Functional changes in patients with internet addiction disclosed by adenosine stressed cerebral blood flow perfusion imaging ^{99m}Tc-ECD SPET

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Abstract

Objective: To investigate the abnormal cerebral blood flow (CBF) perfusion in patients with internet addiction (IA) and its possible association with IA severity. **Subjects and Methods:** Thirty-five adolescents who met the criteria for IA and 12 matched healthy volunteers were recruited for ^{99m}Tc-ethylcysteinate dimer based CBF perfusion imaging with single photon emission tomography (SPET) both at rest and in adenosine-stressed state. Regional CBF (rCBF) was measured and compared between IA subjects and the controls. Correlation analysis between those abnormal rCBF in adenosine-stressed state and the duration of IA was performed. **Results:** At the resting state, the IA individuals showed significantly increased rCBF in the left mid-frontal gyrus and left angular gyrus, but significantly decreased in the left paracentral lobule, compared to the controls. In adenosine-stressed state, more cerebral regions with abnormal rCBF were identified. Specifically, increased rCBF was identified in the right paracentral lobule, right mid-frontal gyrus and left superior temporal gyrus, while decreased rCBF were demonstrated in right transverse temporal gyrus, left inferior frontal gyrus and left precuneus. Those rCBF in rCBF-increased regions in stress state were positively correlated with the duration of IA, while those in rCBF-decreased regions were negatively correlated with the duration of IA.). **Conclusion:** We present specific functional changes in behaviour that may appear in IA patients related to the CBF findings in IA patients. Adenosine can be used as a pharmacological agent for stress CBF perfusion imaging in patients with IA, by which more cerebral regions of abnormal rCBF can be identified compared to the state at rest. These abnormal rCBF may indicate the neurological mechanism in IA patients.

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Introduction

Internet addiction (IA) is becoming a worldwide problem, with the prevalence estimated 2% to 9% in western countries, and 8% to 26% in Asia [1-3]. It has been reported that IA has brought about substantial hazards to the lives of adolescents, such as decline in academic performance, physical and mental health status and interpersonal relationships [4, 5]. Although cognitive and psychological dysfunctions associated with IA have been observed clinically, the related neurobiological mechanisms remain unclear, which is of great importance in order to prevent and treat the IA patients [6, 7].

Recently, molecular and functional imaging have been increasingly used to study the neurobiological mechanism underlying IA [8-20]. Of which, functional magnetic resonance imaging (fMRI) was most commonly used, and various structural or functional abnormalities were identified, such as reduced orbitofrontal cortical thickness [16], lower gray matter density [11], impaired brain activity [14], decreased functional connectivity [9], decreased ratio of N-acetylaspartate to creatine in frontal lobe [8] etc. Besides, reduced frontal and striatal dopamine transporter and receptor, as well as decreased glucose metabolism in prefrontal, temporal, and limbic systems were observed by nuclear imaging using positron emission tomography (PET) and single photon emission tomography (SPET) [17, 20, 21]. Specifically, using appropriate stimulus, task-state fMRI identified brain abnormalities that were associated with dysfunctions in cognitive control, craving, goal-directed behavior, and working memory in IA subjects [12-14, 18, 19]. Tian et al. (2014) [21] even conducted task-state PET scans right after a 30 minutes task of playing "World of Warcraft" and demonstrated abnormal glucose metabolism and dopamine-D2 receptor level in orbitofrontal cortex, which suggested a mechanism for compulsive behavior in internet gaming disorder subjects. Although

task-state imaging present situations that are closer to the real situation, they are clinically unavailable due to complicated experiment designing and subject training. By contrast, rest-state imaging are easier to perform and more comparable across studies. However, without explicit stimulus, it is impossible for rest-state imaging to obtain brain activity patterns as most task-state imaging do.

The pharmacological stress capacity of adenosine has been well established in stress myocardial perfusion imaging [22, 23]. The mechanism refers to that normal myocardial vessels dilate whereas those in pathological areas fail to when adenosine is administrated. This helps identifying potential lesions that are invisible during imaging at rest. Actually, similar function of adenosine in regulating CBF to achieve stress perfusion imaging has also been reported in several previous studies [24-26]. Compared to event-related task imaging, the pharmacological stress imaging is easier and more convenient for clinical application.

In this study, we attempted to evaluate the changes of blood flow in IA patients, through technetium-99m-ethylcysteinate dimer (^{99m}Tc-ECD) SPET CBF perfusion imaging, both in resting and in adenosine-stressed states. We hypothesize that adenosine-stressed imaging may generate similar efficiency as task-state fluorine-18-flurodeoxyglucose (^{18}F-FDG) PET imaging performed by Tian et al. (2014) [21] in identifying additional brain abnormalities compared to their rest-state counterparts. This study helps in determining: 1) if IA patients present any cerebral regions with abnormal blood flow; 2) whether these regions correspond to those abnormalities identified by previous functional imaging studies that indicate the neurological mechanism underlying IA; and 3) whether adenosine-stressed CBF perfusion imaging shows any additional benefit in IA patients as in myocardial perfusion imaging.

Subjects and Methods

Participants

This study was approved by the institutional ethics committee of our hospital. All procedures performed were in accordance with the ethical standards of the institutional research committee and with the Helsinki declaration. Written informed consent was obtained from all participants.

