term ketamine users suffer from depression (Liang *et al.*, 2014), and a longitudinal study found that ex-users still suffered from depression at 1-year follow up (Morgan *et al.*, 2010). Liang *et al.* (2015) recruited participants from substance abuse clinics in Hong Kong. The 129 ketamine users had used ketamine at least 24 times over 6 months within the past 2 years, and 65.1% of them had comorbid psychiatric disorders, most commonly substance-induced psychotic disorder (31.8%) followed by depressive disorder (27.9%). Chan *et al.* (2013) and Liang *et al.* (2014) reported significantly higher depression symptoms among chronic ketamine users than healthy controls (15.32 versus 7.47 and 22.2 versus 9.1, respectively), as measured by the Beck Depression Index. Ketamine can induce psychotic symptoms in healthy volunteers and recreational users with dissociative and schizotypal symptomatology (Curran & Morgan, 2000). Daily ketamine users scored higher on measures of delusions, dissociation and schizotypy than infrequent users and poly ketamine users (Curran & Morgan, 2000, Morgan *et al.*, 2010).

1.2.3 Dependence-related problems

Ketamine addiction is related to the interaction between NMDA receptor, μ-opioid receptor and non-opioid δ receptor effects (Herman *et al.*, 1995, Tanda *et al.*, 1997). Large-scale ketamine dependence studies are rare but there are some case reports. In a study of 90 ketamine users, 57% of frequent users, 43% of infrequent users and 60% of ex-ketamine users were assessed as addicted to ketamine (Muetzelfeldt *et al.*, 2008). Withdrawal symptoms following abstinence and tolerance are referred to as dependence-related problems. Withdrawal symptoms may exist for ketamine users (Morgan & Curran, 2012). Cravings are not usually reported in ketamine users, but a study showed that craving was a central problem for 28 out of 30 daily users (Morgan *et al.*, 2008). Studies have shown that repeated anaesthesia use of ketamine induced tolerance (Kissin *et al.*, 2000, Reich & Silvay, 1989). Increasing dosage was found in frequent ketamine users compared with the first use dosage (Morgan *et al.*, 2008).

1.2.4 Social harm of ketamine

Ketamine intoxication is associated with increased risk of accidental injuries. Ketamine impairs psychomotor performance such as hand-eye movement coordination and balance (Lofwall *et al.*, 2006), decreases attention and induces psychic sensations and sometimes hallucinations (Malhotra *et al.*, 1996). Thirty-six percent of partygoers admitted to driving after ketamine use (Riley *et al.*, 2001). A study examining the prevalence of ketamine use in fatal car-crash victims found that 9% of the drivers tested positive for ketamine (Cheng *et al.*, 2005).

2. Ketamine-induced cognitive impairment

2.1 Ketamine and cognitive function

Cognition is a process of acquiring knowledge and understanding through thoughts, experiences and the senses (Meyer *et al.*, 2010), including the mental processes of attention, language, memory, perception, problem solving, creativity and thinking (Tabassum & Oliveira, 2015). Ketamine use and the NMDA receptor hypofunction are associated with a range of effects on cognition and behaviour in both animals and humans, and preferentially affect the neural mechanisms that regulate the efficacy of memory encoding and consolidation into long-term storage (Newcomer & Krystal, 2001). Memory is the process by which

information is encoded, stored and retrieved (Morrison, 1998). The NMDA receptor is considered to be of vital importance in synaptic plasticity and neuronal learning such as long-term potentiation (LTP) (Newcomer & Krystal, 2001). LTP is involved in memory encoding and storage and also plays an important role in consolidation (Rison & Stanton, 1996). In summary, the hypofunctioning of NMDA receptors can produce specific forms of memory dysfunction.

2.2 Ketamine-induced cognitive impairment

The acute effects of ketamine have often been studied in healthy volunteers. In experimental settings, acute sub-anaesthetic doses of ketamine produce a reliable transient decrease in cognitive performance (Morgan & Curran, 2006). A study of 14 healthy subjects tested their memory before, during and after ketamine administration and found that ketamine had a dose-dependent effect on immediate recall of episodic memories (Krystal *et al.*, 1994). Morgan et al. 2004 tested 54 healthy volunteers after two doses (0.4, 0.8 mg/kg) of ketamine and found impairments in episodic, working and procedural memory.

Previous studies have shown impairments in episodic memory, working memory and executive function in chronic ketamine users (Curran & Morgan, 2000, Morgan *et al.*, 2004a, Morgan *et al.*, 2004b). Morgan et al. (2009) found that frequent ketamine users had impaired spatial working memory and pattern recognition memory compared with infrequent users, ex-users and poly drug users. Another study in Hong Kong found that verbal fluency, cognitive processing speed and verbal learning were impaired in ketamine users (Chan *et al.*, 2013). In poly drug ketamine users, our team found impairments predominantly in verbal and visual memory (Liang *et al.*, 2013).

According to a review by Morgan and Curran (2006), the cognitive impairments caused by ketamine may be reversible. A longitudinal study found that ex-ketamine users had no memory impairments after a year of abstinence from ketamine (Morgan *et al.*, 2010). Another 3-year longitudinal investigation observed that semantic memory impairments were reversible following a marked reduction in use (Morgan *et al.*, 2004b). However, the sample sizes were small and there were no normal control groups in these studies. The abstinence from ketamine use was based on self-report and was unsupervised. In Morgan's study (2004b), the ketamine users only reduced their usage rather than achieving actual abstinence.

3. Biomarkers of ketamine-induced neurotoxicity and cognitive impairment

3.1 Brief introduction to neurotrophic factors

The first member of the neurotrophin family, nerve growth factor (NGF), was identified in the early 1950s as a target-derived protein that promotes the survival and growth of neurons (Levi-Montalcini, 1987). Brain derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF), two other members of the neurotropic family, are widely accepted as regulating cell growth and the survival and maintenance of neurons during nervous system development (Li & Wolf, 2015).

BDNF is synthesised as a precursor in the endoplasmic reticulum, sorted in the Golgi and cleaved into mature BDNF (Bolaños & Nestler, 2004). The biological effect of BDNF exertion binds to both high-affinity tropomyosin receptor kinase B receptors (TrkB) and low-affinity p75 neurotrophin receptors (p75NTR), and the major synaptic functions are mediated by the TrkB receptor (Carvalho *et al.*, 2008, Lessmann *et al.*, 2003). BDNF is one of the most active neurotrophins in the hippocampus, cortex and basal forebrain – areas vital to learning, memory and higher thinking – and an important molecule for synaptic plasticity, learning and memory (Bekinschtein *et al.*, 2008, Tirassa, 2015). BDNF has the ability to cross the blood–brain barrier (Pan *et al.*, 1998). Animal studies suggest that BDNF concentrations in the central nervous system and serum are closely correlated (Halepoto *et al.*, 2014), offering the possibility that peripheral blood concentrations can be used as a possible biologic marker for ketamine abuse.

NGF is critical for the survival and maintenance of sympathetic and sensory neurons. Without it, these neurons undergo apoptosis (the process of cell death) (Chaldarov *et al.*, 2009). NGF could also be related to various psychiatric disorders such as dementia, depression, schizophrenia, autism, Rett syndrome, anorexia nervosa and bulimia nervosa (Chaldarov *et al.*, 2009).

The function of GDNF is to support the survival of dopaminergic and motor neurons in peripheral and central neurons (Airaksinen & Saarma, 2002). GDNF plays an essential role in the development of sympathetic and sensory neurons (Moore *et al.*, 1996), and has also been shown to promote the survival and re-growth of dopamine neurons in the adult brain following injury (Carnicella & Ron, 2009). GDNF is essential for the maintenance and survival of adult dopamine neurons (Pascual *et al.*, 2008). GDNF may be related to depression, bipolar disorder, schizophrenia and obsessive compulsive disorder (Tunca *et al.*, 2015).

Plasma GDNF levels were found to be decreased in depression and bipolar disorder, but were normal in obsessive compulsive disorder and schizophrenia (Tunca *et al.*, 2015).

3.2 Neurotrophic factors in substance abuse

Serum BDNF levels were found to be significantly increased in heroin-dependent patients during early withdrawal (1-7 days) (Zhang *et al.*, 2014). A study of 17 chronic ketamine users and 11 healthy controls found that BDNF levels were significantly higher in ketamine users (Ricci *et al.*, 2011). However, another study of 93 chronic ketamine dependence users showed that serum BDNF levels were lower than in 39 healthy controls who had never used any drugs (Ke *et al.*, 2014).

Zhang *et al.* (2014) found an increase in BDNF levels following 1 month of abstinence among heroin-dependent users. Another study found that BDNF serum levels increased after 2 weeks of abstinence in cocaine users, and there was a positive correlation between BDNF levels and the number of days of abstinence (Corominas-Roso *et al.*, 2013). Significant correlations have also been found between serum BDNF levels and the number of days of abstinence from alcohol, cocaine and methamphetamine (Nejtek *et al.*, 2011).

In a study of 17 chronic ketamine users who used ketamine more than 4 times a week, NGF serum levels 1.5 days after their last use were no different from those of 11 healthy controls (Ricci *et al.*, 2011). Another study of 93 ketamine users who used ketamine almost every day (6.1 days per week) found the NGF levels of ketamine users 9.3 days after their last use to be significantly lower than those of 39 healthy controls (Ke *et al.*, 2014). NGF serum levels were found to be decreased in both heroin and cocaine users (Angelucci *et al.*, 2007b). A study of 104 women with cocaine dependence found that NGF levels were still reduced after 3 weeks of detoxification (Viola *et al.*, 2014).

Long-lasting molecular and structural changes that occur in the mesolimbic dopaminergic neurons as a result of chronic exposure to drugs and alcohol are thought to underlie adverse behaviour such as compulsive drug seeking and relapse (Koskela *et al.*, 2016). Recent studies suggest that a subset of these changes may be prevented or reversed by GDNF (Ron & Janak, 2005). GDNF also regulates alcohol

consumption (Carnicella *et al.*, 2008). GDNF levels in heroin-dependent patients were not found to differ from those of healthy controls (Heberlein *et al.*, 2011).

GDNF blood levels were found to be significantly reduced in alcohol-dependent patients, and did not change during alcohol withdrawal (days 1, 7 and 14) (Heberlein *et al.*, 2010b). In patients with depressive disorder and comorbid benzodiazepine dependence, GDNF plasma levels did not differ from those of healthy controls at baseline and 7 days after abstinence, and did not change significantly after 7 days of abstinence (Heberlein *et al.*, 2010a). However, GDNF plasma levels increased following 3 weeks of detoxification in crack cocaine-dependent users (Viola *et al.*, 2014).

3.3 Neurotrophic factors and cognitive impairment

BDNF levels are considered to be related to cognitive dysfunction (Carlino *et al.*, 2013). In healthy subjects, BDNF was found to be involved in the regulation of psychomotor speed, working memory and executive function, measured by tasks that engage visuo-perceptual processes (Wiłkość *et al.*, 2016). BDNF was observed to be increased in patients with Alzheimer's disease and mild cognitive impairment, suggesting the up-regulation of BDNF in these two disease groups (Angelucci *et al.*, 2010b). Another study of patients with Alzheimer's disease also showed that higher BDNF serum levels predicted slower cognitive decline (Laske *et al.*, 2011). Vinogradov et al. (2009) found that serum BDNF levels served as a biomarker for cognitive enhancement in schizophrenia; although BDNF levels were lower in the patients than the healthy controls at baseline, they were significantly increased after 10 weeks of computerised cognition training. There has been no published study on the relationship between neurotrophic factors and cognitive impairments in chronic drug users, including ketamine users.

4. Possibility of reversibility of cognitive impairments induced by ketamine use

A 1-year longitudinal study tested frequent ketamine users and ex-ketamine users and found that ketamine-induced cognitive impairments were reversible (Morgan *et al.*, 2010). Another study found that semantic memory impairments were reversible after 3 years of reduced ketamine use (Morgan *et al.*, 2004b). Cognitive impairments induced by other drugs also show reversibility. Former cannabis smokers who were

abstinent for at least 3 months showed no cognitive impairments compared with never-users (Fried *et al.*, 2005), indicating full recovery. Cocaine abusers showed recovery of cognitive impairments following different lengths of abstinence (Bell *et al.*, 2011). Cognitive task performance in methamphetamine-dependent patients improved compared with controls after 4-9 days of abstinence (Simon *et al.*, 2010).

5. Hypotheses

We hypothesised that ketamine users would show cognitive impairments compared with healthy controls at baseline. We predicted that ketamine users would have lower levels of serum BDNF, GDNF and NGF than healthy controls. We predicted correlations between cognitive functions and serum levels of BDNF, GDNF and NGF.

After 12 weeks of supervised abstinence, cognitive function was predicted to improve in ketamine users (Aharonovich *et al.*, 2003). The serum BDNF, GDNF and NGF levels were predicted to increase in ketamine users. In addition, correlations were expected between changes in cognitive function and changes in serum BDNF, GDNF and NGF levels.

Methods

1. Design

The participants in this longitudinal study were recruited to three groups – primary ketamine, poly ketamine and healthy control – according to their drug abuse patterns. All ketamine users were staying in residential centres that offer 3-6 months of supervised detoxification for drug abusers. Cognitive functioning was compared between groups while controlling for common confounding factors such as age, gender and education level. The procedure for the study is illustrated in Figures 1 and 2. Participants in all groups were given a $250 coupon as compensation for attending the baseline assessment and a further $250 coupon for attending the same assessment at the 12-week follow up; the ketamine users were given a $100 coupon for two urine tests, one during the baseline assessment and one at the 12-week follow up. This study was approved by the Survey and Behavioural Research Ethics Committee of the Chinese University of Hong Kong.

2. Participants

2.1 Participant recruitment sites

The participants were recruited from non-governmental organisations in Hong Kong. Drug abusers were staying in residential detoxification centres that provide supervised abstinence from all drugs, while the normal controls were recruited from the Chinese University of Hong Kong, based on the inclusion and exclusion criteria. The participating organisations were as follows:

a. Caritas Wong Yiu Nam Center;
b. Christian New Being Fellowship - Life Training Base;
c. Drug Addicts Counselling and Rehabilitation Services (DACARS) - Enchi Lodge;
d. Hong Kong Christian Service - Jockey Club Lodge of the Rising Sun;
e. Hong Kong Christian Service - The Barnabas Charitable Service Association Limited;
f. Hong Kong Christian Service - Yuen Long District Youth Outreaching Social Work Team;
g. Hong Kong Lutheran Social Service Cheer Lutheran Center;

h. Operation Dawn Girl Center;
i. The Evangelical Lutheran Church of Hong Kong-Ling Oi Tan Ka Wan Centre;
j. The Evangelical Lutheran Church of Hong Kong, Enlighten Centre – Yuen Long;
k. The Society for the Aid and Rehabilitation of Drug Abusers - Adult Female Rehabilitation Centre; and
l. Shek Kwu Chau Treatment & Rehabilitation Centre;

2.2 Inclusion and exclusion 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. for the primary ketamine group, use of ketamine at least 24 times over 6 months within the last 2 years; the use of other illicit psychotropic drugs less than 24 times over 6 months within the last 2 years; use of ketamine in the previous month;
e. for the ketamine poly drug group, ketamine use together with another illicit psychotropic drug such as ecstasy, marijuana or methamphetamine at least 24 times during a 6-month period within the past 2 years; use of ketamine in the previous month; and
f. for the healthy group, no history of neurological or severe medical diseases, substance abuse or mental disorders.

