Adults with a history of illicit amphetamine use exhibit abnormal substantia nigra morphology and parkinsonism

Gabrielle Todd [a, *], Verity Pearson-Dennett [a], Robert A. Wilcox [a, b, c], Minh T. Chau [d], Kerry Thoirs [d], Dominic Thewlis [d], Adam P. Vogel [e, f], Jason M. White [a]

[a] School of Pharmacy and Medical Sciences, University of South Australia, GPO Box 2471, Adelaide, SA 5001, Australia
[b] Department of Neurology, Flinders Medical Centre, Flinders Drive, Bedford Park, SA 5042, Australia
[c] Human Physiology, Medical School, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia
[d] School of Health Sciences, University of South Australia, GPO Box 2471, Adelaide, SA 5001, Australia
[e] Centre for Neuroscience of Speech, 550 Swanston Street, Parkville, The University of Melbourne, VIC 3010, Australia
[f] Department of Neurodegeneration, Hertie Institute for Clinical Brain Research, Eberhard Karls Universidad Tübingen, Germany

ARTICLE INFO

ABSTRACT

Introduction: The sonographic appearance of the substantia nigra is abnormally bright and enlarged (hyperechogenic) in young adults with a history of illicit stimulant use. The abnormality is a risk factor for Parkinson's disease. The aim of the current study was to identify the type of illicit stimulant drug associated with substantia nigra hyperechogenicity and to determine if individuals with a history of illicit stimulant use exhibit clinical signs of parkinsonism. We hypothesised that use of amphetamines (primarily methamphetamine) is associated with substantia nigra hyperechogenicity and clinical signs of parkinsonism.
Methods: The area of echogenic signal in the substantia nigra was measured in abstinent human amphetamine users (n = 27; 33 ± 8 years) and in three control groups comprising a) 'ecstasy' users (n = 19; 23 ± 3 years), b) cannabis users (n = 30; 26 ± 8 years), and c) non-drug users (n = 37; 25 ± 7 years). A subset of subjects (n = 55) also underwent a neurological examination comprising the third and fifth part of the Unified Parkinson's Disease Rating Scale.
Results: Area of substantia nigra echogenicity was significantly larger in the amphetamine group (0.276 ± 0.080 cm^2) than in the control groups (0.200 ± 0.075, 0.190 ± 0.049, 0.191 ± 0.055 cm^2, respectively; P < 0.002). The score on the clinical rating scale was also significantly higher in the amphetamine group (8.4 ± 8.1) than in pooled controls (3.3 ± 2.8; P = 0.002).
Conclusion: Illicit use of amphetamines is associated with abnormal substantia nigra morphology and subtle clinical signs of parkinsonism. The results support epidemiological findings linking use of amphetamines, particularly methamphetamine, with increased risk of developing Parkinson's disease later in life.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The substantia nigra (SN) is a midbrain structure with a high concentration of dopaminergic neurons. The sonographic appearance of the SN is abnormally bright and enlarged (hyperechogenic) in young adults with a history of use of multiple illicit stimulant drugs (amphetamine, methamphetamine, ecstasy, and/or cocaine) [1]. This observation has clinical significance because the abnormality is a well-established risk factor for Parkinson's disease. Healthy older adults with the abnormality are 17 times more likely to develop Parkinson's disease over a three year period [2].

The aim of the current study was to identify the type of illicit stimulant drug associated with abnormal SN morphology and risk of Parkinson's disease. We hypothesised that use of illicit amphetamine and/or methamphetamine ('amphetamines') is associated with SN hyperechogenicity, but that use of ecstasy or cannabis is not. We also hypothesised that individuals with a history of illicit amphetamine use would exhibit clinical signs of parkinsonism. Our hypothesis is based on several lines of evidence.

Use of amphetamines at recreational doses is toxic to dopaminergic neurons and retrospective analysis of hospital records suggest that methamphetamine use is associated with increased risk of developing Parkinson's disease later in life (2.65 hazard ratio) [3,4]. Furthermore, the brains of chronic methamphetamine users and Parkinson's disease patients both exhibit reduced dopamine re-uptake transporters [3,5], and abnormal iron deposition and increased intracellular inclusions in the SN [6,7]. The results of the current study are important for the 14–56 million people (global estimate) with a history of recent use of illicit amphetamines and the provision and cost of future healthcare services [8]. Some of the ultrasound data has been published previously in a different form [1].