Forty-seven right handed individuals participated in this study, 35 adolescents with IA (24 boys and 11 girls; age, 19.6±8.6 years) and 12 education-matched healthy volunteers (9 boys and 3 girls; age, 18.9±7.9 years). The IA patients were recruited from the study done by Tao et al. (2010) [27]. The diagnostic criteria proposed by Tao was used as inclusion criteria, which comprised 7 symptomatic criteria as follows [27]: 1) Preoccupation: a strong desire for the internet. Thinking about previous online activity or anticipation of the next online session. Internet use is the dominant activity in daily life. 2) Withdrawal: manifested by a dysphoric mood, anxiety, irritability and boredom after several days without internet activity. 3) Tolerance: namely, marked increase in internet use required to achieve satisfaction. 4) Difficult to control: persistent desire and/or unsuccessful attempts to control, cut back or discontinue internet use. 5) Disregard of harmful consequences: continued excessive use of internet despite knowledge of having a persistent or recurrent physical or psychological problems likely to have been caused or exacerbated by internet use. 6) Social communications and interests are lost: loss of interests, previous hobbies, entertainment as a direct result of, and with the exception of, internet use. 7) Alleviation of negative emotions: uses the internet to escape or relieve a dysphoric mood (e.g. feelings of helplessness, guilt, anxiety). Subjects were interviewed individually by experienced psychiatrists who used the diagnostic criteria as a checklist. Respondents who have symptom 1 through 2 and at least any one of the remaining 5 symptoms were considered as suffering from IA. Besides, duration of internet addiction must have lasted for an excess of 3 months, with at least 6 hours of internet usage every day. The diagnostic accuracy, sensitivity and specificity of this criteria, were tested to be 99.26%, 89.66% and 100%, respectively, while the inter-rater reliability was 98% [27]. The control subjects were recruited from the local community using advertisements, and only those who spent less than 2 hours per day on the internet were included, according to previous IA research [10]. The controls were also tested with Tao's IA criteria to ensure they were not suffering from IA.

The exclusionary criteria included: 1) a history of psychiatric disorders, such as anxiety disorder, depression, compulsivity, schizophrenia, autism, or bipolar disorder; 2) a history of substance abuse or dependency; 3) a history of physical disorders related to the motion, digestive, nervous, respiratory, circulation, endocrine, urinary, and reproductive systems; 4) pregnancy or menstrual period in women during the day of scanning; 5) contraindications to adenosine including allergies, congenital heart diseases, severe hypertension (systolic blood pressure ≥180mmHg and/or diastolic blood pressure ≥100mmHg), acute coronary syndrome, second or third degree atrioventricular block, sick sinus syndrome, severe cardiac arrhythmia (e.g., frequent ventricular premature beat, ventricular tachycardia, etc.), severe cardiac insufficiency (classification of New York Heart Association ≥3), and bronchial asthma. This exclusionary procedure is important to ensure the participants in this study are not affected by other physical, neurological or neuropsychiatric disorders and hence reduce possible biases in the findings obtained. A basic information questionnaire was used to collect demographic information such as gender, age, education, time spend in internet use, and duration of IA. The duration of IA was estimated via requesting subjects to recall their life-style when they were initially addicted to the internet. The length of education was calculated from the first year when the subjects went to the primary school to the last year they acted as students.

Imaging techniques

All SPET, CBF perfusion imaging (CBFPI) were performed on an IRIX tri-head-detector SPET scanner (Philips, Medical Sys-

tems, Netherlands), according to standard institutional clinical protocols. The ^{99m}Tc-ECD (Shanghai Global Medical Solutions Pharmaceutical Co., Ltd, Shanghai, China) with radiochemical purity of more than 95% was used as imaging agent. Resting and stress SPET CBFPI were conducted in a 2 day protocol, with the former done in the first day while the latter in the second.

For rest-state imaging, participants were placed in a silent circumstance of gentle light for resting at least 30 minutes with eyes closed, before intravenous (i.v.) injection of ^{99m}Tc-ECD at a dose of 1110MBq (30mCi) per subject. The SPECT images were acquired with an elliptical 360° acquisition mode using tri-head detectors, which were placed as close to participant's head as possible. The detectors were positioned in a shape of equilateral triangle, with inter-detector angles being 60°. Every detector rotated 120° (360° in total for the three detectors) during acquisition for each subject, at a speed of 6° each frame; and thus a total of 60 frames were acquired, with each frame lasting 30 to 40 seconds and collecting 60k of radiation counts. The imaging matrix was set at 64×64, and a zoom ratio of 1 was applied.

Before the stress-state imaging, adenosine with a dose of 0.8mg/kg was i.v. injected to subjects at a uniform speed that should be accomplished in 6 minutes. Three minutes later, the same dose of ^{99m}Tc-ECD as applied in rest-state imaging (1110MBq each) was i.v. injected. At the time right before, 3 minutes and 6 minutes after the beginning of ECD injection, participants' heart rate, blood pressure and electrocardiogram were examined with the electrocardiogram monitor. Besides, all subjects were observed and asked if they had any adverse reaction at these time points. All adverse reactions were recorded if occurred, and the injection was immediately aborted. The process and techniques in relation to the following stress-state SPET CBFPI acquisition were the same as those applied in the rest-state imaging.

Imaging analysis

Prior to imaging evaluation, raw data were used to reconstruct stress and resting datasets using a reconstructive mode of filtered reverse projection with slice thickness of 7mm. Coronal, sagittal and axial planar images were reconstructed for imaging review, with the axial images being set parallel to the canthomeatal line. The perfusion images were firstly interpreted visually, and quantitative analysis with statistical parametric mapping (SPM) was performed to supplement the visual analysis.