Participants were not recruited if they met the following exclusion criteria:

a. unable to provide valid consent;
b. pregnant;
c. had a history of neurological, endocrinal or any other medical diseases or treatments known to affect the brain;

d. mental retardation;

e. reported history of a seropositive test for the human immunodeficiency virus; and

f. for the control subjects, a positive illicit drug urine test (all control subjects were screened).

3. Data collection

All ketamine users were assessed four times: at baseline, 4 weeks, 8 weeks and 12 weeks. The normal controls were assessed twice: at baseline and 12 weeks. At baseline, all participants were interviewed by trained research assistants who collected demographic and clinical data, conducted psychiatric and cognitive assessments and collected blood samples. The research assistants also performed a rapid urine test to detect any drug use. Each interview lasted 90–120 minutes, and included an assessment of substance use behaviour, screening for psychiatric comorbidity and a cognitive assessment. At the 4- and 8-week follow-up assessments, urine tests were performed in ketamine subjects. At the 12-week follow up, all ketamine subjects were assessed using the same cognitive battery as at baseline; blood samples were collected and a rapid urine test was performed (Table 1).

Table 1. An overview of the data collected from ketamine users

	Baseline	Week 4	Week 8	Week 12
Psychiatric assessment, demographic and clinical data collection	√			
Cognitive assessment	√			√
Urine rapid test	√	√	√	√
Blood collection	√			√

3.1 Demographic information

Demographic information included:

a. age,

b. sex,
c. level of education,
d. marital status,
e. employment status,
f. monthly income,
g. district of residence,
h. housing type, and
i. smoking status.

3.2 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 and support, drug and alcohol, legal, family and 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 problems in these areas.

The psychiatrists and trained RA 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.

3.3 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 (Lee, Yip, Chiu, Leung, & Chung, 2001).

The anxiety subscale of the Hospital Anxiety Depression Scale (HADSA) (Leung, Ho, Kan, Hung, & Chen, 1993) was used 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.

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. The psychiatrists and RA screened the same 20 participants with a kappa of 1.0, indicating that they were highly consistent in their assessment of whether a participant displayed possible psychiatric symptoms.

3.4 Cognition function evaluation

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

a. Executive function: the Stroop Test (Stroop, 1992), Go/NoGo (Rubia et al., 2001) and the Wisconsin Card Sorting Test (WCST; Heaton, Chelune, Talley, Kay, & Curtiss, 1993).
b. Attention and working memory: Digit Span Forward and Digit Span Backward (Wechsler, 1997).
c. Verbal memory: WMS–III logical memory immediate recall, delayed recall and recognition (Hua et al., 2005; D. Wechsler, 1997b).
d. Visual memory: the Rey-Osterrieth Complex Figure (ROCF) (ROCF; Osterrieth, 1944; Taylor, 1959).

A simple version of the Stroop Test (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: colour dot naming (part D), neutral coloured words (part W) and incongruently coloured words (part C). Each condition consists of 24 items and participants are required to name the colour of the dots in part D, the colour of the unrelated words in part W and the printed colour of the incongruently coloured words in part C. The number of errors and reaction times are recorded for each condition. Participants tend to take longer and make more errors in part C due to the activation of inhibitory processing or the interference effect. The

additional time in part C is attributable to the need to inhibit word reading or to resolve interference (Ludwig, Borella, Tettamanti, & de Ribaupierre, 2010), whereas the increased error rates are seen as an index of the temporal maintenance of the task goal (Kane & Engle, 2003). After standardising the scores, the number of errors in part C and the difference in reaction times between parts C and D provide the Stroop score for executive function.

The Go/NoGo test developed by Rubia et al. (2001) is an adaptation of Schacher and Logan's (1990) task. A motor response (pressing a button as fast as possible) is either initiated (Go) or inhibited (NoGo) depending on whether the stimulus that appears on the computer screen is a green square (Go) or a red square (NoGo). The visual stimuli appeared in a random order for 200 ms, with an inter-trial interval of 1600 ms. To increase the difficulty of the task, 70% of the stimuli were green squares (Go stimulus) and 30% red squares (NoGo stimulus). The task was administered in two blocks of 90 trials following an initial practice block to ensure adequate understanding of the task. Errors of omission (misses), errors of commission (false alarms) and the reaction time needed to correct trials during the experimental condition were recorded.

The WCST is composed of 4 stimulus cards and 64 response cards. The response cards differ in three dimensions: colour (red, green, yellow and blue), pattern (triangle, star, cross and circle) and number (one, two, three and 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 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 strings of 2 to 9 digits presented by the examiner and immediately repeat them back in the same order. In the backward mode, the 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. Sub-scores and a total score were generated for this test. The backward mode requires more working memory effort than the forward mode, and thus is more sensitive in detecting deficiencies (Tulsky *et al.*, 2003). The sub-scores for 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 logical memory subtests of the Wechsler Memory Scale – Third edition (WMS-III). The subtests consist of two stories (Stories A and B) and include three elements: immediate recall, delayed recall and recognition. 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 was asked 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 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 after 3 minutes (immediate recall) and again after 30 minutes (delayed recall), 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 (Taylor, 1959). After completing the delayed recall, the participant was shown 24 geometric items and asked to identify which of them were 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. The cognitive tests and maximum scores are listed in Table 2.

Table 2 Cognitive battery.

	Tests	Maximum score
Executive Function	Stroop	
	Reaction Time (seconds)	
	Color Dots	---
	Chinese Characters	---
	Color Words	---
	Number of Errors	
	Color Dots	---
	Chinese Characters	---
	Color Words	---
	Wisconsin Card Sorting Test	
	Number of Attempts Administered	6
Reward – or Impulse- related Function	Go/No Go	---
Attention and Working Memory	Digit Span	
	Forward	16
	Backward	14
	Total	30
Verbal Learning and Memory	Wechsler Memory Scale – III Logical Memory	
	Logical Memory I	
	Total Immediate Recall	50
	Logical Memory II	
	Delayed Recall	50
	Recognition	30
	Percent Retention	---
Visual Learning and Memory	Rey-Osterrieth Complex Figure	
	Copy	36
	Immediate Recall	36
	Delayed Recall	36
	Recognition Total Correct	24

3.5 Blood collection and biomarker analysis

Self-reports of no substance use and a negative urine test were used at baseline and each assessment (4, 8 and 12 weeks) to confirm abstinence status. Blood collection was performed at baseline and 12 weeks. To investigate the serum biomarker levels (BDNF, NGF, GDNF), blood from each subject was collected

between 10:30 am and 11:00 am. A total of 10 ml of blood was drawn from each subject into an EDTA-coated tube. The levels of serum biomarkers were determined using an ELISA protocol according to the manufacturers' instructions (DBD00; R&D Systems, Europe).

4. Statistical methods

Data analyses were performed using IBM SPSS 22.0. Continuous variables are described as mean ± SD, categorical variables as n (%) and skewed continuous variables as median (Quantile 1, Quantile 3). The primary ketamine and poly ketamine user groups were combined to form a group of all ketamine users (all ketamine group) in the analyses. Demographic characteristics, patterns of ketamine use and patterns of psychiatric problems at baseline were compared between all ketamine users and healthy control groups using independent t-tests, chi-square tests, Fisher's exact tests and Mann-Whitney U tests.

Demographic characteristics, patterns of ketamine and other drug and alcohol use, psychiatric problems among primary ketamine users, poly ketamine users and healthy controls at baseline were compared using ANOVA, post hoc comparisons with Bonferroni correction, the Kruskal-Wallis H test, chi-square test, Fisher's exact test and Mann-Whitney U test.

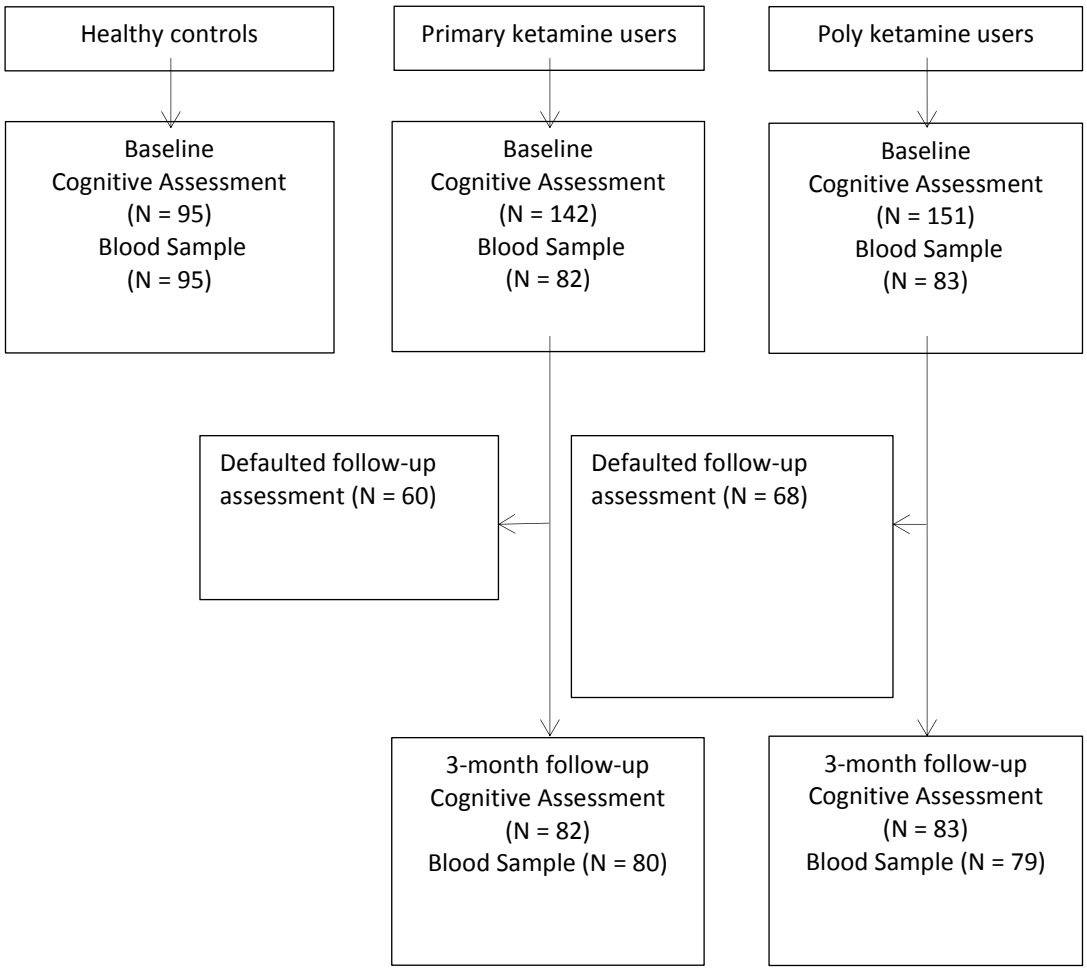
Psychiatric problems at baseline were compared between all ketamine users and healthy controls using independent t-tests, the Mann-Whitney U test, chi-square test and Fisher's exact test. BDI and HADSA scores for all ketamine users at baseline and 12 weeks were compared using paired t-tests. BDI and HADSA scores at baseline were compared between all ketamine users at 12 weeks and the healthy control group using independent t-tests. The above analyses were repeated for the primary ketamine users and poly ketamine users. Repeated measures ANOVA was used to compare primary and poly ketamine users at baseline and follow up. The post hoc analyses were performed with Bonferroni correction.

Cognitive function scores for the all ketamine group at baseline and 12 weeks were compared using paired t-tests and Wilcoxon signed rank tests. Cognitive function scores for the healthy control group at baseline and all ketamine group at baseline and 12 weeks were compared using independent t-tests and Mann-Whitney U tests. ANCOVA was used to analyse potential confounding influences including age, sex

and education. The above analyses were repeated for the primary ketamine users and poly ketamine users. Repeated measures ANOVA was used to compare primary and poly ketamine users at baseline and follow up. The post hoc analyses were performed with Bonferroni correction. The significance level was set at 0.05.

The serum levels of biomarkers (BDNF, NGF and GDNF) for the all ketamine group at baseline and 12 weeks were compared using paired t-tests. The serum levels of biomarkers (BDNF, NGF and GDNF) for the healthy control and all ketamine groups at baseline and 12 weeks were compared using independent t-tests. ANCOVA was used to analyse potential confounding influences including age, sex and BDI score. The above analyses were repeated for the primary ketamine users and poly ketamine users. Repeated measures ANOVA was used to compare primary and poly ketamine users at baseline and follow up. The post hoc analyses were performed with Bonferroni correction. Partial correlations were performed between serum levels of biomarkers (BDNF, NGF and GDNF) and cognitive task scores in the all/primary/poly ketamine user groups separately.

Figure 1. Study procedure.



Results

1. Demographics

The demographic characteristics of the all ketamine group and healthy control group are summarised in Table 3a. One hundred and sixty-five ketamine users and ninety-five healthy controls were recruited for the study. Despite effort of matching, there were differences in the demographic background between the ketamine users and healthy controls. Ketamine users were older than healthy controls (27.1 ± 4.3 versus 24.6 ± 5.7, $p<0.001$). There were more males in the ketamine group than the control group (72.1% versus 46.8%, $p<0.001$). The ketamine group also had significantly lower levels of education (9.6 ± 1.5 versus 14.2 ± 2.3, $p<0.001$) than the control group.

The demographic characteristics of the primary ketamine, poly ketamine and healthy control groups are summarised in Table 3b. Primary ketamine users were significantly older than controls (27.9 ± 3.7 versus 24.6 ± 5.7, $p<0.001$). There were significantly more males in the primary and poly ketamine groups than the control group (64.6% versus 46.3%, $p = 0.015$ and 79.3% versus 46.3%, $p<0.001$). The primary ketamine and poly ketamine groups had significantly lower levels of education (9.9 ± 1.3 versus 14.2 ± 2.3, $p<0.001$, 9.3 ± 1.6 versus 14.2 ± 2.3, $p<0.001$) than the control group.

Table 3a. Descriptive statistics of demographic characteristics between all ketamine users and healthy controls.

	All Ketamine Users (N = 165)	Healthy Control (N = 95)	p
Age	27.1 ± 4.3	24.6 ± 5.7	<0.001[a]
Sex (male), n (%)	119 (72.1)	44 (46.8)	<0.001[b]
Education (year)	9.6 ± 1.5	14.2 ± 2.3	<0.001[a]
Marital status (single)	131 (79.4)	83 (87.4)	0.176[c]

[a] independent t test; [b] chi-square test; [c] Fisher`s exact test; [d] Mann-Whitney U test.

Table 3b. Descriptive statistics of demographic characteristics among primary ketamine users, poly ketamine users and healthy controls.