2. Methods

One hundred and sixteen subjects aged 18–50 years were recruited into the study via community advertisement. The target group comprised 27 individuals with a history of amphetamine and/or methamphetamine use (≥5 occasions; termed 'amphetamine' group) and the control groups comprised a) 19 individuals with a history of ecstasy use (≥5 occasions) but minimal use of amphetamines (<5 occasions; termed 'ecstasy' group), b) 31 individuals with a history of cannabis use (≥5 occasions) but no stimulant use (termed 'cannabis' group), and c) 39 individuals with no history of illicit drug use (termed 'non-drug' group). All experimental procedures were approved by the University of South Australia and Southern Adelaide Clinical Human Research Ethics Committees. Experimental procedures were conducted according to the Declaration of Helsinki and written informed consent was obtained.

2.1. Subject screening

Subjects underwent the following screening tests prior to participation: a) brief medical history questionnaire, b) urine drug test (PSCupA-6MBAU, US Diagnostics Inc., Huntsville, Alabama, USA), c) neuropsychological assessment involving Logical Memory I and II [9], Verbal Trails and Verbal Fluency [10,11], and Digit Span forwards and backwards [12], d) Beck Depression Inventory-II [13], e) Edinburgh Handedness Inventory [14], and f) drug history questionnaire to document recent and lifetime use of alcohol, tobacco, and illicit drugs. The drug history questionnaire listed 20 illicit drugs and requested information on other illicit drugs not listed. Items on the questionnaire included age of first use, age of regular use, duration of use, frequency of use (current and lifetime), average dose if known (current and lifetime), and time since last use for each drug and the number of drug overdoses.

Exclusion criteria included a) history of neurological damage and/or neurological illness prior to illicit drug use, b) use of antipsychotic medications, c) frequent illicit opioid use (>2 times per year during period of illicit drug use), and d) positive urine test for amphetamine, methamphetamine, 'ecstasy' (3,4-methylenedioxymethamphetamine), cocaine, opioids, and/or benzodiazepines. Subjects who returned a positive urine test for cannabis were allowed to participate if use was greater than 12 h prior to the experiment. This exemption was necessary because tetrahydrocannabinol can remain in the body for up to 80 days after last use [15].

2.2. Experimental protocol

Transcranial sonography was performed by one researcher (GT) using published methodology [1] and a Philips iU22 ultrasound system (manufactured June 2004, refurbished November 2011 with software level 6.0.2.144, Philips Healthcare, Best, Netherlands). A 1–5 MHz transducer (model s5-1, Philips Healthcare, Best, Netherlands) was positioned over the pre-auricular acoustic bone window located above the ear. The B-mode setting was used and the dynamic range and penetration depth were set at 60 dB and 14–16 cm, respectively. A qualitative rating of the bone window was made (1-excellent, 2-good, 3-poor, 4-very poor) and the area of echogenicity at the anatomical site of the SN was measured at its greatest extent according to international guidelines [16]. Other parameters that were measured include the internal diameter of the third ventricle, area of the red nucleus at its greatest extent (right and left side), and qualitative rating (normal, abnormal-interrupted, abnormal-absent) of the raphii nucleus (on the clearest side) [16]. Inter-rater reliability and reproducibility of the ultrasound procedure and operator has been previously published [1].

A subset of subjects (n = 55) also underwent a neurological examination performed by an experienced neurologist who specialises in movement disorders (RW). The neurologist was blinded to the subject's drug history and the examination involved the third and fifth part of the Unified Parkinson's Disease Rating Scale (UPDRS) [17].

2.3. Data analysis

Group data are presented as mean ± SD. Between-group comparison of subject characteristics, neuropsychological performance, and ultrasound parameters was made with one-way analysis of variance (ANOVA). One-way ANOVA was also used to compare a) cannabis use in the amphetamine, ecstasy, and cannabis groups and b) UPDRS Part III score in the three control groups (non-drug, ecstasy, and cannabis). Non-parametric data were transformed to ranks and ANOVA on ranks was performed. Post-hoc discrimination between means was made with Bonferroni procedure. Unpaired Student's t-test with sequential Bonferroni correction was used to compare a) use of ecstasy in the amphetamine and ecstasy groups and b) clinical data in the amphetamine and pooled control group. Pearson Product Moment or Spearman Rank Order correlation was used to investigate the relationship between area of SN echogenicity and subject characteristics, drug–use parameters, and clinical parameters (SigmaPlot Version 11.0, Systat Software Inc, San Jose, USA). Significance was set at P < 0.05.

3. Results

3.1. Subject characteristics

Three subjects were excluded due to insufficient bone window for transcranial sonography. Table 1 shows the characteristics for the remaining 113 subjects. The groups significantly differed in age ($F_{3,112}$ = 13.313, P < 0.001). The average age of the amphetamine group was 6–10 years older than the other groups (P < 0.001). This was expected given that the onset of cannabis and ecstasy use tends to occur at an earlier age than amphetamine use. The groups did not differ in years of education, hand dominance (laterality quotient), or neuropsychological performance. The groups tended to differ in recent symptoms of depression (BDI-II score), but the effect did not reach statistical significance (P = 0.053). Thirteen subjects across the drug-using groups had received a formal diagnosis of depression after commencement of illicit drug use (three-cannabis, three-ecstasy, seven-amphetamine) and two were currently medicated (one-cannabis, one-amphetamine).

