Altered Brain Network Degree Centrality in Major Depressive Disorder via Electro-Acupuncture Stimulation at Baihui(gv20)

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Altered Brain network Degree Centrality in Major Depressive Disorder via Electro-acupuncture Stimulation at Baihui(GV20)

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Abstract:

Background: Although the acupuncture treatment of major depressive disorder(MDD) has been recognized by the latest clinical practice guidelines of the American Academy of Internal Medicine, complex therapeutic mechanisms need further to clarify. The aim of the study is investigate whether the aberrant resting state brain network in MDD patients could be regulated by acupuncture at GV20 using functional magnetic resonance imaging(fMRI) combined with degree centrality(DC) method.

Results: Compared to healthy subjects, MDD patients exhibited significantly aberrant DC in widely brain regions, including cortical(PFC, precuneus, temporal, insula) and sub-cortical (thalamus, putamen and caudate) structures. Furthermore, results showed that acupuncture at GV20 induced down-regulation the DC of abnormal brain regions in MDD patients.

Conclusions: Our findings provide imaging evidence to support that GV20-related acupuncture stimulation may modulate the abnormal brain function state in MDD patients by using fMRI technique combined with DC analysis. This study may partly interpret the neural mechanisms of acupuncture at GV20 which is used to treat patients with MDD in clinical.

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Keywords：degree centrality, major depressive disorder, Baihui, acupuncture

Background

Major depressive disorder (MDD) refers to a frequently psychiatric mood disorder characterized by affective, cognitive, and somatic symptoms[1], which has become a global public health problem in virtue of its significant negative impact on individual physical health and a heavy socioeconomic burden[2]. The second generation antidepressants (selective serotonin reuptake inhibitor, serotonin norepinephrine

reuptake inhibitor, selective serotonin noradrenaline reuptake inhibitor) are commonly used in clinical for treating MDD. Even though it works very well, its high rate of adverse reactions, delayed onset and lack of response to drug therapy in some patients limited further application[3,4]. It is thus necessary to seek a new therapeutic approach for treatment of MDD.

As a complementary and alternative therapy method, acupuncture could improve microcirculation, balance organ function, and adjust mental activities, which has been increasingly and widely accepted by western countries[5]. The acupuncture treatment of MDD has the advantages of significant curative effect and less side effects, which has been recognized by the latest clinical practice guidelines of the American Academy of Internal Medicine[6-10]. According to traditional chinese medicine theory, Baihui(GV20) is a commonly acupoint used to relief of dizziness, headache and anxiety by acupuncture stimulation, which attributes to the effect of acupuncture at GV20 on modulating vascular, endocrine, immune and/or nervous systems[11]. Researches has confirmed that GV20 was indentified to be involved in the treatment of MDD[12,13]. Up to date, the exactly mechanism of acupuncture treatment for MDD is still insufficient.

Resting-state functional MRI (Rs-fMRI) can reveal the intrinsic functional connectivity in the human brain by measuring spontaneous brain activity[14,15], which has been successfully used to monitor the acupuncture-related neural response patterns in human and explored an opportunity for exploring the neural mechanism underlying acupuncture. Neuroimaging technologies have been used to

investigating neural mechanisms of acupuncture, and it has been found that acupuncture stimulation may modulate the default mode network(DMN) in healthy subjects[16,17], and patients with certain psychiatric disorders, such as stroke[18], migraine[19], Alzheimer's disease[20], and depression[21,22]. Our previous study also indicated that modulatory effect on the intrinsic connectivity within the DMN in MDD patients induced by acupuncture at GV20[22].

As a frequently fMRI data analysis approach, the degree centrality (DC) can be applied to characterize functional connectivity within the whole-brain network by graph theory-based network measures assessing the centrality or functional importance (A node will have a high DC if it has numerous direct connections to other nodes, vice versa)[23,24], which has been employed to reveal the mechanisms of neuropsychiatric diseases, including AD[25], Parkinson's disease[26], schizophrenia[27] and depressive disorder[28]. These findings give us reason to believe that DC analysis of Rs-fMRI data is promising to address the mechanism of acupuncture for treating MDD.

The aim of the study is employed the Rs-fMRI combined with DC approach to investigate the aberrant brain connectivity in MDD patients, and whether these regions could be regulated induced by acupuncture at GV20. Based on works by others and our previous work, we hypothesized that: (i) the abnormal DC value of the nodes in resting state brain networks could be found in patients with MDD; (ii) the aberrant brain regions might be down-regulated by acupuncture at GV20.