	Primary Ketamine Users N = 82	Poly Ketamine Users N = 83	Healthy Control N = 95	p	p^1	p^2	p^3
Age	27.9 ± 3.7	26.4 ± 4.8	24.6 ± 5.7	<0.001[a]	<0.001[a]	0.042[a]	0.122[a]
Sex (male), n (%)	53 (64.6)	66 (79.5)	44 (46.3)	<0.001[b]	0.015[b]	<0.001[b]	0.037[b]
Education (year)	9.9 ± 1.3	9.3 ± 1.6	14.2 ± 2.3	<0.001[a]	<0.001[a]	<0.001[a]	0.077[a]
Marital status (single)	66 (80.5)	65 (78.3)	83 (87.4)	0.316[b]	-	-	-

[a] ANOVA, post hoc comparison with Bonferroni correction; [b] Chi-square test; [c] Fisher`s exact test; [d] Kruskal-Wallis H test; [e] Mann-Whitney U test.

p: comparisons among three study groups; p^1: primary ketamine users versus healthy control; p^2: poly ketamine users versus health control; p^3: primary ketamine users versus poly ketamine users.

2. Pattern of ketamine use

Among the 165 ketamine users, 94.5% and 70.9% were diagnosed with lifetime and current ketamine dependence. The average duration of ketamine use was 84.4 ± 46.7 months and the mean number of days of use was 9.5 ± 9.5 per month, or 581.9 ± 198.0 in the past 2 years. The mean SDS score was 8.8 ± 3.1 (Table 4a).

Poly ketamine users had a significantly earlier age of first exposure to ketamine use than primary users (16.4 ± 3.9 versus 17.8 ± 3.5, p = 0.017). No other differences were found between the ketamine groups' patterns of use (Table 4b).

Table 4a. Pattern of ketamine use between all ketamine group and healthy control group.

	All Ketamine Users (N = 165)	Healthy Control (N = 95)	p
Age of first ketamine use	17.1 ± 3.7	-	-
Duration of ketamine use (months)	84.4 ± 46.7	-	-
Days of ketamine use in the past two year	581.9 ± 198.0	-	-
Days of ketamine use in previous month (days)	9.5 ± 9.5	-	-
Lifetime diagnosis of ketamine dependence, n (%)	156 (94.5)	-	-
Lifetime diagnosis of ketamine abuse, n (%)	4 (2.4)	-	-
Current diagnosis of ketamine dependence, n (%)	117 (70.9)	-	-
Current diagnosis of ketamine abuse, n (%)	4 (2.4)	-	-
SDS score	8.8 ± 3.1	-	-

[a] independent t test; [b] chi square test; [c] Mann-Whitney U test.
ASI = The Addiction Severity Index – Lite Version; SDS = Severity of Dependence Scale.
Abuse refers to a less severe form of addiction, whereas dependence is a more severe form of addiction

Table 4b. Pattern of ketamine, alcohol use among among primary ketamine users, poly ketamine users and healthy controls.

	Primary Ketamine Users N = 82	Poly Ketamine Users N = 83	Healthy Control N = 95	p	p^1	p^2	p^3
Age of first ketamine use	17.8 ± 3.5	16.4 ± 3.9	-	0.016[a]	-	-	-
Duration of ketamine use (months)	84.3 ± 45.3	84.6 ± 48.4	-	0.965[a]	-	-	-
Days of ketamine use in the past 2 year	554.3 ± 213.3	609.1 ± 178.9	-	0.083[a]	-	-	-
Days of ketamine use in previous month (days)	8.6 ± 9.4	10.3 ± 9.7	-	0.267[a]	-	-	-
Lifetime diagnosis of ketamine dependence, n (%)	77 (93.9)	79 (95.2)	-	0.746[e]	-	-	-
Lifetime diagnosis of ketamine abuse, n (%)	3 (3.7)	1 (1.2)	-	0.367[e]	-	-	-
Current diagnosis of ketamine dependence, n (%)	56 (68.3)	61 (73.5)	-	0.496[b]	-	-	-
Current diagnosis of ketamine abuse, n (%)	3 (3.7)	1 (1.2)	-	0.367[e]	-	-	-
SDS	8.6 ± 3.2	9.0 ± 3.1	-	0.405[c]	-	-	-

[a] independent t test; [b] Chi-Square test; [c] Mann-Whitney U test; [d] Kruskal-Wallis H test; [e] Fisher exact test..

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

p: comparisons among three study group; p^1: primary ketamine users versus healthy control; p^2: poly ketamine users versus healthy control; p^3: primary ketamine users versus poly ketamine users.

Abuse refers to a less severe form of addiction, whereas dependence is a more severe form of addiction

3. Patterns of other drug use

The patterns of other drug use among all ketamine users are shown in Table 5. Any use of alcohol, cocaine, cannabis, ecstasy, hypnotics, methamphetamine and cough medicine was reported by 87.3%, 82.4%, 67.9%, 59.4%, 55.8%, 46.7% and 17.6% of all 165 ketamine users, respectively. The mean years of alcohol use was 9.6 and the mean days of alcohol use in the past month was 2.1. A lifetime diagnosis of dependence on cocaine, cannabis, ecstasy, hypnotics, methamphetamine and cough medicine was reported in 44.8%, 17.0%, 16.4%, 13.3%, 14.5% and 8.5% of ketamine users, respectively. The mean duration of other drug use ranged from 19.8 to 39.9 months, and the mean number of days of other drug use was 0.1–2.3 in the past month or 1.8–165.9 in the past 2 years. The most heavily misused drug in the past 2 years was cocaine (165.9 days), followed by cough medicine (39.3 days), ecstasy (28.5 days), cannabis (17.8 days) and hypnotics (12.8 days).

The patterns of other drug use among primary ketamine users are shown in Table 5. Lifetime use of alcohol, cocaine, cannabis, ecstasy, hypnotics, methamphetamine and cough medicine was reported by 90.2%, 74.4%, 63.4%, 54.9%, 46.3%, 42.7% and 9.8%, respectively. The mean duration of other drug use (other than alcohol) ranged from 9.9 to 34.3 months, and the mean number of days of drug use was 0 to 0.5 days in the past month or 0 to 20.9 days in the past 2 years. The most heavily used drugs in the past 2 years other than ketamine were ecstasy (28.5 days), cocaine (20.9 days), cannabis (5 days), methamphetamine (1.8 days) and hypnotics (0.8 days). Among primary ketamine users, 30.5%, 11.0%, 9.8%, 7.3%, 7.3% and 2.4% had a lifetime dependency on cocaine, ecstasy, cannabis, methamphetamine, hypnotics and cough medicine, respectively.

Any use and lifetime and current diagnoses of cocaine dependence were more common in the poly ketamine than the primary ketamine group (90.2% versus 74.4%, $p = 0.007$, 59.0% versus 30.5%, $p<0.001$ and 37.3% versus 6.1%, $p<0.001$). Poly ketamine users also had significantly more days of cocaine consumption in the past 2 years (268.6 ± 263.5 versus 20.9 ± 100.1, $p<0.001$) and past month (4.0 ± 7.0 versus 0.5 ± 2.5, $p<0.001$). Similarly, more poly ketamine users had lifetime diagnoses of cannabis dependence (24.1% versus 9.8%, $p = 0.013$) and abuse (22.9% versus 7.3%, $p = 0.008$), and current abuse (7.2% vs 0, $p = 0.028$). Poly ketamine users were also more likely to have a lifetime dependence on hypnotics (19.3% versus 7.3%, $p = 0.024$). Poly ketamine users were more likely to have a lifetime diagnosis of methamphetamine dependence (21.7% versus 7.3%, $p = 0.009$). Primary ketamine users had significantly longer duration of methamphetamine use than poly ketamine users (31.5 ± 27.6 vs 23.1 ± 16.8, $p = 0.013$). More poly ketamine users were diagnosed with any use (25.3% versus 9.8%, $p = 0.009$), lifetime (14.5% versus 2.4%, $p = 0.010$) and current dependence on cough medicine (7.2% versus 0%, $p = 0.028$). Finally, poly ketamine users had younger age of onset (18.4 ± 4.0 vs 18.6 ± 2.5, $p = 0.006$), longer duration (36.3 ± 33.1 vs 29.2 ± 47.5, $p = 0.009$) and more days of use of cough medicine in the past 2 years (60.8 ± 151.8 versus 0, $p = 0.002$). (Table 5)

Table 5. Patterns of other drug use in ketamine users.

	All Ketamine Users (N = 165)	Primary Ketamine Users (N = 82)	Poly Ketamine Users (N = 83)	p[a]
Any use of alcohol (Yes)	144 (87.3)	74 (90.2)	70 (85.3)	0.340[b]
Duration of alcohol use (years)	9.6 ± 6.2	9.9 ± 6.1	9.3 ± 6.4	0.506
Days of alcohol use in previous month (days)	2.1 ± 4.8	1.5 ± 3.7	2.7 ± 5.6	0.113
Any use of Cocaine (Yes)	136 (82.4)	61 (74.4)	74 (90.2)	0.007[b]
Age of first use	20.5 ± 4.3	21.2 ± 3.6	20.0 ± 4.8	0.091
Duration of use (months)	39.9 ± 35.1	32.4 ± 34.7	44.3 ± 34.8	0.097
Days of use in past 2 year	165.9 ± 244.0	20.9 ± 100.1	268.6 ± 263.5	<0.001
Days of use in past month	2.3 ± 5.5	0.5 ± 2.5	4.0 ± 7.0	<0.001
Lifetime diagnosis of dependence	74 (44.8)	25 (30.5)	49 (59.0)	<0.001[b]
Lifetime diagnosis of abuse	17 (10.3)	7 (8.5)	10 (12.0)	0.458[b]
Current diagnosis of dependence	36 (21.8)	5 (6.1)	31 (37.3)	<0.001[b]
Current diagnosis of abuse	8 (4.8)	4 (4.9)	4 (4.8)	1.000[c]
Any use of Cannabis (Yes)	112 (67.9)	52 (63.4)	60 (72.3)	0.222[b]
Age of first use	17.4 ± 3.7	17.6 ± 4.6	16.9 ± 3.5	0.147
Duration of use (months)	30.5 ± 35.0	34.3 ± 47.4	28.8 ± 28.2	0.661
Days of use in past 2 year	17.8 ± 87.5	5.0 ± 29.3	28.9 ± 115.8	0.177
Days of use in past month	0.2 ± 1.6	0 ± 0.2	0.3 ± 2.3	0.218
Lifetime diagnosis of dependence	28 (17.0)	8 (9.8)	20 (24.1)	0.014[b]
Lifetime diagnosis of abuse	25 (15.2)	6 (7.3)	19 (22.9)	0.008[b]
Current diagnosis of dependence	4 (2.4)	2 (2.1)	2 (2.4)	1.000[b]
Current diagnosis of abuse	6 (3.6)	0	6 (7.2)	0.028[b]

	All Ketamine Users (N = 165)	Primary Ketamine Users (N = 82)	Poly Ketamine Users (N = 83)	p[a]
Any use of Ecstasy (Yes)	98 (59.4)	45 (54.9)	53 (63.9)	0.269[b]
Age of first use	19.6 ± 4.7	19.3 ± 4.9	19.8 ± 4.6	0.635
Duration of use (months)	19.8 ± 28.7	9.9 ± 10.9	25.8 ± 34.2	0.454
Days of use in past 2 year	28.5 ± 114.9	11.3 ± 52.4	43.3 ± 148.1	0.162
Days of use in past month	0.1 ± 0.9	0.2 ± 1.3	0	0.320
Lifetime diagnosis of dependence	27 (16.4)	9 (11.0)	18 (22.0)	0.063[b]
Lifetime diagnosis of abuse	12 (7.3)	4 (4.9)	8 (9.8)	0.370[b]
Current diagnosis of dependence	2 (1.2)	1 (1.2)	1 (1.2)	1.000[b]
Current diagnosis of abuse	2 (1.2)	1 (1.2)	1 (1.2)	1.000[b]
Any use of Hypnotics (Yes)	92 (55.8)	38 (46.3)	54 (65.1)	0.015[b]
Age of first use	18.4 ± 3.6	19.1 ± 3.3	17.8 ± 3.8	0.112
Duration of use (months)	28.2 ± 27.4	28.3 ± 31.9	28.2 ± 25.4	0.984
Days of use in past 2 year	12.8 ± 78.9	0.8 ± 3.9	21.1 ± 102.4	0.015
Days of use in past month	0.2 ± 1.5	0.2 ± 1.3	0.3 ± 1.8	0.513
Lifetime diagnosis of dependence	22 (13.3)	6 (7.3)	16 (19.3)	0.024[b]
Lifetime diagnosis of abuse	16 (9.7)	5 (6.1)	11 (13.3)	0.120[b]
Current diagnosis of dependence	0	0	0	-
Current diagnosis of abuse	5 (3.0)	0	5 (6.0)	0.059[c]
Any use of Methamphetamine (Yes)	77 (46.7)	35 (42.7)	42 (50.6)	0.308[b]
Age of first use	16.8 ± 3.0	17.0 ± 2.8	16.7 ± 3.1	0.209
Duration of use (months)	26.4 ± 21.8	31.5 ± 27.6	23.1 ± 16.8	0.013
Days of use in past 2 year	1.8 ± 13.8	0.2 ± 1.5	2.9 ± 18	0.965
Days of use in past month	0.2 ± 1.2	0 ± 0.3	0.3 ± 1.6	0.319
Lifetime diagnosis of dependence	24 (14.5)	6 (7.3)	18 (21.7)	0.009[b]

	All Ketamine Users (N = 165)	Primary Ketamine Users (N = 82)	Poly Ketamine Users (N = 83)	p[a]
Lifetime diagnosis of abuse	20 (12.1)	7 (8.5)	13 (15.7)	0.161[b]
Current diagnosis of dependence	4 (2.4)	2 (2.4)	2 (2.4)	1.000[b]
Current diagnosis of abuse	4 (2.4)	1 (1.2)	3 (3.6)	0.620[c]
Any use of Cough medicine (Yes)	29 (17.6)	8 (9.8)	21 (25.3)	0.009[b]
Age of first use	18.4 ± 3.7	18.6 ± 2.5	18.4 ± 4.0	0.006
Duration of use (months)	35.0 ± 34.4	29.2 ± 47.5	36.3 ± 33.1	0.009
Days of use in past 2 year	39.3 ± 124.9	0	60.8 ± 151.8	0.002
Days of use in past month	0.5 ± 3.5	0	1.0 ± 5.0	0.065
Lifetime diagnosis of dependence	14 (8.5)	2 (2.4)	12 (14.5)	0.010[b]
Lifetime diagnosis of abuse	5 (3.0)	1 (1.2)	4 (4.8)	0.367[c]
Current diagnosis of dependence	6 (3.6)	0	6 (7.2)	0.028[b]
Current diagnosis of abuse	1 (0.6)	0	1 (1.2)	1.000[c]

[a] independent t test; [b] Chi-Square test; [c] Fisher`s exact test; [d] Kruskal-Wallis H test.