Table 1
Subject characteristics for the non-drug, amphetamine, ecstasy, and cannabis groups.

	Non-drug	Amphetamine	Ecstasy	Cannabis
Age (yrs)	25 ± 7	33 ± 8 *§†	23 ± 3	26 ± 8
Gender	17M, 20F	15M, 12F	11M, 8F	18M, 12F
Handedness	0.5 ± 0.6	0.7 ± 0.3	0.7 ± 0.4	0.7 ± 0.3
Educ (yrs)	16 ± 2	15 ± 3	15 ± 2	16 ± 3
BDI-II score	6 ± 7	8 ± 6	10 ± 8	7 ± 8
Depression	0	7	3	3
Head injuries	2	5	6	1
Overdose	0	4	1	1
Alcohol	539 ± 1757	14,786 ± 16,336*§†	4,956 ± 7374*	2,841 ± 4,070*
Tobacco	134 ± 810	86,666 ± 109,065*§†	5,696 ± 16,591*	12,010 ± 42,423*

Data are mean ± SD. * Significantly different from non-drug group (P < 0.05). § Significantly different from ecstasy group (P < 0.05). † Significantly different from cannabis group (P < 0.05). Educ: education, Depression: number of subjects that have received a formal diagnosis of depression after commencing use of illicit drugs, Handedness: laterality quotient for the Edinburgh Handedness Questionnaire (−1 = strongly left-handed, 1 = strongly right-handed), Alcohol: estimated lifetime drinks, Tobacco: estimated lifetime cigarettes.

3.2. Drug history

Table 1 shows that the groups significantly differed in lifetime use of alcohol ($F_{3,105}$ = 49.060, P < 0.001) and tobacco ($F_{3,109}$ = 40.907, P < 0.001). Lifetime use of alcohol (estimated total drinks) and tobacco (estimated total cigarettes) was greatest in the amphetamine group and least in the non-drug group.

Table 2 shows the average lifetime use of illicit drugs in the amphetamine, ecstasy, and cannabis groups. Poly-drug use was most common in the amphetamine group and less common in the cannabis group. All subjects in the amphetamine and ecstasy groups had used cannabis and use of hallucinogens (primarily LSD) was common.

In the amphetamine group, the average age of onset of amphetamine use was 19.7 ± 3.9 years and the most common type of amphetamine consumed was methamphetamine (643 ± 860 occasions). The average duration of abstinence from amphetamine use was 3.6 ± 4.7 years (median: 1.3 years). In the ecstasy group, lifetime use of ecstasy (occasions) was significantly lower than in the amphetamine group (P = 0.022; Table 2) and the average duration of abstinence from ecstasy was 1.1 ± 1.6 years (median: 0.4 years). Lifetime use of cannabis (occasions) significantly differed

Table 2
Lifetime history of illicit drug use in the amphetamine, ecstasy, and cannabis groups.

Drug class	Amphetamine	Ecstasy	Cannabis
Stimulant	100% (744 ± 903)	100% (29 ± 50)	0%
Methamph	96% (643 ± 860)	42% (1 ± 1)	0%
Ecstasy	93% (125 ± 174)	100% (27 ± 49)	0%
Cocaine	74% (8 ± 11)	37% (2 ± 2)	0%
Pharma	37% (6 ± 8)	11% (2 ± 1)	0%
Cannabis	100% (3,428 ± 3,512)	100% (524 ± 1,107)	100% (919 ± 2,305)
Hallucinogens	85% (105 ± 157)	68% (26 ± 43)	27% (4 ± 3)
Inhalants	63% (86 ± 163)	37% (46 ± 74)	13% (16 ± 26)
Sedatives	56% (25 ± 68)	21% (2 ± 2)	3% (5)
Opioids	56% (4 ± 7)	5% (1)	0%