All original images were retrieved from the SPET scanner system and were converted into SPM format (e.g., Analyze) using MRIcro. Subsequent image processing and statistical analyses were performed using SPM8 package (Wellcome Department of Cognitive Neurology, London, UK) run on MATLAB R2009b (MathWorks Inc., Sherborn, MA, USA). The SPET images were normalized to the SPM homemade template for SPET imaging. The threshold of intensity was set at 80% of the average intensity of the whole brain. This helps excluding interference from the background radioactivity, and removing blurred halos surrounding images caused by partial volume effects. Other parameters for normalization were set with the default parameters of SPM8: no weighting; 25mm cutoff; 12 linear affine and non-linear iterations; one non-linear regularization; matrix 79×95×68; voxel size 2mm×2mm×2mm. An 10mm×10mm×10mm full-width-half-maximum Gaussian kernel was used to smooth the data for statistical analysis. The standardized Talairach coordinates and the Brodmann classification for brain regions were used to locate and depict abnormalities of rCBF.

Statistical analysis

Comparisons between IA and control groups were performed using a two-sample t test with SPM8 for imaging data and SPSS 20.0 (IBM SPSS Inc., Chicago, IL, USA) for demographics. Comparisons between resting and stress states within the IA or the control group were performed using paired t tests with SPM8. The Chi-square test was used to compare inter-group difference for categorical variables. The Pearson correlation analysis was performed to identify the relationships between the ratios of rCBF in abnormal cerebral regions divided by rCBF of ipsilateral cerebellums and the durations of participants' internet addiction. All hypothesis tests were two sided with significance level being set at 0.01 for SPM8 analysis and 0.05 for SPSS analysis. Data analysts were blinded to subject identity and diagnostic grouping.

Results

Demographic and basic information

The demographic and baseline information of all participants were summarized in Table 1. The age, gender and education were comparable between the IA group and the control group, with P-values being all larger than 0.05. The IA subjects spent significantly more hours every day (4.6±1.7h vs. 1.2±0.5h, P<0.001) and more days every week (6.3±0.4d vs. 4.3±0.3d, P<0.001) on internet use than the healthy controls.

Changes of rCBF: between-group differences (IA vs. control)

The IA individuals showed significantly increased rCBF in the left mid-frontal gyrus and left angular gyrus, but significantly decreased rCBF in left paracentral lobule, compared to the healthy controls (Table 2, Figure 1). To identify the immediate effects of adenosine on rCBF changes, the stress-state images were compared between IA participants and the healthy controls. Interestingly, more abnormal cerebral regions were identified; namely, significant rCBF increases were found in the mid-frontal gyrus, the precentral gyrus, and the postcentral gyrus on the right side, as well as the inferior frontal gyrus, the superior temporal gyrus, and the anterior cingulate gyrus on the right side; while significant rCBF decreases were demonstrated in the right precentral gyrus, the right inferior parietal lobule and the left cuneus (Table 3).

Table 1. *Demographic and baseline information of the participants included in this study*

Variables	IA (n=35)	Control (n=12)	P-Value
Age (years)	19.6±8.6	18.9±7.9	0.805
Gender (male/female)	24/11	9/3	0.957*
Education (years)	10.6±1.1	11.2±0.7	0.085
Hours of internet used (/day)	4.6±1.7	1.2±0.5	<0.001
Days of internet used (/week)	6.3±0.4	4.3±0.3	<0.001
Duration of IA (years)	3.8±2.2	NA	NA

**: Chi-Square test with continuity correction*

Table 2. *Changes of rCBF in resting state: between-group differences (IA vs. control)*

Regions	Talairach coordinate			BA	P-value
	x	y	z		
rCBF increase					
Right mid-frontal gyrus	28	2	44	6	0.002
Left angular gyrus	-48	-72	34	39	<0.001
rCBF decrease					
Left paracentral lobule	0	-30	50	5	0.008

rCBF, regional cerebral blood flow; BA, Brodmann area.