Comorbid psychiatric problems

Ketamine users had significantly higher BDI (14.6 ± 9.0 versus 4.8 ± 5.7, p<0.001) and HADSA (4.1 ± 3.4 versus 2.9 ± 2.9, p = 0.004) scores than healthy controls at baseline. Ketamine users were more likely to have current psychiatric disorders (20.0% versus 6.3%, p = 0.002), current or past mood disorder (20.0% versus 5.3%, p = 0.001), current depressive disorder (11.5% versus 2.1%, p = 0.007) and current dysthymia (6.1% versus 0%, p = 0.015). (Table 6a)

The BDI scores of ketamine users decreased at follow up (14.6 ± 9.0 versus 8.7 ± 8.0, p<0.001), but remained higher than those of the healthy control group (8.7 ± 8.0 versus 4.8 ± 5.7, p<0.001). The HADSA scores of ketamine users also decreased at follow up (4.1 ± 3.4 versus 3.3 ± 3.2, p = 0.002), and were similar to those of the healthy controls (Table 6a).

The primary ketamine users had significantly higher BDI scores than the healthy control group at baseline (14.4 ± 8.9 versus 4.8 ± 5.7, p<0.001). The primary ketamine users were more likely to have a current psychiatric diagnosis (22.5% versus 6.3%, p = 0.002), current or past mood disorder (22.0% versus 5.3%, p = 0.001), current depressive disorder (14.6% versus 2.1%, p = 0.002) and current dysthymia (6.1% versus 0%, p = 0.02) than the healthy controls (Table 6b). The BDI scores of the primary ketamine users decreased at follow up (14.4 ± 8.9 versus 8.8 ± 7.4, p<0.001), but remained higher than those of the healthy control group (8.8 ± 7.4 versus 4.8 ± 5.7, p<0.001) (Table 6b).

The poly ketamine users had significantly higher BDI (14.8 ± 9.1 versus 4.8 ± 5.7, p<0.001) and HADSA (4.5 ± 3.3 versus 2.9 ± 2.9, p<0.001) scores than the healthy control group at baseline. The poly ketamine users were more likely to have a current psychiatric diagnosis (18.1% versus 6.3%, p = 0.013), current or past mood

disorder (18.1% versus 5.3%, p = 0.006) and current dysthymia (6.0% versus 0%, p = 0.020) than the healthy controls. The BDI (14.8 ± 9.1 versus 8.8 ± 8.6, p<0.011) and HADSA (4.5 ± 3.3 versus 3.4 ± 3.4, p<0.001) scores of poly ketamine users decreased at follow up but remained higher than those of the healthy control group (8.7 ± 8.6 versus 4.8 ± 5.7, p<0.001) (Table 6c).

There was no significant difference between the primary and poly ketamine users in terms of BDI scores, HASDA scores or psychiatric diagnoses. The group effect was not significant whereas the time effect on BDI and HADSA scores was significant (p<0.001 and p = 0.002, respectively) (Table 6d).

Table 6a. Psychiatric problems across all ketamine users group and healthy control group.

	All Ketamine Users (N = 165)	p^1	Healthy Control (N = 95)	p^2	p^3
BDI score		<0.001[e]		<0.001[a]	<0.001[a]
Baseline	14.6 ± 9.0		4.8 ± 5.7		
12 weeks	8.7 ± 8.0		-		
HADSA score		0.002[e]		0.004[a]	0.393[a]
Baseline	4.1 ± 3.4		2.9 ± 2.9		
12 weeks	3.3 ± 3.2		-		
Previous visit in a psychiatric outpatient setting	0.5 ± 1.7		0.2 ± 1.0	0.185[b]	
Previous visit in a psychiatric inpatient setting	0.02 ± 0.1		0	0.159[b]	
Psychiatric screening with SCID					
Current psychiatric diagnosis, n (%)	33 (20.0)		6 (6.3)	0.002[c]	
Current or past mood disorder, n (%)	33 (20.0)		5 (5.3)	0.001[c]	
Current depressive disorders, n (%)	19 (11.5)		2 (2.1)	0.007[c]	
Previous depressive disorders, n (%)	9 (5.5)		2 (2.1)	0.337[d]	
Current dysthymia, n (%)	10 (6.1)		0	0.015[d]	
Current generalized anxiety disorders, n (%)	3 (1.8)		1 (1.1)	1.000[d]	

[a] Independent t test; [b] Mann-whiney u test; [c] chi-square test; [d] Fisher`s exact test; [e] pair t test.
p^1 comparisons between baseline and at 12 weeks of all ketamine users group; p^2 comparisons between all ketamine group and healthy control group at baseline; p^3 comparisons between all ketamine users group at 12 weeks and healthy control group at baseline.
BDI = Beck Depression Inventory; HADSA = Anxiety subscale of the Hospital Anxiety Depression Scale.

Table 6b. Psychiatric problems across primary ketamine users group and healthy control group.

	Primary Ketamine Users (N = 82)	p^1	Healthy Control (N = 95)	p^2	p^3
BDI score		<0.001[e]		<0.001[a]	<0.001[a]
Baseline	14.4 ± 8.9		4.8 ± 5.7		
12 weeks	8.8 ± 7.4		-		
HADSA score		0.075[e]		0.064[a]	0.661[a]
Baseline	3.8 ± 3.5		2.9 ± 2.9		
12 weeks	3.1 ± 3.1		-		
Previous visit in a psychiatric outpatient setting	0.4 ± 1.4		0.2 ± 1.0	0.417[d]	
Previous visit in a psychiatric inpatient setting	0.02 ± 0.2		0	0.159[d]	
Psychiatric screening with SCID					
Current psychiatric diagnosis, n (%)	18 (22.5)		6 (6.3)	0.002[c]	
Current or Past mood disorder, n (%)	18 (22.0)		5 (5.3)	0.001[e]	
Current depressive disorders, n (%)	12 (14.6)		2 (2.1)	0.002[c]	
Previous depressive disorders, n (%)	4 (4.9)		2 (2.1)	0.309[d]	
Current dysthymia disorders, n (%)	5 (6.1)		0	0.020[d]	
Current generalized anxiety disorders, n (%)	2 (2.4)		1 (1.1)	0.597[d]	

[a] Independent t test; [b] Mann-whiney u test; [c] chi-square test; [d] Fisher`s exact test; [e] pair t test.
p^1 comparisons between baseline and at 12 weeks of primary ketamine users group; p^2 comparisons between primary ketamine group and healthy control group at baseline; p^3 comparisons between primary ketamine users group at 12 weeks and healthy control group at baseline.
BDI = Beck Depression Inventory; HADSA = Anxiety subscale of the Hospital Anxiety Depression Scale.

Table 6c. Psychiatric problems across poly ketamine users group and healthy control group.

	Poly Ketamine Users (N = 83)	p^1	Healthy Control (N = 95)	p^2	p^3
BDI score		<0.001[e]		<0.001[a]	<0.001[a]
Baseline	14.8 ± 9.1		4.8 ± 5.7		
12 weeks	8.7 ± 8.6		-		
HADSA score		0.011[e]		0.001[a]	0.302[a]
Baseline	4.5 ± 3.3		2.9 ± 2.9		
12 weeks	3.4 ± 3.4		-		
Previous visit in a psychiatric outpatient setting	0.6 ± 2.0		0.2 ± 1.0	0.176[d]	
Previous visit in a psychiatric inpatient setting	0.01 ± 0.1		0	0.320[d]	
Psychiatric screening with SCID					
Current psychiatric diagnosis, n (%)	15 (18.1)		6 (6.3)	0.013[c]	
Current or Past mood disorder, n (%)	15 (18.1)		5 (5.3)	0.006[c]	
Current depressive disorders, n (%)	7 (8.4)		2 (2.1)	0.083[d]	
Previous depressive disorders, n (%)	5 (6.0)		2 (2.1)	0.252[d]	
Current dysthymia disorders, n (%)	5 (6.0)		0	0.020[d]	
Current generalized anxiety disorders, n (%)	1 (1.2)		1 (1.2)	1.000[c]	

[a] Independent t test; [b] Mann-whiney u test; [c] chi-square test; [d] Fisher`s exact test; [e] pair t test.

p^1 comparisons between baseline and at 12 weeks of poly ketamine users group; p^2 comparisons between poly ketamine group and healthy control group at baseline; p^3 comparisons between poly ketamine users group at 12 weeks and healthy control group at baseline.

BDI = Beck Depression Inventory; HADSA = Anxiety subscale of the Hospital Anxiety Depression Scale

Table 6d. Mood status and psychiatric problems across primary and poly ketamine users group.

	Primary Ketamine Users (N = 82)	Poly Ketamine Users (N = 83)	Group effect	Time effect	Group * time effect
BDI score			0.952[a]	<0.001	0.738
Baseline	14.4 ± 8.9	14.8 ± 9.1			
12 weeks	8.8 ± 7.4	8.7 ± 8.6			
HADSA score			0.307[a]	0.002	0.632
Baseline	3.8 ± 3.5	4.5 ± 3.3			
12 weeks	3.1 ± 3.1	3.4 ± 3.4			
Previous visit in a psychiatric outpatient setting	0.4 ± 1.4	0.6 ± 2.0	0.476[b]		
Previous visit in a psychiatric inpatient setting	0.0 ± 0.2	0.0 ± 0.1	0.577[b]		
Psychiatric screening with SCID					
Current psychiatric diagnosis, n (%)	18 (22.5)	15 (18.1)	0.531[c]		
Current or Past mood disorder, n (%)	18 (22.0)	15 (18.1)	0.559[c]		
Current depressive disorders, n (%)	12 (14.6)	7 (8.4)	0.222[c]		
Previous depressive disorders, n (%)	4 (4.9)	5 (6.0)	1.000[d]		
Current dysthymia disorders, n (%)	5 (6.1)	5 (6.0)	1.000[c]		
Current generalized anxiety disorders, n (%)	2 (2.4)	1 (1.2)	1.000[d]		

[a] Repeated measure ANOVA; [b] independent t test; [c] chi-square test; [d] Fisher`s exact test.
p^1 comparisons between baseline and at 12 weeks of primary ketamine users group; p^2 comparisons between primary and poly ketamine group at baseline; p^3 comparisons between primary and poly ketamine users group at 12 weeks.
BDI = Beck Depression Inventory; HADSA = Anxiety subscale of the Hospital Anxiety Depression Scale

4. Cognitive functioning

There were differences between cognitive functioning at baseline and follow up in the all ketamine group. Scores increased at follow up on the WAIS III Digit Span (backward, 9.0 ± 3.0 versus 9.2 ± 3.1, p = 0.017; total, 24.2 ± 3.8 versus 24.7 ± 3.4, p = 0.001); WMS III Logical Memory (immediate recall, 18.1 ± 8.2 versus 21.2 ± 7.5, p<0.001; delayed recall, 15.3 ± 7.8 versus 19.2 ± 7.6, p<0.001; recognition, 21.3 ± 4.1 versus 23.0 ± 3.5, p<0.001 and percent retention, 78.4 ± 27.0 versus 92.0 ± 51.5, p<0.001); ROCF (copy, 32.5 ± 3.1 versus 32.0 ± 3.5, p = 0.043; immediate recall, 19.1 ± 7.0 versus 23.8 ± 6.5, p<0.001, delayed recall, 19.3 ± 6.5 versus 23.4 ± 6.3, p<0.001; recognition, 20.3 ± 2.3 versus 20.9 ± 2.2, p = 0.001); WCST (total attempts, 96.9 ± 22.3 versus 89.4 ± 21.0, p<0.001; categories completed, 5.1 ± 1.7 versus 5.4 ± 1.4, p<0.001; perseverative errors, 12.9 ± 10.8 versus 9.7 ± 8.6, p<0.001); and Stroop Test (total reaction time, 49.7 ± 10.7 versus 45.9 ± 10.7, p<0.001). The most prominent improvements were on the WMS III Logical Memory (percent retention and delayed recall), WCST (perseverative errors) and ROCF (immediate recall), with 25.8%, 25.4%, 24.8% and 24.6% changes in scores, respectively. At baseline, the all ketamine group performed worse than the healthy controls on the WMS III Logical Memory (immediate recall, 18.1 ± 8.2 versus 26.1 ± 6.9, p = 0.001; delayed recall, 15.3 ± 7.8 versus 23.0 ± 7.4, p = 0.029 and recognition, 21.3 ± 4.1 versus 24.3 ± 3.1, p = 0.021) and ROCF (immediate recall, 19.1 ± 7.0 versus 24.4 ± 5.7, p = 0.038). There were no significant differences between the all ketamine group at follow up and healthy controls at baseline in terms of cognitive function, and the difference in ROCF (immediate recall) scores was of borderline significance (Table 7a).

Table 7a. Comparison of cognitive function for all ketamine users group and healthy control group.

	All ketamine users at baseline (N = 165)	All ketamine users at 12 weeks (N = 165)	% Change	p[1]	Healthy control at baseline (N = 95)	p[2]	p[3]
WAIS III Digit Span (Forward)	15.2 ± 1.4	16.1 ± 8.0	+5.9	0.133	15.5 ± 0.9	0.115	0.123
WAIS III Digit Span (Backward)	9.0 ± 3.0	9.2 ± 3.1	+2.1	0.017	10.4 ± 3.4	0.439	0.354
WAIS III Digit Span total	24.2 ± 3.8	24.7 ± 3.4	+1.9	0.001	26.0 ± 3.9	0.247	0.204
WMS III Logical Memory: immediate recall	18.1 ± 8.2	21.2 ± 7.5	+16.9	<0.001	26.1 ± 6.9	0.001	0.001
WMS III Logical Memory: delayed recall	15.3 ± 7.8	19.2 ± 7.6	+25.4	<0.001	23.0 ± 7.4	0.029	0.034
WMS III Logical Memory: recognition	21.3 ± 4.1	23.0 ± 3.5	+8.0	<0.001	24.3 ± 3.1	0.021	0.020
WMS III Logical Memory: percent retention	78.4 ± 27.0	92.0 ± 51.5	+25.8	<0.001	86.9 ± 15. 9	0.267	0.282
ROCF: copy	32.5 ± 3.1	32.0 ± 3.5	-1.6	0.250	33.5 ± 1.8	0.207	0.224
ROCF: immediate recall	19.1 ± 7.0	23.8 ± 6.5	+24.6	<0.001	24.4 ± 5.7	0.038	0.042
ROCF: delayed recall	19.3 ± 6.5	23.4 ± 6.3	+21.6	<0.001	24.6 ± 5.5	0.059	0.065
ROCF: recognition	20.3 ± 2.3	20.9 ± 2.2	+3.1	<0.001	20.7 ± 2.2	0.986	0.972
WCST: total attempts	96.9 ± 22.3	89.4 ± 21.0	-6.0	<0.001	82.4 ± 17.1	0.540	0.601
WCST: categories completed	5.1 ± 1.7	5.4 ± 1.4	+6.3	<0.001	5.8 ± 1.0	0.322	0.336
WCST: perseverative errors	12.9 ± 10.8	9.7 ± 8.6	-24.8	<0.00	8.0 ± 7.3	0.401	0.420

	All ketamine users at baseline (N = 165)	All ketamine users at 12 weeks (N = 165)	% Change	p^1	Healthy control at baseline (N = 95)	p^2	p^3
Stroop Test: interference (seconds)	9.8 ± 5.6	9.0 ± 5.1	-8.9	0.004	9.0 ± 4.8	0.794	0.808
Stroop Test: total reaction time (seconds)	49.7 ± 10.7	45.9 ± 10.7	-7.8	<0.001	45.2 ± 10.2	0.950	0.965
Stroop Test: total errors	2.2 ± 2.5	2.1 ± 2.5	-3.4	0.422	1.6 ± 1.8	0.341	0.371
GO/NOGO Test: reaction time	504.8 ± 110.6	501.3 ± 57.6	+2.2	0.684	499.7 ± 108.6	0.254	0.273
GO/NOGO Test: omission errors	7.6 ± 15.0	5.5 ± 9.5	+54.2	0.134	7.8 ± 16.7	0.627	0.619
GO/NOGO Test: commission errors	7.0 ± 5.3	6.3 ± 5.7	+17.5	0.191	6.8 ± 5.3	0.633	0.614

p^1: comparison of all ketamine users between baseline and 12 weeks, p^2: comparison between all ketamine users at baseline and healthy control group at baseline, p^3: comparison between all ketamine users at 12 weeks and healthy control group at baseline.

p^1: paired t test, p^2 and p^3: independent t test

p^2 and p^3: adjusted for age, gender, education, BDI score by ANCOVA.