Data are percentage of subjects that have consumed that class of illicit drug in their lifetime and mean ± SD for the estimated number of occasions of use (in brackets). The term 'ecstasy' describes MDMA and MDA (3,4-methylenedioxyamphetamine, three subjects). The term 'methamph' describes methamphetamine. The term 'pharma' describes pharmaceutical stimulants such as dexamphetamine and methylphenidate. The term 'hallucinogen' describes LSD (lysergic acid diethylamide), LSA (D-lysergic acid amide), 'magic' mushrooms, ketamine, salvia divinorum, DOI (2,5-dimethoxy-4-iodoamphetamine), DMT (dimethyltryptamine), 2CI/2CB (2,5-dimethoxy-4-iodophenethylamine or 2-(4-bromo-2,5-dimethoxyphenyl)ethanamine), and/or dextromethorphan. The term 'inhalant' describes nitrous oxide, amyl nitrate, and/or glue. The term 'sedative' describes GHB/Fantasy, methaqualone, chelidonium majus, and recreational use of benzodiazepines, antidepressants, and antihistamines. The term 'opioid' describes heroin, opium, poppy tea, and recreational use of methadone, oxycodone, hydrocodone, and/or morphine.
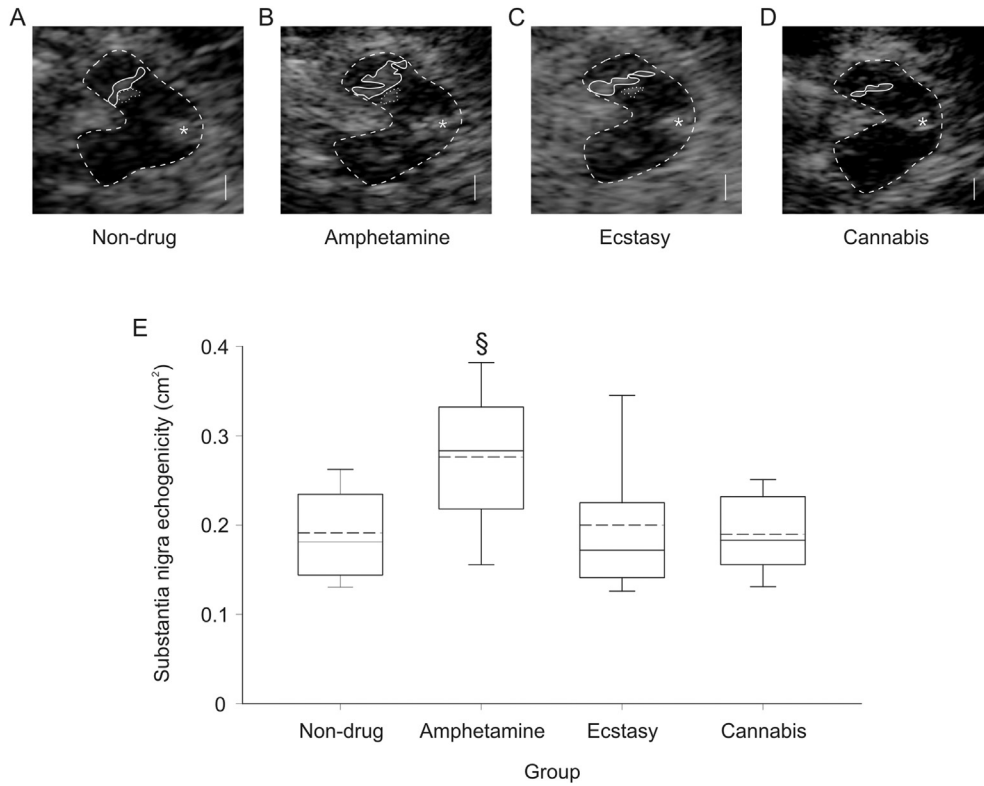
between the drug-using groups ($F_{2,73}$ = 12.514, P < 0.001; Table 2). Lifetime cannabis use was greater in the amphetamine group than in the ecstasy and cannabis groups (P < 0.001), but lifetime cannabis use did not differ between the ecstasy and cannabis groups. The duration of abstinence from cannabis use was 1.0 ± 2.6 years, 0.7 ± 1.8 years, and 1.6 ± 3.5 years in the amphetamine, ecstasy, and cannabis groups, respectively.

3.3. Transcranial ultrasound

Across all subjects, the average maximum subjective rating of the bone window was 1.5 ± 0.7 (good to excellent). All subjects exhibited a normal diameter of the third ventricle (<4.9 mm) and the diameter did not significantly differ between groups.

Fig. 1A–D shows single subject images of the SN and associated landmarks. The area of SN echogenicity is larger in the amphetamine subject than in the non-drug, ecstasy, and cannabis subjects.

Fig. 1E shows group data for the area of SN echogenicity. The area of SN echogenicity significantly differed between groups, in both the largest area (right or left, $F_{3,112}$ = 8.415, P < 0.001) and average area across the left and right side ($F_{3,112}$ = 8.799, P < 0.001). The area of echogenicity was significantly greater in the amphetamine group than in the non-drug (P < 0.001), ecstasy (P = 0.002), and cannabis (P < 0.001) groups. The effect was still evident after accounting for the between-group difference in age (P < 0.009), alcohol (P < 0.020), and tobacco (P < 0.041) (ANCOVA). In the amphetamine group, 56% of subjects exhibited echogenicity that exceeded the 90th percentile for the non-drug group (>0.256 cm^2). SN hyperechogenicity was observed on one or both sides and there was no consistent pattern for the affected side. The area of SN echogenicity did not differ between the non-drug, ecstasy, and cannabis groups.