Results

Demographic and clinical results

One MDD patient was excluded from further data analysis due to incomplete EAS at GV20 during the study. There were no significant differences in terms of age, gender and weight between the patients and healthy subjects. Patients had higher scores in HDRS-17, SDS and SAS compared to healthy subjects **(Table.1).**

Acupuncture Sensations Results

The prevalence of *Deqi* sensations reported by MDD patients was presented as intensity**(Figure.1).** The current results revealed that main *Deqi* sensations included fullness, numbness, dull pain, sharp pain and tingling.

DC analysis rs-fMRI data results

Compared with HCs, MDD patients showed significantly decreased DC value in the bilateral orbitofrontal cortex(OFC), bilateral insula and right middle temporal, but increased DC in widely brain regions, including bilateral dorsolateral prefrontal cortex(DLPFC), bilateral M2(premotor cortex), bilateral precuneus, bilateral caudate, bilateral hippopotamus/para-hippopotamus(HIPP/paraHIPP), right midcingulate cortex(MCC), right putamen, right supplementary motor area(SMA) and left thalamus **(Figure.2 andTable.2).**

In addition, comparison of the data before acupuncture at GV20 in MDD patients, the results indicated that acupuncture could increase the DC values of bilateral OFC induced by acupunture, and also decrease the DC values of several brain

regions, including left DLPFC, bilateral M2, bilateral HIPP/paraHIPP, right MCC, right putamen, and right SMA **(Figure.3 and Table.3).**

Discussion

Utilizing the rs-fMRI technology combined with DC approach, the present study investigated whether the aberrant resting state brain network in MDD patients could be regulated by acupuncture at GV20. The results validated our prior hypothesis that the abnormal DC values of cortex and sub-cortex regions were found in MDD patients compared with HCs, which located in OFC, DLPFC, M2, precuneus, HIPP/paraHIPP, MCC, SMA, insula, middle temporal and sub-cortex regions(caudate, putamen, thalamus). Interestingly, several aberrant brain regions mentioned above can be down-regulated by acupuncture at GV20, mainly involved in OFC, DLPFC, M2, SMA, MCC, HIPP/paraHIPP and putamen. Our findings could provide valuable imaging evidence that the connection status of abnormal brain network nodes could be down-regulate by acupuncture at GV20 for comprehending the mechanism of acupuncture for treatment on MDD via using rs-fMRI combined with DC approach.

Aberrant DC values of brain regions in MDD patients

Accumulating evidence has confirmed that the abnormality of DMN is closely related to the physiopathology of MDD[29]. OFC, Precuneus, HIPP/paraHIPP, DLPFC, and MTG belong to the key components of DMN, which performed functions such as regulating emotion, self-referential activities and planning the

future[30]. OFC is a vital part of the prefrontal cortex, which is responsible for top-down regulation of emotion and attention, and dysfunctions in this area play a role in the pathogenesis of MDD patients[31]. OFC is one of the most connected regions of the brain and participates in multiple brain functional networks[32]. It receives highly processed input from all the sensory modalities, and is connected to other prefrontal areas(e.g. DLPFC), M2, and subcortical areas such as the striatum(e.g. putamen and caudate), amygdala and HIPP[33,34]. This dense connectome suggests that functional interactions with other brain regions are key to the processes that are performed by the OFC. Evidence suggests that OFC partake of the executive control of information processing and behavioral expression by inhibiting neural activity associated with irrelevant, unwanted, or uncomfortable (e.g. painful) information, sensations, or actions[32]. Therefore, we considered that decreased DC values in OFC might results in its inhibitory function decline for other several regions in MDD patients, which including precuneus, HIPP/paraHIPP, putamen, caudate, M2 and DLPFC, and causing these brain regions to be overactive(namely increased DC values). We speculated that OFC might be a key region for the pathophysiological mechanisms of MDD.

As a sub-region of the cingulate gyrus, the MCC(also known as the ‘dorsal’ ACC) constitutes a hub where information about reinforcers can be linked to motor centres responsible for expressing affect and executing goal-directed behaviour, which is commonly activated by imaging studies of negative affect, pain and cognitive control[35]. Anatomically, MCC projects to the spinal cord, striatum and primary motor, PC and SMA[36]. The putamen and caudate, belong to striatum, has

been associated with mood, cognitive processes, motivation and regulation of movement[37]. Meanwhile, the thalamus is an integral part of the emotional salience network, emotion modulation network and cognitive/executive network. Furthermore, the insula has bidirectional connections with the frontal, parietal, and temporal lobes; the cingulate gyrus; and subcortical structures such as the amygdala, brainstem, thalamus, and basal ganglia. These connections serve as the anatomical foundation for the integration of autonomic, viscerosensory, visceromotor, and limbic functions in the insular cortex[38]. We inferred that the altered of DC values in MCC and insula affect the active state of the brain network, which might be involved in the pathogenesis of MDD.