There were differences between cognitive functioning at baseline and follow up in the primary ketamine group. Scores increased at follow up on the WAIS III Digit Span (forward, 15.1 ± 1.7 versus 15.5 ± 1.0, p = 0.030); WMS III Logical Memory (immediate recall, 17.5 ± 7.3 versus 22.1 ± 7.7, p<0.001; delayed recall, 14.5 ± 7.8 versus 20.2 ± 8.0, p<0.001; recognition, 20.7 ± 4.2 versus 23.2 ± 3.8, p<0.001 and percent retention, 76.0 ± 26.7 versus 87.6 ± 21.3, p = 0.001); ROCF (copy, 32.7 ± 2.6 versus 31.9 ± 4.0, p = 0.033; immediate recall, 18.7 ± 7.3 versus 23.8 ± 7.2, p<0.001; delayed recall, 19.1 ± 6.9 versus 23.4 ± 7.2, p<0.001; recognition, 20.2 ± 2.4 versus 21.0 ± 2.2, p = 0.007); WCST (total attempts, 98.0 ± 22.4 versus 91.5 ± 21.8, p = 0.003; categories completed, 5.0 ± 1.8 versus 5.3 ± 1.5, p = 0.018; perseverative errors, 13.2 ± 11.3 versus 10.4 ± 9.8, p = 0.005); and Stroop Test (total reaction time, 48.8 ± 10.8 versus 45.4 ± 10.5, p<0.001). The most prominent improvements were on the WMS III Logical Memory (immediate and delayed recall), ROCF (immediate and delayed recall) and WCST (perseverative errors), with 26.4%, 39.3%, 27.2%, 22.7% and 21.8% changes in scores, respectively. At baseline, the primary ketamine group performed worse than the healthy controls on the WMS III Digit Span total score (24.6 ± 3.9 versus 26.0 ± 3.9, p = 0.031) and WMS III Logical Memory (immediate recall, 17.5 ± 7.3 versus 26.1 ± 6.9, p = 0.004; recognition, 20.7 ± 4.2 versus 24.3 ± 3.1, p = 0.012 and possibly delayed recall (14.5 ± 7.8 versus 23.0 ± 7.4, p = 0.051). The only significant difference between primary ketamine users at follow up and healthy controls at baseline was in WMS III Digit Span total scores (15.1 ± 1.7 versus 15.5 ± 0.9, p = 0.025); the differences in WMS III Logical Memory (percent retention) and ROCF (immediate and recognition) scores were of borderline significance (Table 7b).

Table 7b. Comparison of cognitive function for primary ketamine users group and healthy controls.

	Primary ketamine users at baseline (N = 82)	Primary ketamine users at 12 weeks (N = 82)	% Change	p^1	Healthy control at baseline (N = 95)	p^2	p^3
WAIS III Digit Span (Forward)	15.1 ± 1.7	15.5 ± 1.0	+2.7	0.030	15.5 ± 0.9	0.110	0.025
WAIS III Digit Span (Backward)	9.5 ± 2.9	9.4 ± 3.2	-0.7	0.817	10.4 ± 3.4	0.051	0.140
WAIS III Digit Span total	24.6 ± 3.9	24.9 ± 3.4	+1.3	0.393	26.0 ± 3.9	0.031	0.051
WMS III Logical Memory: immediate recall	17.5 ± 7.3	22.1 ± 7.7	+26.4	<0.001	26.1 ± 6.9	0.004	0.803
WMS III Logical Memory: delayed recall	14.5 ± 7.8	20.2 ± 8.0	+39.3	<0.001	23.0 ± 7.4	0.051	0.463
WMS III Logical Memory: recognition	20.7 ± 4.2	23.2 ± 3.8	+12.2	<0.001	24.3 ± 3.1	0.012	0.379
WMS III Logical Memory: percent retention	76.0 ± 26.7	87.6 ± 21.3	+15.3	0.001	86.9 ± 15. 9	0.326	0.099
ROCF: copy	32.7 ± 2.6	31.9 ± 4.0	-2.5	0.033	33.5 ± 1.8	0.583	0.839
ROCF: immediate recall	18.7 ± 7.3	23.8 ± 7.2	+27.2	<0.001	24.4 ± 5.7	0.108	0.074
ROCF: delayed recall	19.1 ± 6.9	23.4 ± 7.2	+22.7	<0.001	24.6 ± 5.5	0.171	0.119
ROCF: recognition	20.2 ± 2.4	21.0 ± 2.2	+3.6	0.007	20.7 ± 2.2	0.987	0.094
WCST: total attempts	98.0 ± 22.4	91.5 ± 21.8	-6.6	0.003	82.4 ± 17.1	0.596	0.588
WCST: categories completed	5.0 ± 1.8	5.3 ± 1.5	+5.8	0.018	5.8 ± 1.0	0.720	0.369
WCST: perseverative errors	13.2 ± 11.3	10.4 ± 9.8	-21.8	0.005	8.0 ± 7.3	0.448	0.147
Stroop Test: interference (seconds)	9.8 ± 5.8	9.0 ± 4.8	-8.5	0.199	9.0 ± 4.8	0.403	0.425
Stroop Test: total reaction time (seconds)	48.8 ± 10.8	45.4 ± 10.5	-7.0	<0.001	45.2 ± 10.2	0.486	0.228

	Primary ketamine users at baseline (N = 82)	Primary ketamine users at 12 weeks (N = 82)	% Change	p^1	Healthy control at baseline (N = 95)	p^2	p^3
Stroop Test: total errors	2.1 ± 2.5	2.1 ± 2.57	+1.9	0.909	1.6 ± 1.8	0.227	0.387
GO/NOGO Test: reaction time	522.0 ± 164.4	501.4 ± 55.7	+3.3	0.992	499.7 ± 108.6	0.436	0.988
GO/NOGO Test: omission errors	6.5 ± 14.6	5.4 ± 10.2	+40.9	0.325	7.8 ± 16.7	0.976	0.400
GO/NOGO Test: commission errors	6.7 ± 5.4	7.0 ± 6.5	+40.0	0.834	6.8 ± 5.3	0.776	0.354

p^1: comparison of primary ketamine users between baseline and 12 weeks, p^2: comparison between primary ketamine users at baseline and healthy control group at baseline, p^3: comparison between primary ketamine users at 12 weeks and healthy controls at baseline.
p^1: paired t test, p^2 and p^3: independent t test
p^2 and p^3: adjusted for age, gender, education, BDI score by ANCOVA.

There were differences between cognitive functioning at baseline and follow up in the poly ketamine group. Scores increased at follow up on the WAIS III Digit Span total (23.9 ± 3.6 versus 24.5 ± 3.4, p = 0.028), WMS III Logical Memory (delayed recall, 16.0 ± 7.8 versus 18.1 ± 7.0, p = 0.007; recognition, 22.0 ± 3.8 versus 22.8 ± 3.2, p = 0.032; percent retention, 80.8 ± 27.2 versus 96.5 ± 69.6, p = 0.041 and possibly immediate recall (18.8 ± 9.0 versus 20.3 ± 7.2, p = 0.099); ROCF (immediate recall, 19.4 ± 6.6 versus 23.7 ± 5.6, p<0.001; delayed recall, 19.5 ± 6.1 versus 23.5 ± 5.3, p<0.001 and possibly recognition (20.4 ± 2.2 versus 20.9 ± 2.2, p = 0.054); WCST (total attempts, 95.9 ± 22.3 versus 87.4 ± 20.1, p<0.001; categories completed, 5.2 ± 1.6 versus 5.6 ± 1.3, p = 0.007 and perseverative errors, 12.4 ± 10.2 versus 9.0 ± 7.2, p<0.001); Stroop Test (total reaction time, 50.6 ± 10.5 versus 46.2 ± 10.9, p<0.001); and GO/NOGO Test (commission errors, 6.3 ± 5.6 versus 4.7 ± 4.6, p = 0.006). The most prominent improvements were in WMS III Logical Memory (delayed recall), ROCF (immediate recall), WMS III Logical Memory (immediate recall) and ROCF (delayed recall) scores, with 37.0%, 33.9%, 31.4% and 30.4% changes in scores, respectively. At baseline, the poly ketamine users performed worse than the healthy controls on the WMS III Logical Memory (immediate recall; 18.7 ± 9.0 versus 26.1 ± 6.9, p = 0.001; delayed recall, 16.0 ± 7.8 versus 23.0 ± 7.4, p = 0.038 and recognition, 22.0 ± 3.8 versus 24.3 ± 3.1, p = 0.018) and possibly the ROCF (immediate recall, 19.4 ± 6.6 versus 24.4 ± 5.7, p = 0.052; delayed recall, 19.5 ± 6.1 versus 24.6 ± 5.5, p = 0.089) and Stroop Test (total reaction time, 50.6 ± 10.5 versus 45.2 ± 10.2, p = 0.098). There were no significant differences between poly ketamine users at follow up and healthy controls at baseline in terms of cognitive function, except the WMS III Logical Memory (immediate recall, 20.2 ± 7.2 versus 26.1 ± 6.9, p = 0.010) (Table 7c).

Table 7c. Comparison of cognitive function for poly ketamine users group and healthy controls.

	Poly ketamine users at baseline (N = 83)	Poly ketamine users at 12 weeks (N = 83)	% Change	p^1	Healthy controls at baseline (N = 95)	p^2	p^3
WAIS III Digit Span (Forward)	15.3 ± 1.1	16.7 ± 1.3	+8.9	0.273	15.5 ± 0.9	0.202	0.792
WAIS III Digit Span (Backward)	8.5 ± 3.0	9.0 ± 3.0	+14.9	0.070	10.4 ± 3.4	0.761	0.546
WAIS III Digit Span total	23.9 ± 3.6	24.5 ± 3.4	+3.7	0.028	26.0 ± 3.9	0.936	0.303
WMS III Logical Memory: immediate recall	18.7 ± 9.0	20.2 ± 7.2	+31.4	0.099	26.1 ± 6.9	0.001	0.010
WMS III Logical Memory: delayed recall	16.0 ± 7.8	18.1 ± 7.0	+37.0	0.007	23.0 ± 7.4	0.038	0.124
WMS III Logical Memory: recognition	22.0 ± 3.8	22.8 ± 3.2	+5.8	0.032	24.3 ± 3.1	0.018	0.107
WMS III Logical Memory: percent retention	80.8 ± 27.2	96.5 ± 69.6	+28.0	0.041	86.9 ± 15. 9	0.656	0.719
ROCF: copy	32.3 ± 3.5	32.1 ± 3.0	+0.8	0.457	33.5 ± 1.8	0.387	0.407
ROCF: immediate recall	19.4 ± 6.6	23.7 ± 5.6	+33.9	<0.001	24.4 ± 5.7	0.052	0.297
ROCF: delayed recall	19.5 ± 6.1	23.5 ± 5.3	+30.4	<0.001	24.6 ± 5.5	0.089	0.218
ROCF: recognition	20.4 ± 2.2	20.9 ± 2.2	+3.4	0.054	20.7 ± 2.2	0.945	0.689
WCST: total attempts	95.9 ± 22.3	87.4 ± 20.1	-7.2	<0.001	82.4 ± 17.1	0.353	0.624
WCST: categories completed	5.2 ± 1.6	5.6 ± 1.3	+9.9	0.007	5.8 ± 1.0	0.412	0.240
WCST: perseverative errors	12.4 ± 10.2	9.0 ± 7.2	-6.0	<0.001	8.0 ± 7.3	0.935	0.322
Stroop Test: interference (seconds)	9.9 ± 5.5	8.9 ± 5.5	-25.6	0.134	9.0 ± 4.8	0.215	0.937
Stroop Test: total reaction time (seconds)	50.6 ± 10.5	46.2 ± 10.9	-8.2	<0.001	45.2 ± 10.2	0.098	0.831
Stroop Test: total errors	2.2 ± 2.5	2.1 ± 2.4	-8.3	0.621	1.6 ± 1.8	0.821	0.819
GO/NOGO Test: reaction time	512.4 ± 148.8	502.8 ± 59.7	+1.3	0.557	499.7 ± 108.6	0.165	0.372
GO/NOGO Test: omission errors	6.8 ± 12.2	4.9 ± 7.8	+66.9	0.262	7.8 ± 16.7	0.288	0.531

	Poly ketamine users at baseline (N = 83)	Poly ketamine users at 12 weeks (N = 83)	% Change	p^1	Healthy controls at baseline (N = 95)	p^2	p^3
GO/NOGO Test: commission errors	7.2 ± 5.5	5.4 ± 4.5	-7.1	0.004	6.8 ± 5.3	0.596	0.255

p^1: comparison of poly ketamine users between baseline and 12 weeks, p^2: comparison between poly ketamine users at baseline and healthy control group at baseline, p^3: comparison between poly ketamine users at 12 weeks and healthy controls at baseline.
p^1: paired t test, p^2 and p^3: independent t test.
p^2 and p^3: adjusted for age, gender, education and BDI score by ANCOVA.

There was no significant group effect between primary and poly ketamine users. A main effect of time was found on WMS III Logical Memory (immediate recall ($F(1, 162) = 28.26$, $p<0.001$), delayed recall($F(1,161) = 55.34$, $p<0.001$), recognition ($F(1,161) = 29.24$, $p<0.001$), percent retention ($F(1,161) = 10.98$, $p = 0.001$)); ROCF (copy ($F(1, 162) = 4.15$, $p = 0.043$), immediate recall ($F(1, 162) = 114.59$, $p<0.001$), delayed recall ($F(1, 162) = 111.41$, $p<0.001$), recognition ($F(1, 161) = 11.03$, $p = 0.001$)); WCST (total attempts ($F(1, 162) = 26.66$, $p<0.001$), categories completed ($F(1, 162) = 13.39$, $p<0.001$), preservative errors ($F(1, 162) = 13.39$, $p<0.001$)); Stroop Test (total reaction time ($F(1, 161) = 37.43$, $p<0.001$)); and the GO/NOGO test (omission errors ($F(1, 134) = 6.61$, $p = 0.011$)). A significant group x time interaction emerged on the WMS III Logical Memory (immediate recall ($F(1,162) = 7.18$, $p = 0.008$), delayed recall ($F(1,161) = 12.55$,$p = 0.001$), recognition ($F(1, 161) = 7.02$, $p = 0.009$) (Table 7d). Post hoc analyses demonstrated that this was due to significantly lower recognition scores in primary ketamine users ($p = 0.044$) at baseline, but there was no difference between the groups at follow up.