The raphe was rated abnormal-interrupted in 8%, 15%, 16%, and 10% of subjects in the non-drug, amphetamine, ecstasy, and cannabis groups, respectively. The raphe was rated normal in all other subjects.

The red nucleus was clearly delineated bilaterally in 107 subjects and unilaterally in 6 subjects. The largest area of the red nucleus (right or left) significantly differed between groups ($F_{3,112}$ = 4.431, P = 0.006). The area of the red nucleus was significantly smaller in the ecstasy group (0.049 ± 0.020 cm^2) than in the amphetamine group (0.061 ± 0.015 cm^2, P = 0.034) and tended to be smaller than the non-drug group as well (0.059 ± 0.016 cm^2, P = 0.064). However, the average area of the red nucleus (across the right and left side) did not differ between groups.

Fig. 1. Echomorphology of the substantia nigra and mesencephalic brainstem. Images from one subject in the non-drug (A), amphetamine (B), ecstasy (C), and cannabis (D) group are shown. The outline of the substantia nigra (solid line) and red nucleus (dotted line) ipsilateral to the probe (the side at which the planimetric measurement is done) is shown along with the mesencephalic brainstem (dashed line). Asterisk indicates the cerebral aqueduct and calibration bar represents 0.5 cm. E) Group data showing the area of substantia nigra echogenicity. Data represent the largest area across the right and left side. The boundary of each box indicates the 25th and 75th percentile and the whiskers (error bars) indicate the 10th and 90th percentiles. The solid and dashed lines within each box indicate the median and mean values, respectively. § Significantly different from non-drug, ecstasy, and cannabis groups (P < 0.002).
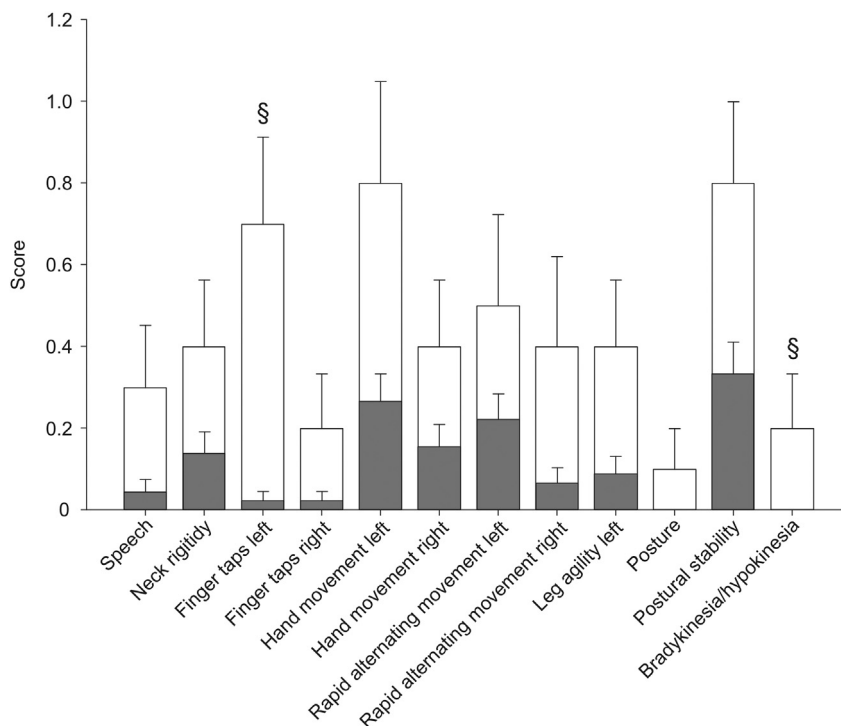

Fig. 2. Average score on sub-items of the Unified Parkinson's Disease Rating Scale. Group data (mean ± SEM) for the amphetamine group (white bars) and pooled control group (grey bars) are shown. § Significant difference between amphetamine and pooled control groups (P < 0.002).