In general, complex brain function activities are performed by multiple brain functional areas, rather than a single region. The brain's functional connectome is necessarily dynamic as it underpins a multitude of brain states involving emotion, cognition, action, perception, and sensation[39,40]. DC measures allow us to capture the complexity of the functional connectome as a whole. Our findings of present work indicated that the abnormal DC values of widely nodes in MDD patients might be the pathogenesis of MDD.

Regulatory effects related to acupuncture at GV20

Our study found that the altered DC values in several brain regions in MDD patients induced by acupuncture at GV20, which mainly embodied in decreasing DC values in bilateral HIPP/paraHIPP, left DLPFC, bilateral M2, right SMA, right MCC and right putamen, and increasing that in bilateral OFC. Exhilaratingly,

184 acupuncture of GV20 could down-regulate the abnormal DC values brain regions
185 in MDD patients.

186 To our knowledge, acupuncture can achieve the purpose of treating diseases
187 by improving microcirculation, balancing organ function, and adjusting mental
188 activities[41]. The safety and efficacy of acupuncture for treating MDD has been
189 proved in numerous clinical studies[42]. Our previous study found that the regulatory
190 effect on DMN in patients with MDD by acupuncture stimulation[22], consistent with
191 the results of previous studies[29]. Applying DC methods in present study, we found
192 that the importance of brain network nodes has changed in MDD patients induced
193 by acupuncture at GV20. OFC, HIPP/paraHIPP, and DLPFC belong to the key
194 components of DMN, which has been confirmed that aberrant activities in MDD
195 patients by neuroimaging studies[29]. Among of them, OFC is a vital part of the
196 prefrontal cortex, which is responsible for top-down regulation of emotion and
197 attention, and dysfunctions in this area play a role in the pathogenesis of MDD
198 patients[43]. We found that raise the DC values in bilateral OFC underwent
199 acupuncture at GV20, could increase the inhibitory effect of OFC on other brain
200 regions. We thus inferred that acupuncture could re-balance the importance of
201 DMN network nodes in MDD patients.

202 In addition, a core characteristic of patients with depression is the loss of
203 interest in pleasurable activities and limitations in multiple dimensions of well-being.
204 OFC, HIPP/paraHIPP, DLPFC, motor cortex(M2 and SMA), and putamen are also
205 formed the reward system, which involved in pleasure and motivation, and have
206 been proven to play important roles in the pathophysiology of MDD[44,45]. Recent

study suggest that the observed activities change of the reward system was associated with clinical improvement in MDD patients induced by acupuncture[46]. The present study indicated that acupuncture could change the DC values in reward system, we thus inferred that acupuncture could re-balance the importance of DMN network nodes in MDD patients.

There were some limitations of our study: (1) Our study aimed to investigate whether the aberrant brain connectivity in MDD patients could be modulated by acupuncture, but not to investigate the specificity of acupoint (GV20). So, there was not a sham acupoint as a control in our experiment paradigm. However, the acupuncture associated with both GV20 and sham acupoint was our another research target in the future. (2) There were not different gender distributions of the two groups. Although gender, as a nuisance covariate, was regressed out from our data, gender factor could still not fully eliminate. Gender differences should be investigated and the present findings should be retested in larger samples in the future. (3) The present results showed a preliminary research about the immediate effect of EAS on the DMN in patients with MDD. Further studies were still needed to confirm whether or not there were the possibilities of improving treatment effect in patients by a long-term EAS at GV20.

Conclusions

We investigated whether the aberrant resting state brain network in MDD patients could be regulated by acupuncture at GV20 by using the rs-fMRI technology

combined with DC approach. The results validated that the abnormal DC values of cortex and sub-cortex regions were found in MDD patients compared with HCs, Interestingly, and several aberrant brain regions mentioned above can be down-regulated by acupuncture at GV20, including OFC, DLPFC, PC, SMA, MCC, HIPP/paraHIPP and putamen. Our findings could provide valuable imaging evidence that the connection status of abnormal brain network nodes could be down-regulate by acupuncture at GV20 for comprehending the mechanism of acupuncture for treatment on MDD via using rs-fMRI combined with DC approach.