Table 7d. Comparison of cognitive function for primary and poly ketamine users groups.

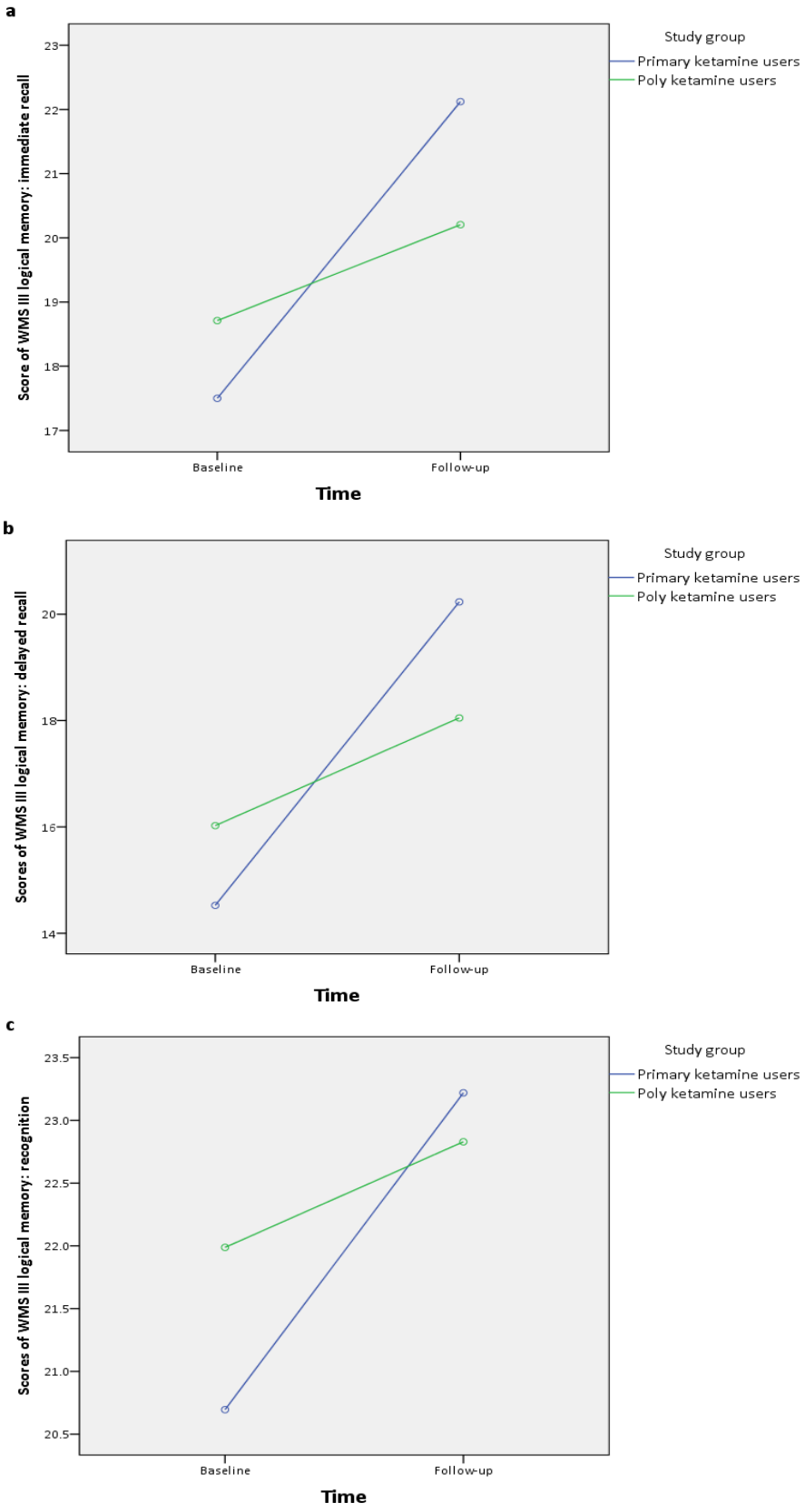
	Primary ketamine users (N = 82)	Poly ketamine users (N = 83)	Group effect	Time effect	Group * time effect	p^1	p^2	p^3	p^4
WAIS III Digit Span (Forward)			0.265	0.161	0.447	-	-	-	-
Baseline	15.1 ± 1.7	15.3 ± 1.1							
12 weeks follow-up	15.5 ± 1.0	16.7 ± 11.3							
WAIS III Digit Span (Backward)			0.130	0.302	0.168	-	-	-	-
Baseline	9.46 ± 2.91	8.5 ± 3.0							
12 weeks follow-up	9.39 ± 3.15	9.0 ± 3.0							
WAIS III Digit Span total			0.288	0.043	0.505	-	-	-	-
Baseline	24.6 ± 3.9	23.9 ± 3.6							
12 weeks follow-up	24.9 ± 3.4	24.5 ± 3.4							
WMS III Logical Memory: immediate recall			0.744	<0.001	0.007	<0.001	0.067	0.345	0.101
Baseline	17.5 ± 7.3	18.7 ± 9.0							
12 weeks follow-up	22.1 ± 7.7	20.2 ± 7.2							
WMS III Logical Memory: delayed recall			0.751	<0.001	0.001	<0.001	0.006	0.220	0.063
Baseline	14.5 ± 7.8	16.0 ± 7.8							
12 weeks follow-up	20.2 ± 8.0	18.1 ± 7.0							
WMS III Logical Memory: recognition			0.366	<0.001	0.008	<0.001	0.058	0.040	0.475
Baseline	20.7 ± 4.2	22.0 ± 3.8							
12 weeks follow-up	23.2 ± 3.8	22.8 ± 3.2							
WMS III Logical Memory: percent retention			0.166	0.001	0.617	-	-	-	-
Baseline	76.0 ± 26.7	80.8 ± 27.3							

	Primary ketamine users (N = 82)	Poly ketamine users (N = 83)	Group effect	Time effect	Group * time effect	p[1]	p[2]	p[3]	p[4]
12 weeks follow-up	87.6 ± 21.3	96.5 ± 69.6							
ROCF: copy			0.909	0.035	0.259	-	-	-	-
Baseline	32.7 ± 2.6	32.3 ± 3.5							
12 weeks follow-up	31.9 ± 4.0	32.1 ± 3.0							
ROCF: immediate recall			0.772	<0.001	0.366	-	-	-	-
Baseline	18.7 ± 7.3	19.4 ± 6.6							
12 weeks follow-up	23.8 ± 7.2	23.7 ± 5.6							
ROCF: delayed recall			0.778	<0.001	0.703	-	-	-	-
Baseline	19.1 ± 6.9	19.5 ± 6.1							
12 weeks follow-up	23.4 ± 7.2	23.5 ± 5.3							
ROCF: recognition total correct			0.852	0.001	0.594	-	-	-	-
Baseline	20.2 ± 2.4	20.4 ± 2.2							
12 weeks follow-up	21.0 ± 2.2	20.9 ± 2.2							
WCST: total attempts			0.288	<0.001	0.796	-	-	-	-
Baseline	98.0 ± 22.4	95.9 ± 22.3							
12 weeks follow-up	91.5 ± 21.8	87.4 ± 20.1							
WCST: categories completed			0.415	<0.001	0.678	-	-	-	-
Baseline	5.0 ± 1.8	5.2 ± 1.6							
12 weeks follow-up	5.3 ± 1.5	5.6 ± 1.3							
WCST: perseverative errors			0.322	<0.001	0.487	-	-	-	-
Baseline	13.2 ± 11.3	12.4 ± 10.2							
12 weeks follow-up	10.4 ± 9.8	9.0 ± 7.2							
Stroop Test: interference (seconds)			0.989	0.049	0.873	-	-	-	-
Baseline	9.8 ± 5.8	9.9 ± 5.5							
12 weeks follow-up	9.0 ± 4.8	8.9 ± 5.5							

	Primary ketamine users (N = 82)	Poly ketamine users (N = 83)	Group effect	Time effect	Group * time effect	p^1	p^2	p^3	p^4
Stroop Test: total reaction time (seconds)			0.413	<0.001	0.405	-	-	-	-
Baseline	48.8 ± 10.8	50.6 ± 10.5							
12 weeks follow-up	45.4 ± 10.5	46.2 ± 10.9							
Stroop Test: total errors			0.849	0.787	0.665	-	-	-	-
Baseline	2.1 ± 2.5	2.2 ± 2.5							
12 weeks follow-up	2.1 ± 2.6	2.1 ± 2.4							
GO/NOGO Test: reaction time			0.601	0.687	0.677	-	-	-	-
Baseline	522.0 ± 164.4	509.8 ± 113.2							
12 weeks follow-up	501.4 ± 55.7	504.0 ± 60.8							
GO/NOGO Test: omission errors			0.822	0.136	0.960	-	-	-	-
Baseline	6.5 ± 14.6	6.3 ± 12.1							
12 weeks follow-up	5.4 ± 10.2	5.5 ± 11.7							
GO/NOGO Test: commission errors			0.491	0.164	0.083	-	-	-	-
Baseline	6.7 ± 5.4	6.3 ± 5.6							
12 weeks follow-up	7.0 ± 6.5	4.7 ± 4.6							

p^1: comparison of primary ketamine users between baseline and 12 weeks, p^2: comparison between poly ketamine users at baseline and 12 weeks, p^3: comparison between primary and poly ketamine users at baseline; p^4: comparison between primary and poly ketamine users at 12 weeks

Figure 2 Plots of performance on WMS III logical memory test between primary and poly ketamine users



5. Biomarkers

Serum GDNF levels in 159 ketamine users and 95 healthy controls were analysed. The serum level of NGF was too low to be detected in 97 ketamine users and 54 healthy controls at baseline. Only four ketamine users and ten healthy controls had a detectable level of serum GNDF at baseline. There were no significant differences in serum BNDF and NGF levels between baseline and follow up in ketamine users, and the difference in GDNF levels was of borderline significance (p = 0.06). There were no significant differences in serum BDNF, NGF and GDNF levels between ketamine users and healthy controls at either baseline or follow up (Table 8a). It is worth noting that the sample size for GDNF was very small.

Table 8a. Comparison of level of biomarkers for all ketamine users group and healthy control group.

	All ketamine users at baseline	All ketamine users at 12 weeks	p^1	Healthy control at baseline	p^2	p^3
Serum BDNF level (ng/L)	N = 159	N = 159		N = 95		
	60432.4 ± 48468.3	58277.7 ± 36212.6	0.385	48468.3 ± 33828.4	0.234	0.351
Serum NGF level (ng/L)	N = 62	N = 67		N = 41		
	13.5 ± 10.3	12.5 ± 8.9	0.661	13.2 ± 10.1	0.483	0.564
Serum GDNF level (ng/L)	N = 4	N = 3		N = 10		
	165.8 ± 86.3	226.9 ± 83.8	0.060	365.6 ± 298.7	0.719	0.824

p^1: comparison of all ketamine users between baseline and 12 weeks, p^2: comparison between all ketamine users at baseline and healthy control group at baseline, p^3: comparison between all ketamine users at 12 weeks and healthy control group at baseline.

p^1: paired t test, p^2 and p^3: independent t test
p^2 and p^3: adjusted for age, gender, BDI score by ANCOVA.

Serum GDNF levels were analysed in 80 primary ketamine users. The level was too low to be detected in 45 of them. Only two primary ketamine users had a detectable level of serum GNDF at baseline. There were no significant differences between baseline and follow up serum BNDF, NGF and GDNF levels in primary ketamine users. At baseline, a borderline difference was found between the serum BDNF levels of primary ketamine users and healthy controls (65916.1 ± 41851.3 versus 48468.3 ± 33828.4, p = 0.068). There was no significant differences in the serum NGF and GDNF levels of primary ketamine users and healthy controls. At follow up, no significant differences were found in serum BNDF, NGF and GDNF levels between primary ketamine users and healthy controls (Table 8b). It is worth noting that the sample size for GDNF was very small.

Table 8b. Comparison of level of biomarkers for primary ketamine users group and healthy control group.

	Primary ketamine users at baseline	Primary ketamine users at 12 weeks	p^1	Healthy control at baseline	p^2	p^3
Serum BDNF level (ng/L)	N = 80	N = 80		N = 95		
	65916.1 ± 41851.3	64583.1 ± 42085.0	0.689	48468.3 ± 33828.4	0.068	0.133
Serum NGF level (ng/L)	N = 35	N = 40		N = 41		
	13.3 ± 9.0	12.6 ± 9.3	0.661	13.2 ± 10.1	0.711	0.381
Serum GDNF level (ng/L)	N = 2	N = 2		N = 10		

	Primary ketamine users at baseline	Primary ketamine users at 12 weeks	p^1	Healthy control at baseline	p^2	p^3
Serum BDNF level (ng/L)	N = 80	N = 80		N = 95		
	230.8 ± 73.5	270.9 ± 49.6	0.255	365.6 ± 298.7	0.831	0.726

p^1: comparison of primary ketamine users between baseline and 12 weeks, p^2: comparison between primary ketamine users at baseline and healthy control group at baseline, p^3: comparison between primary ketamine users at 12 weeks and healthy controls at baseline.
p^1: paired t test, p^2 and p^3: independent t test
p^2 and p^3 adjusted for age, gender and BDI score by ANCOVA.

Serum GDNF levels in 79 poly ketamine users were analysed. The serum level of NGF level was too low to be detected in 52 poly ketamine users at baseline. Only two poly ketamine users had a detectable level of serum GNDF at baseline. There were no significant differences in serum BNDF and NGF levels between baseline and follow up in poly ketamine users. There were no significant differences in serum BDNF, NGF and GDNF levels between poly ketamine users and healthy controls at either baseline or follow up (Table 8c). It is worth noting that the sample size for GDNF was very small.

Table 8c. Comparison of level of biomarkers for poly ketamine users group and healthy control group.

	Poly ketamine users at baseline	Poly ketamine users at 12 weeks	p^1	Healthy control at baseline	p^2	p^3
Serum BDNF level (ng/L)	N = 79	N = 79		N = 95		
	54879.3 ± 35025.5	51892.6 ± 27935.7	0.420	48468.3 ± 33828.4	0.722	0.614
Serum NGF	N = 27	N = 27		N = 41		

	Poly ketamine users at baseline	Poly ketamine users at 12 weeks	p^1	Healthy control at baseline	p^2	p^3
Serum BDNF level (ng/L) level (ng/L)	N = 79	N = 79		N = 95		
	13.8 ± 11.9	12.5 ± 8.4	0.710	13.2 ± 10.1	0.662	0.980
Serum GDNF level (ng/L)	N = 2	N = 1		N = 10		
	100.8 ± 3.5	139.0	-	365.6 ± 298.7	0.603	0.723

p^1: comparison of poly ketamine users between baseline and 12 weeks, p^2: comparison between poly ketamine users at baseline and healthy control group at baseline, p^3: comparison between poly ketamine users at 12 weeks and healthy controls at baseline.
p^1: paired t test, p^2 and p^3: independent t test.
p^2 and p^3: adjusted for age, gender and BDI score by ANCOVA.