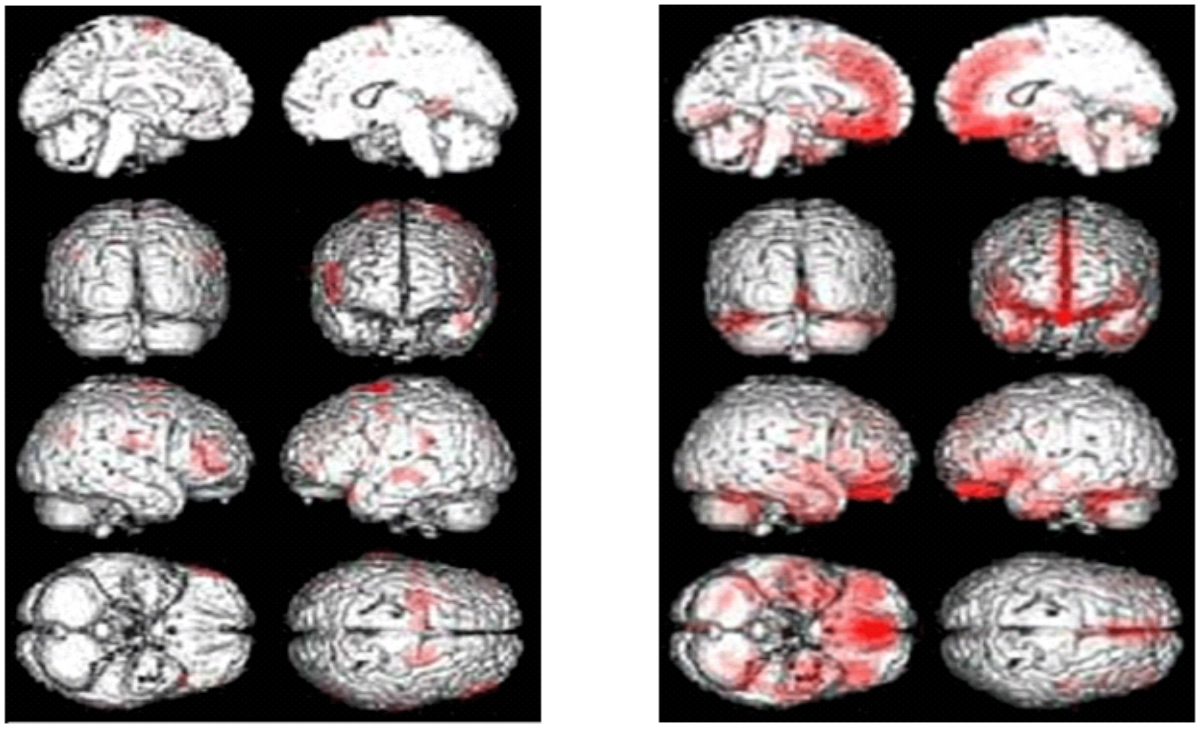

Figure 1. Whole brain three dimensional images generated by SPM showing the abnormal rCBF at resting state. The regions marked in red color denote the rCBF increases (a) and decreases (b) in IA patients compared to the healthy controls (between-group differences).

Changes of rCBF: intra-group differences (resting state vs. stress state)

As compared to resting state, in healthy controls, significant rCBF increases were found in the inferior frontal gyrus, the mid-temporal gyrus, the lingual gyrus and the cuneus on the left side, as well as the inferior frontal gyrus, the precentral gyrus, the superior temporal gyrus, the inferior temporal gyrus and the cingulate gyrus on the right side; while significant rCBF decreases were demonstrated in the superior frontal gyrus, the mid-frontal gyrus, the postcentral gyrus

Table 3. *Changes of rCBF in adenosine-stress state: between-group differences (IA vs. control)*

Regions	Talairach coordinate			BA	P-value
	x	y	z		
rCBF increase					
Right mid-frontal gyrus	58	36	16	46	<0.001
Right precentral gyrus	56	-6	18	4	0.001
Right postcentral gyrus	52	-30	34	2	0.003
Left inferior frontal gyrus	-58	18	4	45	0.009
Left superior temporal gyrus	-44	20	-24	38	0.001
Left anterior cingulate gyrus	-8	-34	-8	32	0.003
rCBF decrease					
Right precentral gyrus	54	-14	42	4	0.001
Right inferior parietal lobule	48	-46	52	40	0.002
Left cuneus	-22	-100	2	18	<0.001

rCBF, regional cerebral blood flow; BA, Brodmann area.

and the uncinus of limbic lobe on the left side, as well as the superior frontal gyrus, the mid-frontal gyrus, the inferior frontal gyrus, the precentral gyrus, the postcentral gyrus, the inferior parietal lobule and the mid-occipital gyrus on the right side (Table 4, Figure 2). By contrast, in IA patients, significant rCBF increases were found in the mid-frontal gyrus, the inferior frontal gyrus, the superior temporal gyrus, the inferior temporal gyrus, the uncinus of the limbic lobe, the cingulate gyrus, and the insular lobe on the left side, as well as the mid-frontal gyrus, the inferior frontal gyrus, the paracentral lobule, the mid-temporal gyrus, the parahippocampal gyrus, the lingual gyrus and the putamen on the right side; while significant rCBF decreases were demonstrated in the superior frontal gyrus, the mid-frontal gyrus, the inferior frontal gyrus, the precentral gyrus, the superior temporal gyrus, the inferior parietal lobule, the postcentral gyrus and the precuneus on the left side, as well as the mid-frontal gyrus, the inferior temporal gyrus, the transverse temporal gyrus and the inferior occipital gyrus on the right side (Table 5, Figure 3).

Changes of rCBF in stress state: between-group difference (IA vs. control)

As some abnormal rCBF in adenosine-stressed state might relate with normal responses to adenosine compared to resting state, we excluded those regions that showed abnormal rCBF in stressed state in healthy controls (Table 4) from those in IA group (Table 5). The rest abnormal regions were compared between the IA group and the control group. The results were summarized in Table 6. After excluding effects of normal response to adenosine, significantly increased rCBF were identified in right paracentral lobule, right mid-frontal gyrus and left superior temporal gyrus, while significant decreased rCBF were demonstrated in right transverse temporal gyrus, left inferior frontal gyrus and left precuneus in IA subjects compared to the controls (Figure 4).

Correlations between rCBF changes and history of internet addiction

Taking the ratio of regional rCBF to ipsilateral cerebellum rCBF as an index to reduce influence from individual variation, regions as shown in Table 6 were selected for further correlation analysis with the duration of IA. Interestingly, those regional-to-cerebellum ratios in rCBF-increased regions in IA patients in stress state were positively correlated with the duration of internet addiction (Figure 5a), while those in rCBF-decreased regions were negatively correlated with the duration of internet addiction (Figure 5b).

Discussion

In this study, we found that IA patients showed abnormal CBF perfusion both in resting and in adenosine-stressed state. Compared to resting state, more cerebral regions of abnormal rCBF were identified in adenosine-stressed state, namely, increased rCBF in right paracentral lobule, right mid-frontal gyrus, left superior temporal gyrus, and decreased rCBF in right transverse temporal gyrus, left inferior frontal gyrus, left precuneus in IA subjects compared to the controls. Abnormal rCBF in these regions significantly correlated with the duration of subjects' internet addiction.