3.4. Neurological examination

The total UPDRS Part III score did not significantly differ between the three control groups. Thus, clinical data for the control groups was pooled. The total UPDRS Part III score was significantly higher in the amphetamine group (8.4 ± 8.1) than in the pooled controls (3.3 ± 2.8; P = 0.002). The average UPDRS Part V score (Modified Hoehn and Yahr Staging) was also significantly higher in the amphetamine group (0.9 ± 0.9) than in the pooled controls (0.1 ± 0.4; P < 0.001). Significant between-group differences were also observed for sub-items of the UPDRS Part III. Fig. 2 shows that the average score on the finger taps (left hand: P < 0.001) and body bradykinesia and hypokinesia (P = 0.002) sub-items were significantly higher in the amphetamine group than in the pooled controls. The amphetamine group also tended to have a higher score on the finger taps (right hand: P = 0.029), speech (P = 0.010), hand movement (left: P = 0.005, right: P = 0.083), rapid alternating movements of the hand (left: P = 0.105, right: P = 0.012), leg agility (left: P = 0.011), posture (P = 0.033), postural stability (P = 0.017), and neck rigidity (P = 0.060) sub-items but the between-group difference did not reach statistical significance after sequential Bonferroni correction.

3.5. Correlations

In the amphetamine group, there was no significant correlation between area of SN echogenicity (largest side) and a) age, b) alcohol use, c) tobacco use, d) characteristics of amphetamine, ecstasy, and cannabis use, e) other illicit drug use, and f) UPDRS. There was also no significant correlation between area of SN echogenicity and age and use of alcohol and tobacco in the non-drug group.

4. Discussion

The results of the current study show for the first time that illicit use of amphetamines is associated with abnormal SN morphology and subtle clinical signs of parkinsonism in conscious humans. The results support epidemiological findings linking methamphetamine use with increased risk of developing Parkinson's disease later in life.

The average area of SN echogenicity in the amphetamine group (0.276 ± 0.080 cm^2) was significantly larger than in the ecstasy, cannabis, and non-drug groups, and was comparable to values obtained in older adults with diagnosed Parkinson's disease ($0.275–0.34$ cm^2) [18,19]. The observed abnormality is likely to be long-lasting because individuals in the amphetamine group had not used amphetamines for an average of 3.6 years and there was no correlation between SN echogenicity and duration of abstinence. There are also no reported instances in the literature of SN hyperechogenicity improving over time, in any population.

There was no significant correlation between SN echogenicity and the age of F onset of amphetamine use and number of occasions of amphetamine use. The former is not surprising given that the average age of onset (19.7 ± 3.9 years) occurred after the major period of brain development [20]. The latter suggests that the dose of amphetamine may be of greater importance than cumulative use. This proposition is supported by a strong dose-dependent relation between amphetamine use and neural toxicity in rodents, primates, and humans [3].

Identifying the mechanism(s) responsible for the abnormal SN morphology in human amphetamine users is difficult. The screening procedure used in the current study enables us to rule out factors such as memory, cognition, symptoms of depression, years of education, head injuries, drug overdoses, changes in gross brain volume (evidenced by the diameter of the third ventricle), and acute drug effects because these parameters did not differ between groups. The abnormality is also not associated with use of ecstasy or cannabis because the appearance of the SN was normal in the ecstasy and cannabis groups. However, methodological limitations associated with all studies on illicit amphetamine use in humans limit further definitive conclusions. Individuals differ in their pattern of drug use and quantification of lifetime drug use is based on self-report in which retrospective quantification of dose and drug composition is not possible. Tables 1 and 2 also demonstrate that poly-drug use and greater use of alcohol and tobacco also occur in this population [21]. Thus, it is more prudent to associate the abnormality with use of a class of drugs (e.g. amphetamines) than to ascribe causation to use of a specific drug.

The abnormal SN morphology observed in the amphetamine group was accompanied by a higher score on a clinical symptom severity scale designed specifically for Parkinson's disease. The total score on the third motor part of the UPDRS was significantly higher in the amphetamine group (8.4 ± 8.1) than in the pooled controls (3.2 ± 2.8). Analysis of sub-items on the scale suggests that abnormalities span several movement domains, including speech, hand movement, movement speed, alternating movements, posture, and postural stability. The abnormalities were detected by an experienced neurologist who specialises in movement disorders and who was blinded to the participant's drug history. The average UPDRS Part V score was also significantly higher in the amphetamine group than in the pooled controls and the average for the amphetamine group (0.9 ± 0.9) approached Hoehn and Yahr Stage 1 (unilateral disease).

The presence of both SN hyperechogenicity and signs of parkinsonism in the amphetamine group suggests that history of amphetamine use may be associated with increased risk of developing Parkinson's disease later in life. SN hyperechogenicity is present in 80–90% of Parkinson's disease patients [22,23] and healthy older adults with this abnormality are 17 times more likely to develop Parkinson's disease over a three year period [2]. Retrospective analysis of hospital records also suggests that use of methamphetamine, but not cocaine, is associated with increased risk of developing Parkinson's disease [4,24].