The comparison of BDNF levels in primary and poly ketamine users showed a significant group effect (p = 0.028), whereas the time effect and group x time interaction effect were not significant. There were no significant group or time effects on NGF and GNDF levels (Table 8d).

Table 8d. Comparison of level of biomarkers for primary and poly ketamine users groups.

	Primary ketamine users	Poly ketamine users	Group effect	Time effect	Group x time effect
Serum BDNF level (ng/L)	N = 80	N = 79	0.028	0.385	0.739
Baseline	65916.1 ± 41851.3	54879.3 ± 35025.5			
12 weeks follow-up	68303.6 ± 41620.5	51708.6 ± 28064.5			
Serum NGF level (ng/L)	N = 28	N = 21	0.754	0.645	0.828
Baseline	14.7 ± 9.5	14.3 ± 13.0			
12 weeks follow-up	14.3 ± 10.4	13.1 ± 9.5			

	Primary ketamine users	Poly ketamine users	Group effect	Time effect	Group x time effect
Serum GDNF level (ng/L)	N = 2	N = 1	0.335	0.235	0.906
Baseline	230.8 ± 73.5	103.3			
12 weeks follow-up	270.9 ± 50.0	139.0			

6. Correlations between biomarkers and cognitive functions

At baseline, the all ketamine group showed a significant partial correlation between serum BDNF levels and the number of categories completed on the WCST ($r = -0.167$, $p = 0.039$) (Figure 3), but not between serum BDNF and scores on any other cognitive tasks. There were no significant partial correlations between serum NGF levels and scores on any cognitive tasks. Due to the small number of ketamine users with a detectable GDNF level, partial correlations were not performed between GDNF level and cognitive task scores.

At follow up, significant partial correlations were found between serum BDNF levels and WCST scores (total attempts, $r = 0.173$, $p = 0.032$, Figure 4; categories completed, $r = -0.226$, $p = 0.005$, Figure 5). There were no significant correlations between serum BDNF levels and scores on any other cognitive tasks. A significant partial correlation was found between serum NGF levels and the GO/NOGO Test reaction times ($r = -0.349$, $p = 0.010$) (Figure 6). There were no significant partial correlations between serum NGF levels and scores on other cognitive tasks.

Figure 3. Partial correlation between serum BDNF level and WCST: categories completed in all ketamine users group at baseline (N = 157) (r = -0.167, p = 0.039).

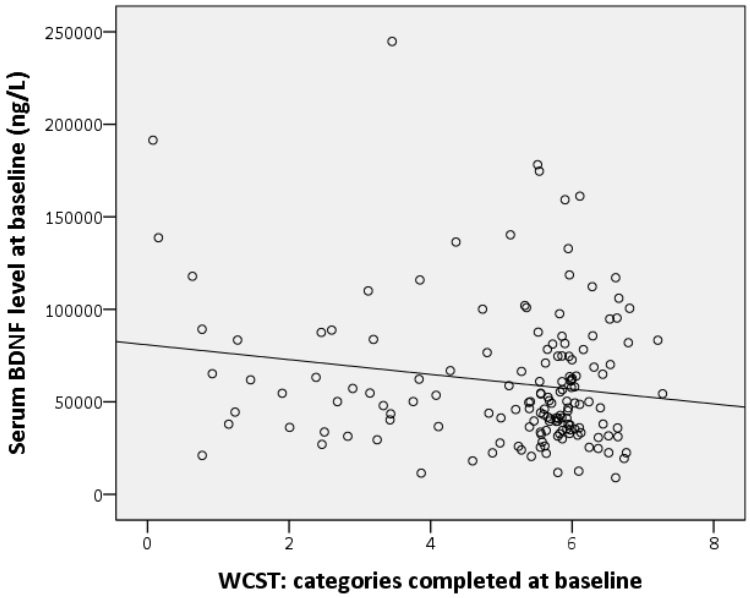


Figure 4. Partial correlation between serum BDNF level and WCST: total attempts in all ketamine users group at follow-up (N = 157) (r = 0.173, p = 0.032)

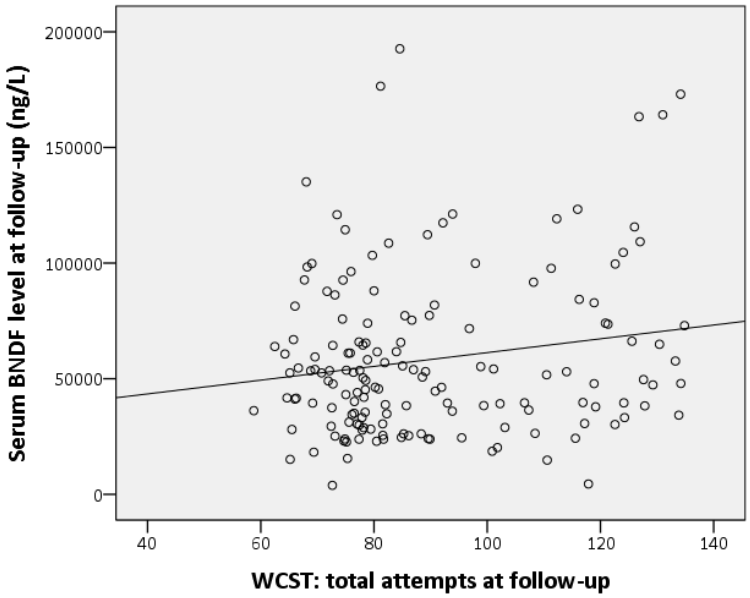


Figure 5. Partial correlation between serum BDNF level and WCST: categories completed in all ketamine users group at follow-up (N = 157) r = -0.226, p = 0.005).

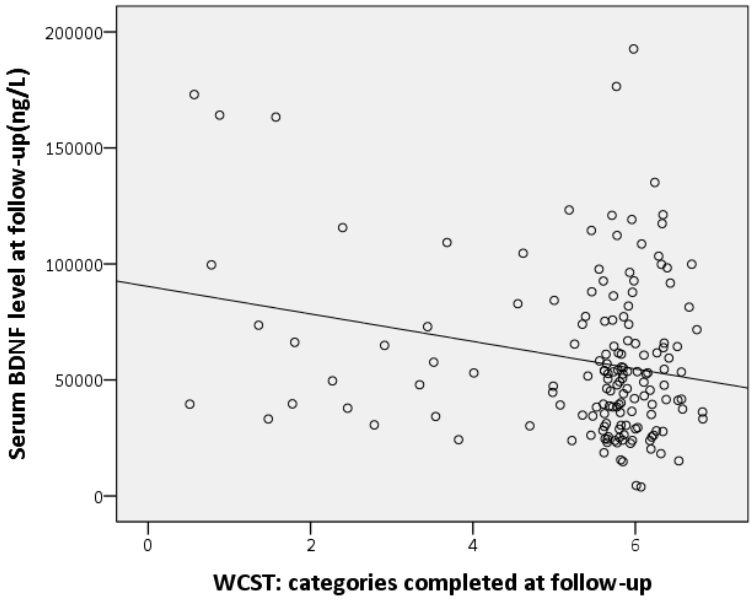
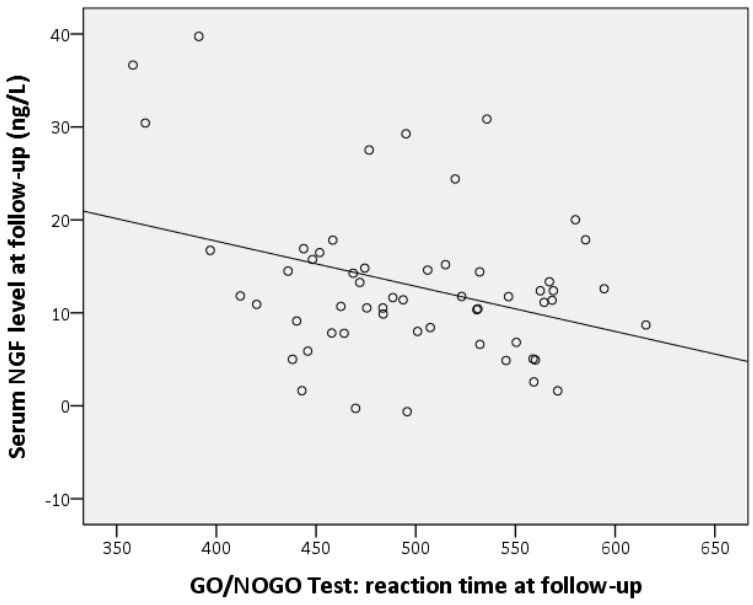


Figure 6. Partial correlation between serum NGF level and GO/NOGO Test: reaction time in all ketamine users group at follow-up (N = 57) (r = -0.349, p = 0.010)



In the primary ketamine group at baseline, a borderline partial correlation was found between serum BDNF levels and the number of categories completed on the WCST (r = -0.202, p = 0.083). There were no significant partial correlations between serum NGF levels and cognitive task scores. At follow up, significant partial correlations were found between serum BDNF levels and the WCST (total attempts, r = 0.247, p = 0.033, Figure 7; categories completed, r = -0.324, p = 0.005, Figure 8). There were no significant partial correlations between serum NGF levels and cognitive task scores.

Figure 7. Partial correlation between serum BDNF level and WCST: total attempts in primary ketamine users group at follow-up (N = 79) (r = 0.247, p = 0.033)

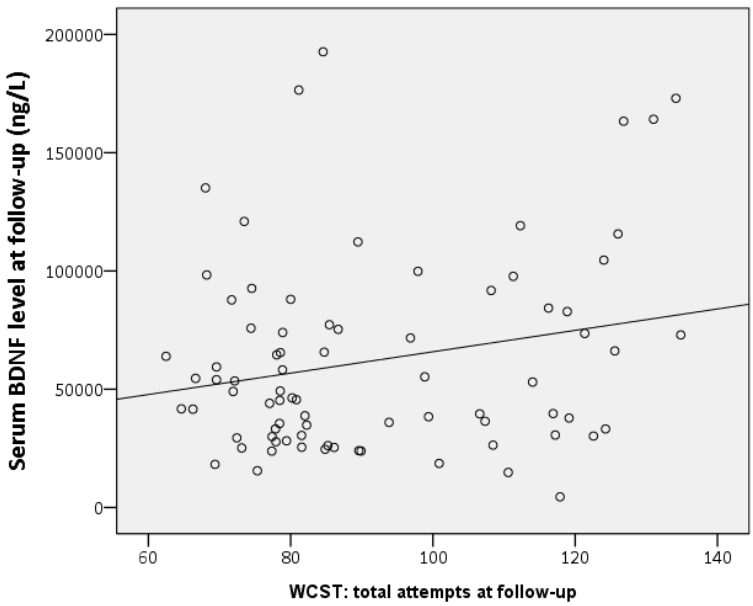
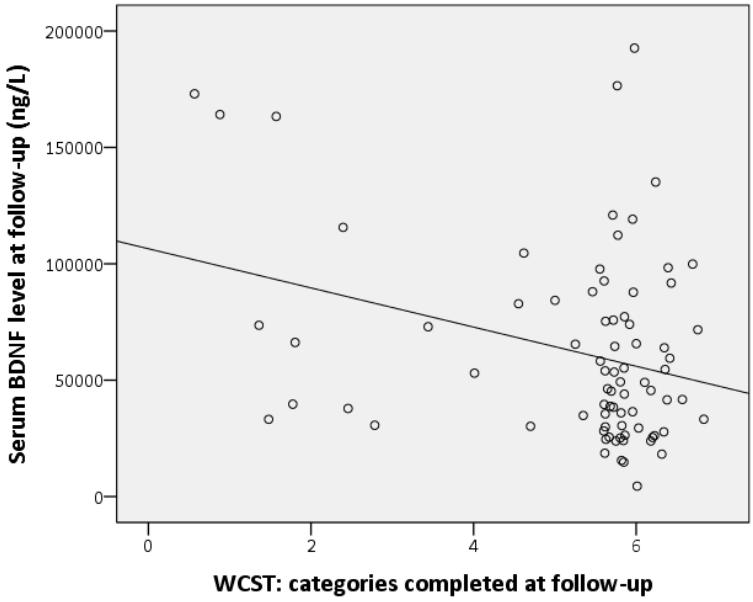
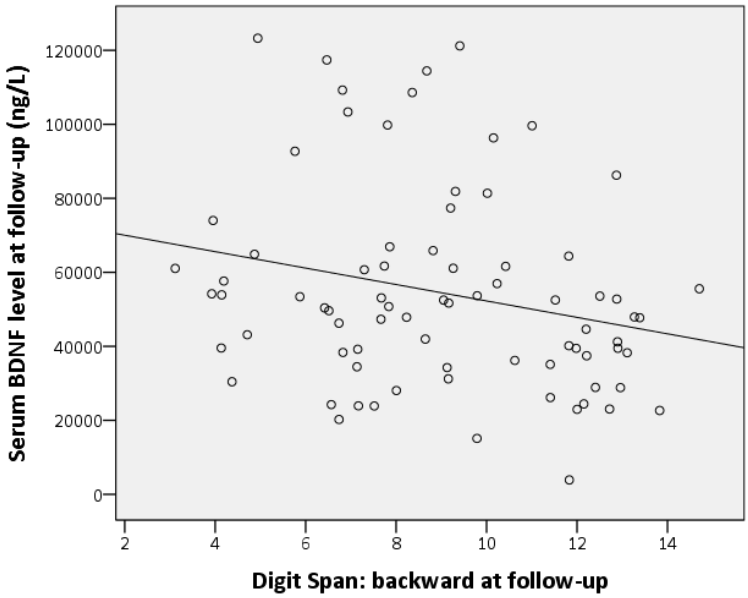


Figure 8. Partial correlation between serum BDNF level and WCST: categories completed in primary ketamine users group at follow-up (N = 79) (r = -0.324, p = 0.005)



In the poly ketamine users group at baseline, there were no significant partial correlations between serum BDNF or NGF levels and cognitive task scores. At follow up, a significant partial correlation was found between Digit Span (backward) and serum BDNF level (r = -0.241, p = 0.039) (Figure 9). There were no significant partial correlations between serum BDNF level and other cognitive task scores. There were no significant partial correlations between serum NGF level and cognitive task scores.

Figure 9. Partial correlation between serum BDNF level and Digit Span: backward in poly ketamine users group at follow-up (N = 78) (r = -0.241, p = 0.039)



Discussion

1. Summary of new findings

This study found that cognitive functions, especially verbal and visual memory, had improved in ketamine users after 12 weeks of abstinence and were comparable to those of normal controls. These findings suggest that ketamine-induced cognitive impairments are reversible.