Table 4. *Changes of rCBF in adenosine-stress state compared to rest state in control group: intra-group differences*

Regions	Talairach coordinate			BA	P-value
	x	y	z		
rCBF increase					
Right inferior frontal gyrus	62	18	26	9	0.001
Right precentral gyrus	28	-14	54	6	0.005
Right superior temporal gyrus	54	6	0	22	0.001
Right inferior temporal gyrus	48	-2	-30	20	0.009
Right cingulate gyrus	4	-52	6	30	<0.001
Left inferior frontal gyrus	-56	26	12	45	0.006
Left mid-temporal gyrus	-66	-20	-8	21	0.001
Left lingual gyrus	16	-100	-4	18	<0.001
Left cuneus	-16	-102	4	18	<0.001
rCBF decrease					
Right superior frontal gyrus	26	54	36	9	<0.001
Right mid-frontal gyrus	40	56	22	10	0.003
Right inferior frontal gyrus	56	32	-6	47	<0.001
Right precentral gyrus	54	-4	10	43	0.008
Right postcentral gyrus	46	-22	18	40	0.002
Right inferior parietal lobule	48	-52	52	40	0.007
Right mid-occipital gyrus	58	-66	-6	19	0.004
Left superior frontal gyrus	-38	50	24	10	<0.001
Left mid-frontal gyrus	-8	8	-18	25	0.005
Left postcentral gyrus	-66	-22	18	40	0.002
Left uncinus	-24	4	-20	28	<0.001

rCBF, regional cerebral blood flow; BA, Brodmann area.

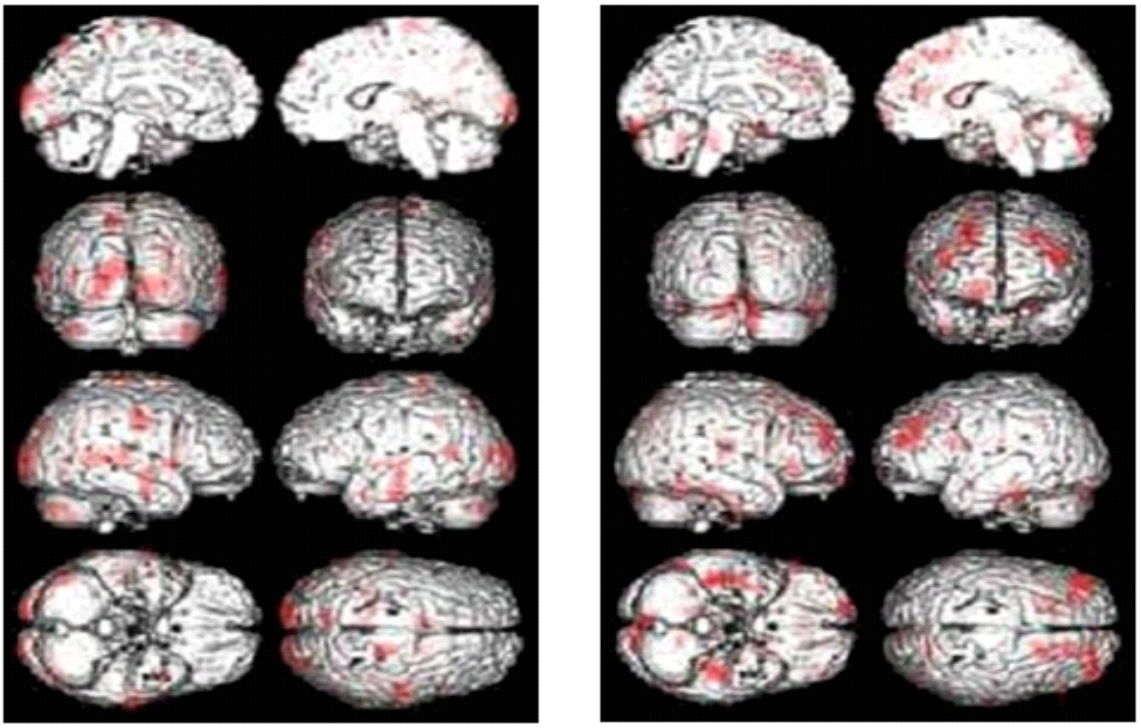

Figure 2. Whole brain three dimensional images generated by SPM illustrating abnormal cerebral regions of rCBF in adenosine-stress state compared to resting state in heathy controls (intra-group differences). The regions with increased (a) and decreased (b) rCBF were marked in red color.

Table 5. *Changes of rCBF in adenosine-stress state compared to rest state in control group: intra-group differences*

Regions	Talairach coordinate			BA	P-value
	x	y	z		
rCBF increase					
Right mid-frontal gyrus	6	4	52	6	0.003
Right inferior frontal gyrus	16	26	-16	47	0.001
Right paracentral lobule	2	-34	52	5	0.005
Right mid-temporal gyrus	50	-78	24	39	0.001
Right parahippocampal gyrus	22	-26	-16	35	0.002
Right lingual gyrus	20	-64	0	19	0.001
Right putamen	24	-12	8	-	0.008
Left mid-frontal gyrus	-44	56	-4	10	0.003
Left inferior frontal gyrus	-58	42	4	46	<0.001
Left superior temporal gyrus	-60	4	0	22	0.005
Left inferior temporal gyrus	-68	-14	8	42	0.002
Left uncinus of the limbic lobe	-38	-14	-28	20	0.005
Left cingulate gyrus	-4	-14	38	24	0.009

(continued)