Methods

Participants

Thirty patients with first-episode, drug-naïve MDD (21 females and 9 males) were recruited for the study in the local hospital via advertising recruitment. every patient was diagnosed by two associate chief physician psychiatrists using the structured clinical interview of the diagnostic and statistical manual of mental disorders-fourth criteria (DSM-V)[47]. The inclusion criteria included: (1) patients is first-episode, untreated; (2) patient is 18-45 years of age; (3) right-handed; (4) the score of at least 18 on 17-items Hamilton Depression Rating Scale (HDRS-17)[48]. The exclusion criteria were: (1) having other neuropsychiatric diseases by DSM-IV criteria; (2) having history of head injury and degenerative diseases, such as movement disorder and Parkinson's disease; (3) having acutely suicidal or

homicidal tendency; (4) having any MRI contraindications; (5) being non-responders in acupuncture needling.

Twenty-nine healthy subjects (14 females and 15 males; mean age: 26.76±1.72 years) were recruited in this study, who were free of depression or other psychiatric or neurological illness, and had no history of head injury and alcohol or drugs abuse. Healthy subjects did not have any family history related to neurological or psychiatric illness in their first-degree relatives.

In addition, it was required that all of the subjects were no smokers， current pregnancy or breast feeding. Meanwhile, each subject completed an identical assessment protocol, which including the HDRS-17, self-rating depression scale (SDS) and self rating anxiety scale (SAS)[49].

Study Procedures

The non-repeated event-related (NRER) paradigm was applied in the study[50,51]. Healthy subject underwent only a 6-minute resting-state MRI scan. Each MDD patient underwent 6-minute resting-state MRI scans respectively before and after the acupuncture stimulation. 20-minute electro-acupuncture stimulate(EAS) at GV20 was operated by the same professional acupuncturist (1Hz, 2mA, continuous-wave, HuaTuo-brand, SDZ-V-type, Shanghai, China). EAS was performed by inserting the sterile stainless steel disposable needle (0.30 mm in diameter and 25 mm in length; Huatuo-brand, Suzhou, Jiangsu, China) into GV 20 at the depth of needling arranging from 1.0 cm to 1.5cm. Another electrode was attached to the acupuncture needle which was shallowly inserted point 1.0 cm

nearby GV20. The flow chart was shown in Figure 1. Every subject was instructed to keep eyes closed, not to think about anything and to stay awake in the MRI scanning. At the end of scanning, each MDD patient was required to recall acupuncture sensations as following: aching, soreness, numbness, fullness, sharp or dull pain, pressure, heaviness, warmth, coolness, tingling, itching, and any others. The intensity of each sensation was measured by using a 100-point visual analogue scale (VAS) (0 = no sensation, 10–30 = mild, 40–60 = moderate, 70–80 = strong, 90 = severe and 100 = unbearable sensation), which was similarly determined by Hui et al[52].

fMRI Data Acquisition and Preprocessing

Images were acquired using a 3.0 Tesla Siemens Magnetom Verio MRI System (Siemens Medical, Erlangen, Germany) in the local hospital. Each subject's head was fixed by foam pads in a standard 8-channel birdcage head coil for reducing head movement. Functional images were acquired with a single-shot gradient–recalled echo planar imaging (EPI) sequence with the parameters: repetition time (TR)/echo time (TE) = 2000ms/30ms, Flip angle = 90°, field of view (FOV) = 240mm×240mm, matrix size = 64×64, slice thickness = 5 mm and slices=31. High resolution T1-weighted images were then collected with a volumetric three-dimensional spoiled gradient recall sequence with the parameters: TR/TE = 1900 ms/2.22ms, FOV = 250 mm×250 mm, matrix size: 250×250, flip angle = 9°, slice thickness = 1 mm and 176 slices).