It is mechanistically plausible that use of methamphetamine contributed to the abnormal SN echogenicity and signs of parkinsonism observed in the current study. Lifetime use of methamphetamine was high (643 ± 860 occasions) in the amphetamine group and accounted for 86% of the total stimulants consumed. Several other lines of evidence support this view. For example, methamphetamine causes damage to dopaminergic nerve terminals and chronic use of methamphetamine is associated with long-lasting dopaminergic dysfunction [3]. In addition, a 2.5 fold increase in the intensity of iron staining in the SN of adult vervet monkeys is present 1.5 years after two doses of methamphetamine (2 mg/kg) and iron accumulation is one mechanism that underlies SN hyperechogenicity in humans [7,25]. Furthermore, pre-synaptic dopaminergic dysfunction, evidenced by reduced [^{18}F]-dopa activity, is also present in the striatum of vervet monkeys 24 weeks after amphetamine administration and pre-synaptic dopaminergic dysfunction is also present in the striatum of healthy adult humans with SN hyperechogenicity [26,27].

The current study has two limitations. First, the average age of the amphetamine group was 6–10 years older than the other groups. This was expected given that onset of cannabis and ecstasy use predates onset of amphetamine use by an average of four and two years, respectively [28]. The between-group difference in age is unlikely to have had an effect on the results of the current study because SN echogenicity was still significantly greater in the amphetamine group after accounting for age and area of SN echogenicity does not vary between 20 and 50 years of age [29].

Second, lifetime consumption of alcohol and tobacco was significantly higher in the amphetamine group than in the other groups. There is no published data on the specific effect of alcohol and tobacco use on SN echogenicity. Our data suggest that the effect is likely to be minimal given that the between group difference in SN echogenicity was still evident after accounting for use of alcohol and tobacco. However, cigarettes contain chemicals that inhibit monoamine oxidase and concurrent use of tobacco and amphetamines may further impair degradation of monoamine neurotransmitters in amphetamine users [30]. Personality traits (e.g. addictive behaviour) were also not controlled for, but are unlikely to play a major role given that the cannabis and ecstasy groups exhibited normal SN echogenicity. Twenty three percent of subjects in the cannabis group had a history of addictive behaviour (daily use) and use of cannabis and ecstasy occurs prior to first use of amphetamines [28].

The results of the current study suggest that history of primarily methamphetamine use is associated with abnormal SN morphology and signs of parkinsonism. Further longitudinal investigation is required to accurately document the relation between the observed abnormalities and risk of Parkinson's disease.

Acknowledgements

References

[1] G. Todd, C. Noyes, S.C. Flavel, C.B. Della Vedova, P. Spyropoulos, B. Chatterton, D. Berg, J.M. White, Illicit stimulant use is associated with abnormal substantia nigra morphology in humans, PLoS One 8 (2013) e56438.

[2] D. Berg, S. Behnke, K. Seppi, J. Godau, S. Lerche, P. Mahlknecht, I. Liepelt-Scarfone, C. Pausch, N. Schneider, A. Gaenslen, K. Brockmann, K. Srulijes, H. Huber, I. Wurster, H. Stockner, S. Kiechl, J. Willeit, A. Gasperi, K. Fassbender, T. Gasser, W. Poewe, Enlarged hyperechogenic substantia nigra as a risk marker for Parkinson's disease, Mov. Disord. 28 (2013) 216–219.

[3] B.K. Yamamoto, A. Moszczynska, G.A. Gudelsky, Amphetamine toxicities: classical and emerging mechanisms, Ann. N. Y. Acad. Sci. 1187 (2010) 101–121.

[4] R.C. Callaghan, J.K. Cunningham, G. Sajeev, S.J. Kish, Incidence of Parkinson's disease among hospital patients with methamphetamine-use disorders, Mov. Disord. 25 (2010) 2333–2339.

[5] J.M. Fearnley, A.J. Lees, Ageing and Parkinson's disease: substantia nigra regional selectivity, Brain 114 (1991) 2283–2301.

[6] L. Quan, T. Ishikawa, T. Michiue, D.R. Li, D. Zhao, S. Oritani, B.L. Zhu, H. Maeda, Ubiquitin-immunoreactive structures in the midbrain of methamphetamine abusers, Leg. Med. (Tokyo) 7 (2005) 144–150.

[7] W.P. Melega, G. Lacan, D.C. Harvey, B.M. Way, Methamphetamine increases basal ganglia iron to levels observed in aging, Neuroreport 18 (2007) 1741–1745.

[8] L. Degenhardt, W. Hall, Extent of illicit drug use and dependence, and their contribution to the global burden of disease, Lancet 379 (2012) 55–70.

[9] D. Wechsler, Wechsler Memory Scale-revised, Psychological Corporation, New York, 1987.