Left insular lobe	-42	-2	10	13	<0.001
rCBF increase					
Right mid-frontal gyrus	38	60	0	10	<0.001
Right inferior frontal gyrus	54	-32	-16	20	<0.001
Right transverse temporal gyrus	66	-18	10	42	<0.001
Right inferior occipital gyrus	46	-84	4	19	0.003
Left superior frontal gyrus	-18	66	14	10	<0.001
Left mid-frontal gyrus	-28	48	-14	11	<0.001
Left inferior frontal gyrus	58	6	26	9	<0.001
Left precentral gyrus	-50	-4	36	6	<0.001
Left superior temporal gyrus	-44	-54	24	39	<0.001
Left inferior parietal lobule	-56	-32	40	40	0.001
Left postcentral gyrus	-44	-22	62	3	0.001
Left precuneus	-26	-54	52	7	0.002

rCBF, regional cerebral blood flow; BA, Brodmann area

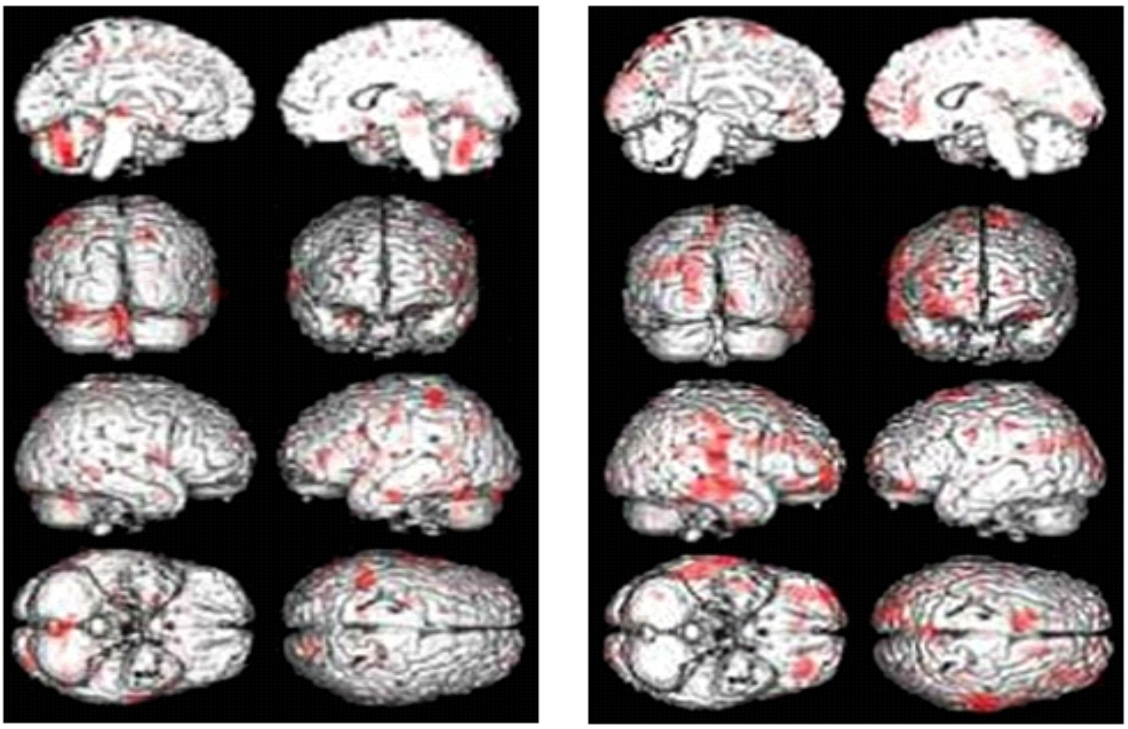

Figure 3. Whole brain three dimensional images generated by SPM illustrates abnormal cerebral regions of rCBF in adenosine-stress state compared to resting state in IA patients (intra-group differences). The regions with increased (a) and decreased (b) rCBF were marked in red color.

Table 6. *Changes of rCBF in adenosine-stress state excluding effects of normal response to adenosine: between-group differences (IA vs. control).*

Region	rCBF (mean ± SD)		Talairach coordinate			BA	P-value
	IA	Control	x	y	z		
rCBF increase							
Right paracentral lobule	1.01±0.08	0.94±0.06	2	-34	52	5	0.008
Right mid-frontal gyrus	1.02±0.08	0.91±0.16	6	4	52	6	0.003
Left superior temporal gyrus	0.98±0.11	0.88±0.07	-60	4	0	22	0.005
rCBF decrease							
Right transverse temporal gyrus	0.82±0.10	0.94±0.11	66	-18	10	42	<0.001
Left inferior frontal gyrus	0.80±0.10	0.92±0.09	58	6	26	9	<0.001
Left precuneus	0.79±0.11	0.90±0.05	-26	-54	52	7	0.002