After 12 weeks of abstinence, verbal memory, visual memory and executive function performance improved in the all ketamine group. There were no significant differences in cognitive function between ketamine users at follow up and healthy controls at baseline. This finding indicates the recovery of cognitive functions among ketamine users. Similarly, working memory, verbal memory, visual memory and executive function improved in the primary ketamine and poly ketamine groups. The only significant differences in cognitive function between primary ketamine and poly ketamine users at follow up and healthy controls at baseline were on the digit span test and immediate recall in the memory test, respectively.

There were no significant differences between ketamine users' serum BNDF and NGF levels at baseline and follow up, and the difference in GDNF levels was of borderline significance. There was no significant difference in serum BDNF, NGF and GDNF levels between ketamine users and healthy controls at baseline and follow up.

Serum BDNF levels were negatively correlated with the number of categories completed and positively correlated with the total number of attempts on the WSCT, in the all ketamine group and primary ketamine group. These results suggest that a higher serum BDNF level is associated with poorer executive function. Serum NGF

levels were negatively correlated with reaction times in the GO/NOGO test in ketamine users, suggesting that a higher NGF level is associated with better executive function.

2. Demographics and drug use pattern

Most drug users recruited in the study were male and the average age was 27, with an average of 9.6 years of education, which is equivalent to lower secondary level. These findings are consistent with the data from previous local reports (Liang *et al.*, 2013, Narcotics Division, 2015). The mean SDS score for the all ketamine group was 8.8, which indicates a severe level of dependence (Gossop *et al.*, 1995). An SDS score of 8 can detect DSM IV ketamine dependence diagnoses with 99.1% specificity (Fernández-Calderón *et al.*, 2016).

The duration of ketamine use in the study was 7 years, which was slightly longer than the 5.1 years reported in another local study (Liang *et al.*, 2013). The recruited ketamine users were frequent chronic ketamine users and the administration route was always by nasal inhalation. The participants in this study preferred to use ketamine at home alone, which is consistent with local reports (Narcotics Division, 2015). Many of the ketamine users also used other substances, including cocaine, cannabis, ecstasy and alcohol. This finding is also in line with other studies (Morgan *et al.*, 2009, Narcotics Division, 2015).

3. Effects of ketamine on psychological health

The ketamine users had more depressive symptoms than the healthy control group at baseline. The mean BDI score for the ketamine users at baseline was 14.6, which

suggests mild depression (Beck *et al.*, 1988). Depressive symptoms have been reported in other studies on ketamine users (Liang *et al.*, 2013, Liang *et al.*, 2015, Morgan *et al.*, 2010). However, the BDI score in this study was lower than that in the previous local report, with an average score of 22.2 (Liang *et al.*, 2013), and higher than that in UK with an average of 11.5 (Morgan *et al.*, 2009). It is reported that men in general have lower BDI scores than women (Beck *et al.*, 1988), and the number of years of education is inversely related to BDI scores (Beck *et al.*, 1988). In the present study, 72% of the participants were men compared with only 57% in the local study by Liang *et al.* (2013), which may explain the difference in BDI scores. The mean education of our study sample was 9.6 years, compared with 12.3 years in Morgan's (2009) study, which may explain the higher BDI scores found in the present study.

At the 12-week follow-up assessment, the mean BDI score for all/primary/poly ketamine users was significantly reduced and within the normal range of 0–10 (Beck *et al.*, 1988). However, the mean BDI scores for the all/primary ketamine groups were still significantly lower than that of the healthy controls. BDI scores were also found to be reduced in users who abstained from opiates, marijuana, alcohol, cocaine and methamphetamine (Dodge *et al.*, 2005, Glasner-Edwards *et al.*, 2009).

The association between ketamine and depression has been reported previously (Antagonism, 1996, Morgan & Curran, 2012, Price *et al.*, 2009). Ketamine is considered to have an anti-depressant effect, particularly for treatment-resistant depression (Price *et al.*, 2009). The anti-depressant effect is considered to be due to blocking of the N-methyl-d-aspartate (NMDA) receptor in the brain, which interacts with the major excitatory neurotransmitter glutamate (Antagonism, 1996). However, chronic ketamine users suffer more depressive symptoms (Morgan *et al.*, 2009). A

possible reason is that recreational ketamine users take considerably more than the medical dosage for an anti-depressant effect (Morgan & Curran, 2012), and the effect of ketamine on depression may be dose dependent. In a 1-year follow up study by Morgan (2010), the BDI scores of frequent ketamine users increased over the year (Morgan *et al.*, 2010). One explanation for the increase in depressive symptoms is that ketamine users may experience more negative life events that generate depressive symptoms. Several studies have reported that substance users have more negative life events (Nordfjærn *et al.*, 2010, Dodge *et al.*, 2005, Glasner-Edwards *et al.*, 2009, Morgan *et al.*, 2009).

4. Effect of ketamine on cognitive function

The all ketamine users and primary ketamine users had significant impairments in verbal and visual memory compared with the healthy control group at baseline. The poly ketamine users had impaired verbal memory compared with the healthy control group at baseline. Verbal and visual memory impairments at baseline were found in ketamine users, consistent with the results of previous studies (Liang *et al.*, 2013, Liang *et al.*, 2014, Morgan *et al.*, 2004c). An animal study demonstrated that ketamine impairs the acquisition of information by disrupting long-term potentiation in the hippocampus (Morris *et al.*, 1994). In healthy volunteers, ketamine caused dose-dependent impairments in verbal memory performance (Honey *et al.*, 2003, Newcomer *et al.*, 1999, Parwani *et al.*, 2005, Rowland *et al.*, 2005).

After 12 weeks of abstinence, we found that the verbal and visual memory performance of all ketamine users improved and was comparable to that of the healthy controls at baseline. This result suggests that ketamine-related memory impairments are reversible following abstinence. No previous longitudinal study has

examined the recovery of cognitive functions following supervised abstinence. A cross-sectional study showed that ex-ketamine users who had been abstinent for 120-2,980 days performed no differently from healthy controls on cognitive function tests, including verbal and visual memory, while current ketamine users had impaired verbal memory (Morgan *et al.*, 2009). The lack of cognitive function impairments in the ex-ketamine users in Morgan's study (2009) implies that cognitive functions may improve upon the cessation of ketamine use, which is consistent with our findings. Cocaine, opiate and marijuana users showed a non-significant trend of verbal memory improvement after 6 weeks of abstinence (Bates *et al.*, 2005). In contrast, a study compared alcohol users who had been abstinent for <6 months and >6 months and found that both groups performed worse on visual memory tests than controls, suggesting that the alcoholics' visual memory impairment did not recover within 6 months (Munro *et al.*, 2000).

The improvements in cognitive function were different between the primary and poly ketamine users following 12 weeks of abstinence from all substances. The primary ketamine users showed a greater improvement in verbal memory than the poly ketamine users, possibly due to the latters' use of other drugs. For instance, poly ketamine users had co-abused significantly more drugs, especially cocaine. Previous studies have shown verbal memory impairments in cocaine-dependent users (Ardila *et al.*, 1991, Bolla *et al.*, 1999, Mittenberg & Motta, 1993), with only minor improvement observed after 45 days of abstinence (Van Gorp *et al.*, 1999) and only partial improvement even after 1 year of abstinence (Vonmoos *et al.*, 2014).

5. Levels of biomarkers in ketamine users

At baseline, our study found no significant differences in the levels of biomarkers (BDNF/NGF) between ketamine users and healthy controls. In a study of chronic cannabis users, BDNF levels were similar to those of normal controls, whereas NGF levels were decreased (Angelucci et al., 2008). A study of chronic ketamine users found increased BDNF levels compared with healthy controls (Ricci *et al.*, 2011), and similar results were reported in heroin-dependent users (Luan *et al.*, 2017) and ecstasy addicted subjects (Angelucci *et al.*, 2010a). In contrast, other studies have found lower BDNF and NGF levels in chronic ketamine abusers (Ke et al., 2014) and cocaine-dependent users (Angelucci et al., 2007a). In summary, the literature on the changes in biomarkers in ketamine and other substance users are conflciting. Various factors may have contributed to these inconsistent results among studies, including recruitment of patients with different doses, frequencies, durations and stages (active use versus withdrawal) of ketamine use (Ke et al 2014) and differences in the techniques for measuring BDNF/NGF levels.

After 3 months of abstinence, we again found no significant differences in the levels of biomarkers (BDNF/NGF) between ketamine users and healthy controls. No previous study has examined the change in BDNF/NGF levels following ketamine abstinence. In a study of alcohol-dependent patients, BDNF levels did not change during 14 days of alcohol withdrawal (Heberlein et al., 2010). In another cross-sectional study of cocaine users, BDNF levels were not associated with length of abstinence (Pedraz et al., 2015). In contrast, increased BDNF levels were found in cocaine users after 12 days of abstinence (Corominas-Roso et al., 2013), and in alcohol users after 7 days (Huang et al., 2008) and 6 months of abstinence (Costa et al., 2011). Increased serum BDNF levels have been reported in heroin-dependent

patients had after 26 weeks of abstinence (Zhang *et al.*, 2016) and cocaine users during early abstinence (Viola *et al.*, 2015). The differences between the results obtained in various studies may be partly explained by differences in the substance of abuse, phases of abstinence and detoxification treatments.

6. Correlations between biomarkers and cognitive functions in ketamine users

Higher serum BDNF levels in ketamine users were related to more severe executive dysfunction at baseline and follow up. Similarly, after 3 months of abstinence, poly ketamine users with higher serum BDNF levels had more severely impaired working memory. These findings are consistent with a study of methamphetamine-dependent users, in which higher serum BDNF levels were correlated with more severe cognitive impairment (Su et al., 2015a). Another study of alcohol-dependent patients also found a negative correlation between BDNF levels and executive function (Han *et al.*, 2015). Finally, higher BDNF levels were found in cocaine users with memory impairment (Viola et al., 2015). Neurotrophic factors have an effect on both synaptic activity and neuronal survival (Vinogradov *et al.*, 2009). Cognitive disturbance in dementia is related to altered trophic support of neuronal activity and survival by neurotrophic factors (Arancio & Chao, 2007). BDNF plays a critical role in modulating synaptic activity and plasticity and has been considered a marker of cognitive impairments (Carlino *et al.*, 2013). Moreover, BDNF plays a pivotal role in synaptic remodelling during cognitive processing (Schinder & Poo, 2000). In alcohol-dependent users, the BDNF genotype (Val homozygotes) was related to brain grey matter volume recovery after short-term abstinence (Mon et al., 2013), suggesting that BDNF is involved in the process of brain tissue recovery. BDNF's role in cognitive recovery was also

suggested by a study that found that serum BDNF levels increased in patients with schizophrenia following cognitive training (Vinogradov et al., 2009). Animal studies suggest that upregulating BDNF expression in the brain might improve cognitive function (Pilc, 2010). Prolonged exposure to ketamine in rat pups led to increased BDNF levels in the brain, and this increased expression of BDNF may be a response to ketamine-induced injury (Ibia et al., 2009). Hence, it is possible that the increase in BDNF levels in ketamine users is a compensatory response to cognitive impairment.

Our study found that increased NGF levels correlated with better executive functioning in ketamine users at follow-up. No previous study has examined the relationship between NGF and cognitive function in ketamine or other substance abuse. NGF is a well-established neurotrophin acting on cholinergic neurons (Lad *et al.*, 2003), which are important for cognitive functions (Browne *et al.*, 2001). NGF is important for learning and memory (Budni *et al.*, 2015), and provides neuroprotection by upregulating the NMDA receptor to promote synaptic plasticity (Bai & Kusiak, 1997). Similarly, GDNF protects against NMDA-induced excitotoxicity (Bonde et al., 2000).

7. Limitations

First, as a longitudinal study, the second assessment was conducted within a relatively short interval, so learning effects cannot be excluded. The practice effect on cognitive functioning is related to the difficulty of the task, the length of the test-retest interval, the individual's age and general ability and the disease being assessed (Falleti *et al.*, 2006). There is no consensus on the impact of the practice effect. A study that involved repeated assessment of cognitive functions including executive function and

working memory at 10-minute, 1-week and 1-month test–retest intervals observed no practice effects at an interval of 1 month (Falleti, Maruff, Collie, & Darby, 2006). Other studies have found a practice effect (Bates *et al.*, 2005, Morgan *et al.*, 2010), but the effect may be limited (Morgan *et al.*, 2010). Second, there were differences between the ketamine users and controls in terms of age, sex and education level that were only partially solved by statistical adjustments. Third, we did not administer a general intelligence test so the possibility that the groups differed in terms of general intelligence could not be excluded, even though we adjusted for the education level in the analyses. Fourth, the genetics of BDNF and other neurotrophic factors were not analysed. In heroin- and methamphetamine-dependent patients, BDNF Val^{66}Met polymorphism did not affect the peripheral blood BDNF level (Chen *et al.*, 2015, Su *et al.*, 2015b). Similarly, a meta-analysis showed no relationship between Val^{66}Met polymorphism and serum BDNF levels in different samples, including healthy subjects and patients with schizophrenia, bipolar disorder and Alzheimer's disease (Mandelman & Grigorenko, 2012). In alcohol withdrawal patients, serum NGF levels were positively correlated with the mean methylation of the Cytosin-phosphatidyl-Guanin island promoter methylation of the NGF gene (Heberlein *et al.*, 2013). No published study has investigated the correlation between GDNF genotype and serum level.

It has been reported that the BDNF Met allele predicted better perceptual speed and better working memory performance in an elderly population (Brooks *et al.*, 2014, Ghisletta *et al.*, 2014). The BDNF Val allele was associated with better visuospatial/constructional performance in both schizophrenic patients and healthy controls (Zhang *et al.*, 2012) The BDNF Met allele was associated with poorer executive function in the healthy adult children of alcoholics (Benzerouk *et al.*, 2013).

No published study has investigated the correlation between NGF or GDNF genotypes and cognitive function.

Finally, 12 weeks was chosen as the endpoint, which represents short-term recovery of cognitive function (Aharonovich *et al.*, 2003). Other studies have examined changes in cognitive function after longer periods of abstinence (12 to 36 months) (Morgan *et al.*, 2004b, Morgan *et al.*, 2010, Rourke & Grant, 1999). Ketamine users often leave the detoxification centres after 3 months of treatment, and there is a high chance of relapse once they return to the community. Therefore, to ensure supervised abstinence and to reduce the dropout rate, we chose 3 months (12 weeks) as the endpoint in this study. Finally, the levels of NGF and GDNF were not detectable in many ketamine users.

8. Implications

Cognitive impairments caused by the effect of ketamine on the brain are not permanent. The knowledge that memory function improves after short-term cessation of ketamine might encourage ketamine users to quit.

9. Future research

First, a longer period of abstinence is needed to measure the long-term trend of cognitive function recovery. Second, this study should be repeated using a control group matched in terms of age, gender and education level. Further research on other potential biomarkers of brain damage in ketamine users is warranted.

Conclusion

This study provides evidence that ketamine-induced memory impairment is reversible. Knowing that impaired memory can be reversed by abstinence may encourage ketamine users to quit.

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