Statistical parametric mapping software (SPM12, http://www.fil.ion.ucl.ac.uk/spm) and data processing and analysis of brain imaging toolbox (DPABI, http://rfmri.org/dpabi) (http://rfmri.org/DPARSF) were used to preprocess the MRI data. The initial 5 functional volumes were discarded for stabilization of the initial signal. The remaining volumes were then corrected by slice timing and realigned to correct for head motion. Data with maximum displacement in head rotation of larger than 2°or any directions of larger than 2 mm were excluded from further analysis. The datasets were further spatially normalized to the Montreal Neurological Institute (MNI) template and resampled to 3×3×3 mm3 isotropic voxels. The normalized data were smoothed with a 4-mm full width at half maximum (FWHM) Gaussian kernel. Nuisance covariates were regressed out from our data, including the 24 head motion parameters, white matter signal and cerebrospinal fluid (CSF) signal. As global signal regression may cause a negative shift in the distribution of correlations[53-55], global signal was not regressed in our study. The data were then detrended and bandpass filtered from 0.01 to 0.1 Hz to reduce the effect of low-frequency drifts and high-frequency noise.

DC calculation

We used voxel-based whole-brain correlation analysis on the preprocessed fMRI data to calculate the DC, which mentioned in the previous studies. Firstly, pearson's correlation coefficient was calculated between all pairs of brain voxels in the gray matter mask. Secondly, we converted the Pearson's correlationdata to

normally distributed Fisher's Z-scores and constructed the whole-brain functional network by thresholding each correlation at >0.25. The DC for a given voxel was calculated as the sum of the significant connections in at the individual level. Finally, the voxel-wise DC value was transformed into a Z-score map using the Fisher-Z transformation to improve normality. The z-score map was finally smoothed by a Gaussian kernel of 4 mm full-width at half-maximum (FWHM). Because of the uncertainty of interpretation and detrimental effects on test-retest reliability, only positive correlations were considered in the DC calculations.

Statistical analysis

Demographic and clinical data were compared by using two-sample t-test and Chi-square test. The threshold level in all statistical analysis for significance criterion was determined at $p<0.05$. Main acupuncture sensations were described with each sensation intensity in patients.

The two-sample t-test was used for the imaging-related group differences in the HCs and MDD patients, and the paired t-test was used for alteration after and before the acupuncture treatment in the MDD patients at a significant level $p<0.05$ (false discovery rate (FDR) corrected).

List of abbreviations

MDD=Major depressive disorder

Rs-fMRI=Resting-state functional MRI

DMN=default mode network

DC=degree centrality

OFC=orbitofrontal cortex

DLPFC=dorsolateral prefrontal cortex

M2=premotor cortex

HIPP/paraHIPP=hippopotamus/para-hippopotamus

MCC=midcingulate cortex

SMA=supplementary motor area

Declarations

Ethics approval and consent to participate

All procedures of the study are conducted in accordance with the Declaration of Helsinki. Each participant is clearly told the whole experiment procedure and signed an informed consent in the study, which is allowed by the Medicine Ethics Committee of the First Affiliated Hospital of Guangxi University of Chinese Medicine. The present study was registered at http://www.chictr.org.cn, and the Clinical Trial Registration Number is ChiCTR-IOR-15006357.

Consent for publication

All paticipants have consent for publication.

Availability of data and materials

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Competing interests

The authors declare that they have no conflicts of interest.

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Author Contributions

Conceptualization, D.D. and Y.P.; writing—original draft preparation,S.L.; writing—review and editing, G.D. and Y.C. and H.L. Data curation—P.S. and S.L. and L.L. All authors have read and agreed to the published version of the manuscript.

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Tables

Table 1 Demographic and clinical characteristics for the study.

Variable	HSs(n=29)	DPs(n=29)	*p* value
Gender(male/female)	15/14	9/20	0.182[a]
Age(years)	26.76±1.72	28.69±6.69	0.138[b]
Weight(kg)	59.55±12.95	55.10±10.50	0.157[b]
SDS	42.17±7.74	62.72±9.81	<0.001[b]
SAS	43.00±7.59	62.14±8.79	<0.001[b]
HDRS-17	4.48±3.22	21.31±2.58	<0.001[b]

Abbreviations: SDS: Self Rating Depression Scale; SAS: self rating anxiety scale; HDRS: Hamilton Depression Rating Scale; HSs: healthy subjects; DPs: major depressive disorder patients. Except for gender, all values are mean ± standard deviation (SD).

a The p-value was obtained by Chi-square.

b The p-value was obtained by two sample t-test.