rCBF, regional cerebral blood flow; BA, Brodmann area

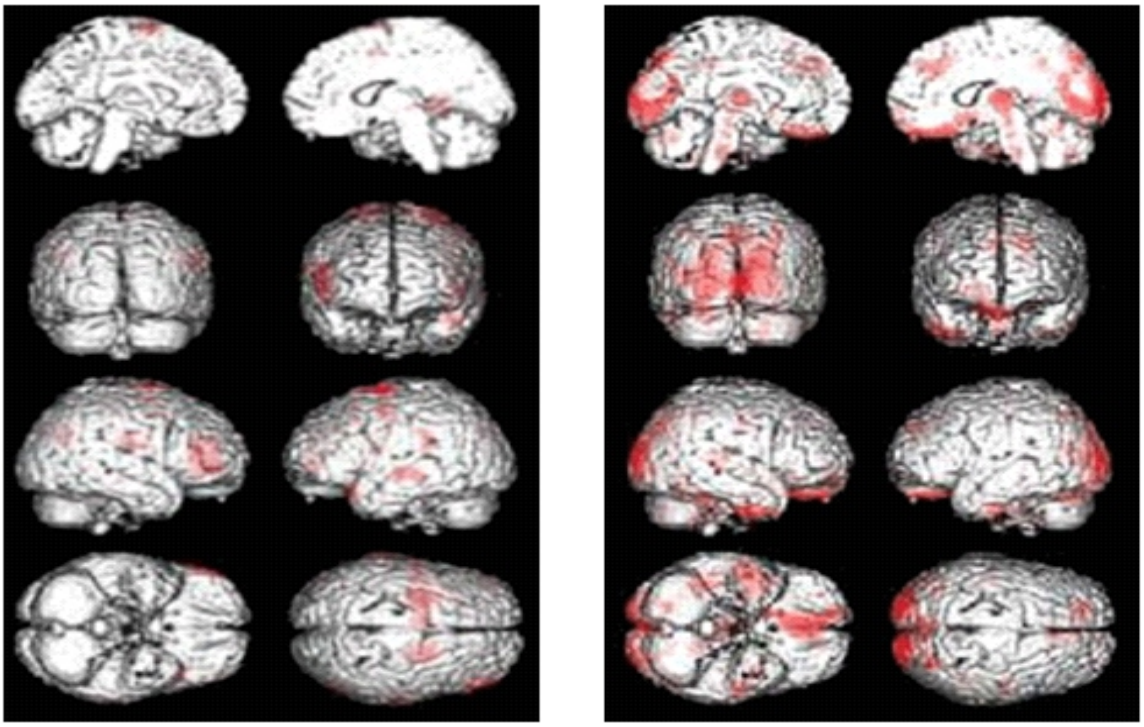

Figure 4. Whole brain three dimensional images generated by SPM presents abnormal rCBF in adenosine-stressed state. The regions marked in red color denote the rCBF increases (a) and decreases (b) in IA patients compared to the healthy controls (between-group differences).

To the best of our knowledge, this study is first to investigate abnormal cerebral blood flow perfusion in patients with IA.

The brain has the capacity to modulate CBF during changes in blood pressure when facing morbidity. This phenomenon is known as autoregulation. The mechanism responsible for the regulation of cerebral arteriolar tone during autoregulation is unclear, but a number of theories have been proposed, including the myogenic, neural, and metabolic hypotheses [24, 28]. All these hypotheses entail biochemical signals whose action leads to a change in arterial diameter. A number of biochemical factors have been proposed to have such a role, one of which refers to a purine nu-

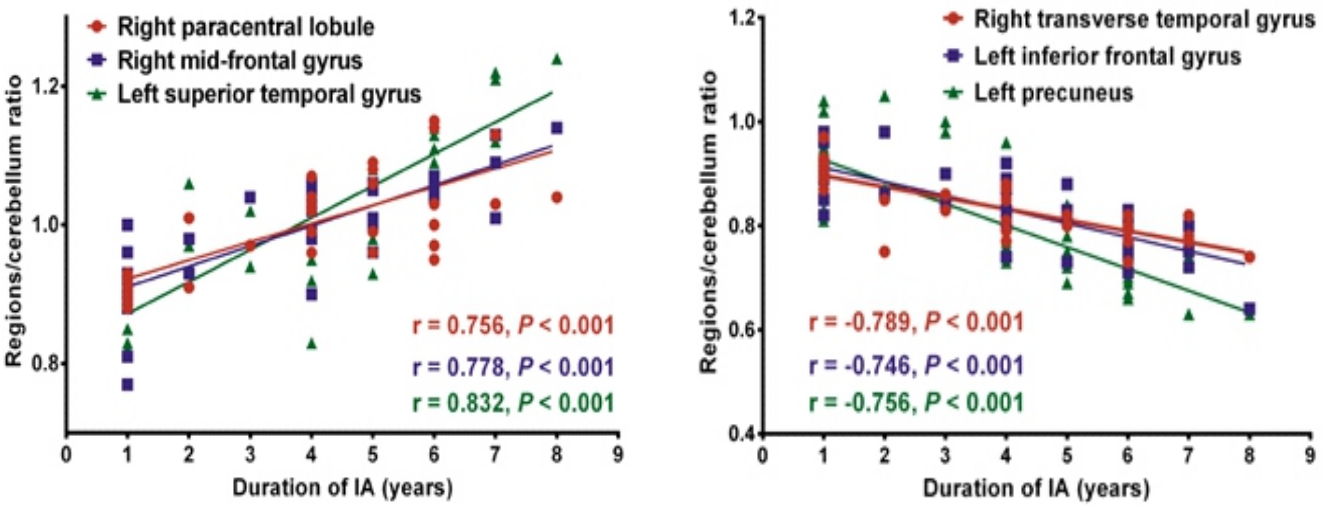

Figure 5. Correlation analysis between the abnormal regional to ipsilateral cerebellar rCBF ratio and the duration of internet addiction. a) In rCBF increased regions, namely, right paracentral lobule, right middle frontal gyrus and left superior temporal, the regional to cerebellar rCBF ratios are positively correlated with the duration of internet addiction, with r being 0.756, 0.778, and 0.832, respectively, and the P values being all less than 0.001. b) In rCBF decreased regions, i.e., in right transverse temporal gyrus, left inferior frontal gyrus and left precuneus, the regional to cerebellar rCBF ratios are negatively correlated with the duration of internet addiction, with r being -0.789, -0.746, and -756, respectively, and the P values being all less than 0.001.