[10] A.L. Benton, K. Hamsher, Multilingual Aphasia Examination, AJA Associates, Iowa City, 1983.

[11] J. Grigsby, K. Kaye, Alphanumeric sequencing and cognitive impairment among elderly persons, Percept. Mot. Ski. 80 (1995) 732–734.

[12] D. Wechsler, Wechsler Adult Intelligence Scale - Revised, Psychological Corporation, New York, 1981.

[13] A.T. Beck, R.A. Steer, G.K. Brown, Manual for the Beck Depression Inventory-ii, The Psychological Corporation, San Antonio, 1996.

[14] R.C. Oldfield, The assessment and analysis of handedness: the Edinburgh inventory, Neuropsychologia 9 (1971) 97–113.

[15] F. Grotenhermen, Pharmacokinetics and pharmacodynamics of cannabinoids, Clin. Pharmacokinet. 42 (2003) 327–360.

[16] D. Berg, J. Godau, U. Walter, Transcranial sonography in movement disorders, Lancet. Neurol. 7 (2008) 1044–1055.

[17] S. Fahn, R.L. Elton, The Unified Parkinson's Disease Rating Scale, in: S. Fahn, C.D. Marsden, D.B. Calne, M. Goldstein (Eds.), Recent Developments in Parkinson's Disease, MacMillan Healthcare Information, Florham Park, New York, 1987, pp. 153–163, 293-304.

[18] Y.W. Huang, J.S. Jeng, C.F. Tsai, L.L. Chen, R.M. Wu, Transcranial imaging of substantia nigra hyperechogenicity in a Taiwanese cohort of Parkinson's disease, Mov. Disord. 22 (2007) 550–555.

[19] J.Y. Kim, S.T. Kim, S.H. Jeon, W.Y. Lee, Midbrain transcranial sonography in Korean patients with Parkinson's disease, Mov. Disord. 22 (2007) 1922–1926.

[20] S.J. Blakemore, Imaging brain development: the adolescent brain, NeuroImage 61 (2012) 397–406.

[21] AIHW, 2010 National Drug Strategy Household Survey Report, in: Drug Statistics Series No. 25. Cat. No. PHE 145, Australian Institute of Health and Welfare, Canberra, 2011, pp. 1–323.

[22] D. Berg, C. Siefker, G. Becker, Echogenicity of the substantia nigra in Parkinson's disease and its relation to clinical findings, J. Neurol. 248 (2001) 684–689.

[23] C.F. Tsai, R.M. Wu, Y.W. Huang, L.L. Chen, P.K. Yip, J.S. Jeng, Transcranial color-coded sonography helps differentiation between idiopathic Parkinson's disease and vascular parkinsonism, J. Neurol. 254 (2007) 501–507.

[24] R.C. Callaghan, J.K. Cunningham, J. Sykes, S.J. Kish, Increased risk of Parkinson's disease in individuals hospitalized with conditions related to the use of methamphetamine or other amphetamine-type drugs, Drug. Alcohol. Depend. 120 (2012) 35–40.

[25] D. Berg, C. Grote, W.D. Rausch, M. Maurer, W. Wesemann, P. Riederer, G. Becker, Iron accumulation in the substantia nigra in rats visualized by ultrasound, Ultrasound. Med. Biol. 25 (1999) 901–904.

[26] D. Berg, W. Roggendorf, U. Schroder, R. Klein, T. Tatschner, P. Benz, O. Tucha, M. Preier, K.W. Lange, K. Reiners, M. Gerlach, G. Becker, Echogenicity of the substantia nigra: association with increased iron content and marker for susceptibility to nigrostriatal injury, Arch. Neurol. 59 (2002) 999–1005.

[27] W.P. Melega, J. Quintana, M.J. Raleigh, D.B. Stout, D.C. Yu, K.P. Lin, S.C. Huang, M.E. Phelps, 6-[18F]fluoro-L-DOPA-PET studies show partial reversibility of long-term effects of chronic amphetamine in monkeys, Synapse 22 (1996) 63–69.

[28] SAMHSA, Results from the 2012 National Survey on Drug Use and Health: Summary of National Findings, in: NSDUH Series H-46, Substance Abuse and Mental Health Services Administration, Rockville, 2013.

[29] D. Berg, G. Becker, B. Zeiler, O. Tucha, E. Hofmann, M. Preier, P. Benz, W. Jost, K. Reiners, K.W. Lange, Vulnerability of the nigrostriatal system as detected by transcranial ultrasound, Neurology 53 (1999) 1026–1031.

[30] A.J. Lewis, P. Truman, M.R. Hosking, J.H. Miller, Monoamine oxidase inhibitory activity in tobacco smoke varies with tobacco type, Tob. Control 21 (2012) 39–43.