cleoside, namely, adenosine, which is a potent vasodilator. There are four adenosine receptors among vertebrates, which have been denoted adenosine A_1, A_{2A}, A_{2B} and A_3 receptors [29]. Adenosine is a full agonist at all these receptors. A_1 and A_3 receptors are not involved in cerebral vasodilation whereas the A_2 receptor is the main adenosine receptor involved in cerebral vasodilation with the A_{2A} receptor having a higher affinity for adenosine than the less sensitive A_{2B} receptor [29]. Adenosine helps in identifying potential lesions with abnormal blood supply that are invisible during resting state, due to its capacity in enlarging the contrast between lesions and normal tissue, taking advantage of its function of vasodilation. This capacity has been well established in stress myocardial perfusion imaging [22, 23]. Furthermore, similar efficacy of adenosine in regulating the cerebral blood flow for the purpose of achieving stress perfusion imaging has also been reported in several previous studies [24-26]. Moeller et al. (2012) has even found increased orbitofrontal activation after administration of a selective adenosine A_{2A} antagonist in cocaine dependent subjects [30]. In the current study, more cerebral regions of rCBF were identified in adenosine-stressed blood flow perfusion imaging, as compared to rest-state imaging, also confirmed the stress and regulatory function of adenosine in IA patients.

Some previous fMRI studies have found that the dysfunction of frontal lobe, either functional or structural, are associated with IA [8, 13, 16, 30, 31]. For example, imaging results have demonstrated that brain regions, such as the left orbitofrontal cortex (OFC), and the entorhinal cortex are associated with executive function; reduced cortical thickness of OFC is correlated with the impaired cognitive control ability in internet game disorder [32], and with the severity of internet addiction [16]. Besides, the prefrontal cortex, which is implicated in planning complex cognitive behavior, personality expression, and decision making, has also been brought to light great role in internet addiction [31]. Furthermore, the prefrontal cortex is also implicated in the pathology of drug and behavioral addictions, which share similar neurobiological mechanism with internet addiction [33, 34]. All these explained the decreased rCBF in the left inferior frontal gyrus in IA patients compared to the healthy controls in this study. The precuneus is associated with visual imagery, attention, and memory retrievals, while the inferior and transversal temporal cortex have been shown to engage in craving induced by drug cues [35]. Therefore, the reduced rCBF in the right transversal temporal gyrus and in the left precuneus in IA patients in the current study suggest that these areas in IA patients may be associated with their craving of using internet.

It is obvious that internet surfing, especially online game playing, is a complex activity that necessitates high-intensity cooperation of multiple sensor-motor cerebral regions. Regional homogeneity is a widely used method in fMRI studies to measure the functional coherence of a given voxel with its nearest neighbors, and thereby evaluating activities based on the hypothesis that spatially neighboring voxels should have similar temporal patterns [35]. By using this method, Dong et al. (2013) found a significant increase in regional homogeneity in the inferior parietal lobe, left posterior cerebellum, and left middle frontal gyrus and decreased regional homogeneity in temporal, occipital, and parietal brain regions, in patients with internet game disorder compared with healthy controls [36]. Based on this finding, the authors advocated that the long-time online game playing had enhanced brain synchronization in sensory-motor coordination related brain regions and decreased excitability in visual and auditory related brain regions. In this study, the increased rCBF in the right paracentral lobule, right middle frontal gyrus and left superior temporal gyrus in IA patients compared to the healthy controls may also indicate an enhanced brain synchronization and coordi-

nation between sensory and motor related brain regions.

Several limitations in this study should be highlighted. First, the SPET scanner used in this study belongs to a relatively old generation and therefore the spatial resolution is not favorable. For this reason, we could only determine cerebral regions to the level of gyrus. Fortunately, the new generation of SPET scanner, especially those registered with CT has improved the spatial resolution to an acceptable level, which means future studies with this kind of equipment will present higher-quality images with more accurate lesion localization for CBF perfusion imaging. Second, our study is limited by the imbalanced gender proportion of the participants (33 males and 14 females), which might reduce the statistical power and generalizability of the findings. However, we have controlled the gender proportion of participants to be statistically comparable between the IA group and the control group. Future experiments using cohorts with more balanced gender ratio are required to better investigate the abnormal cerebral blood flow perfusion. Third, the striatum is one of the main cerebral regions where adenosine A_{2A} receptors localized and form functional units with dopamine receptors known as heteromers. Through heteromer formation, A_{2A} receptor activation blocks D_2 receptor-mediated decrease in excitability of the striatopallidal neuron [37]. Reduced striatal dopamine D_2 receptor has been previously reported in IA patients [17, 20], which means the normal striatal rCBF as shown in adenosine-stressed images in this study might not represent the real situation of blood flow in striatum, probably affected by the reduced A_{2A}-D_2 heteromer. Therefore, future studies that simultaneously carry out adenosine-stressed CBF perfusion imaging and dopamine-D_2-receptor specified imaging are needed to better illustrate the relationship between cerebral blood flow and dopamine-D_2-receptor levels in patients with IA.

In conclusion, our results show specific functional changes in behaviour that may appear in IA patients related to the CBF findings. Adenosine can be used as a pharmacological stress agent for cerebral blood perfusion imaging for patients with IA, by which more cerebral regions of abnormal rCBF were identified compared to resting state. These abnormal rCBF may indicate the neurological mechanism of IA patients.

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The Silence

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The words of the silence
Are solemn and nice
Like the sun's golden rays
Behind white clouds in skies

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The words of the silence
Are plenty of preaches
And meaningful feelings
Like the eternity's greetings