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533 **Table 2** Main localization of DC maps by comparing MDD patients with Healthy Subjects

Regions	BA	MNI			T-Value	Vol
		X	Y	Z		
left DLPFC	46	-33	36	42	4.48	38
right DLPFC	46	33	42	39	3.91	49
right SMA	6	6	-12	60	4.20	51
left M2	6	-33	-3	58	4.04	34
right M2	6	33	-6	60	5.31	40
left precuneus	7	-6	-63	45	5.49	90
right precuneus	7	9	-75	45	5.32	72
right MCC	32	6	21	36	5.98	60
left thalamus		-15	-12	18	4.88	21
right putamen		15	12	-6	4.72	18
left caudate		-12	-9	21	4.80	49
right caudate		15	-12	21	3.47	30
left HIPP/paraHIPP		-30	-12	-18	4.66	55
right HIPP/paraHIPP		36	-24	-15	4.41	35
left OFC	11	-45	27	-12	-3.45	35
right OFC	11	30	42	-12	-3.45	49
right middle temporal	21	57	-21	-9	-4.71	60
left insula	48	-33	3	15	-3.55	39
right insula	48	36	3	15	-4.00	53

Abbreviations: DLPFC:dorsolateral prefrontal cortex; SMA:supplementary motor area; M2:premotor cortex; MCC: midcingulate cortex; HIPP:hippopotamus; OFC:orbitofrontal cortex.

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536 **Table 3** Altered DC maps in MDD patients by comparing Pre-EAS and Post-EAS

Regions	BA	MNI			T-Value	Vol
		X	Y	Z		
left OFC	11	-9	54	-15	5.56	61
right OFC	11	6	54	-12	5.42	71
left DLPFC	46	-39	36	30	-3.88	50
left M2	6	-46	0	33	-3.87	77
right M2	6	42	6	33	-4.32	81
right SMA	6	6	15	54	-4.44	60
right MCC	32	6	27	36	-4.47	65
left HIPP/paraHIPP		-33	15	-18	-3.74	21
right HIPP/paraHIPP		30	15	-18	-4.34	28
right putamen		18	12	-6	-3.82	25

Abbreviations: DLPFC:dorsolateral prefrontal cortex; SMA:supplementary motor area; M2:premotor cortex; MCC: midcingulate cortex; HIPP:hippopotamus; OFC:orbitofrontal cortex.

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Figures

Figure.1 Results of acupuncture sensations in patients with MDD.

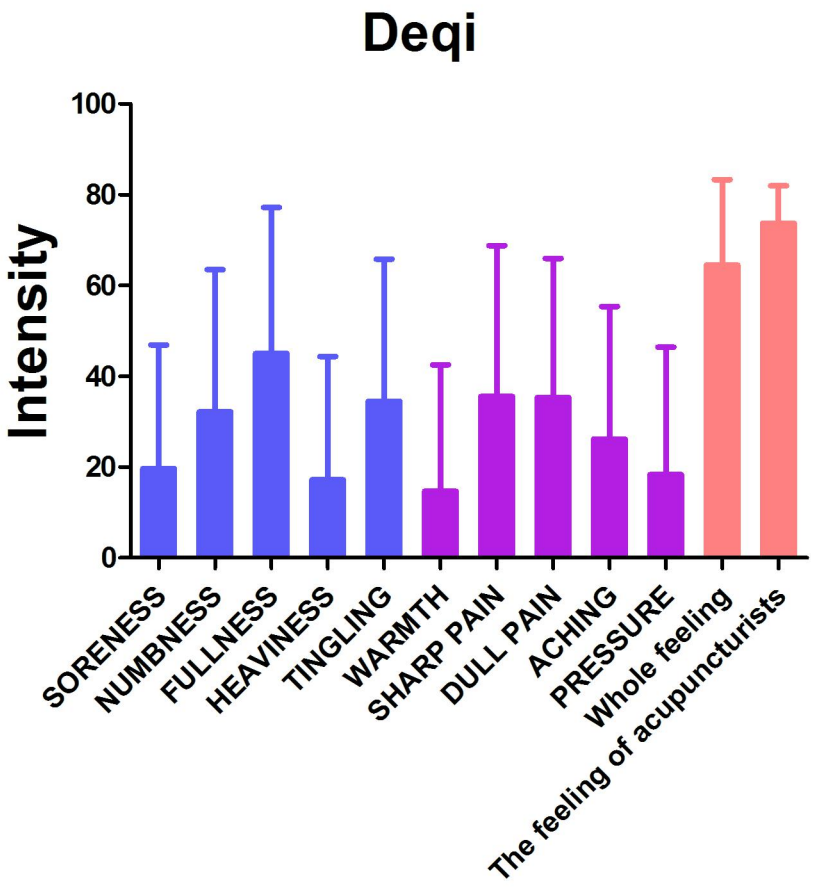


Figure.2 Altered DC values in MDD patients compared with HC.

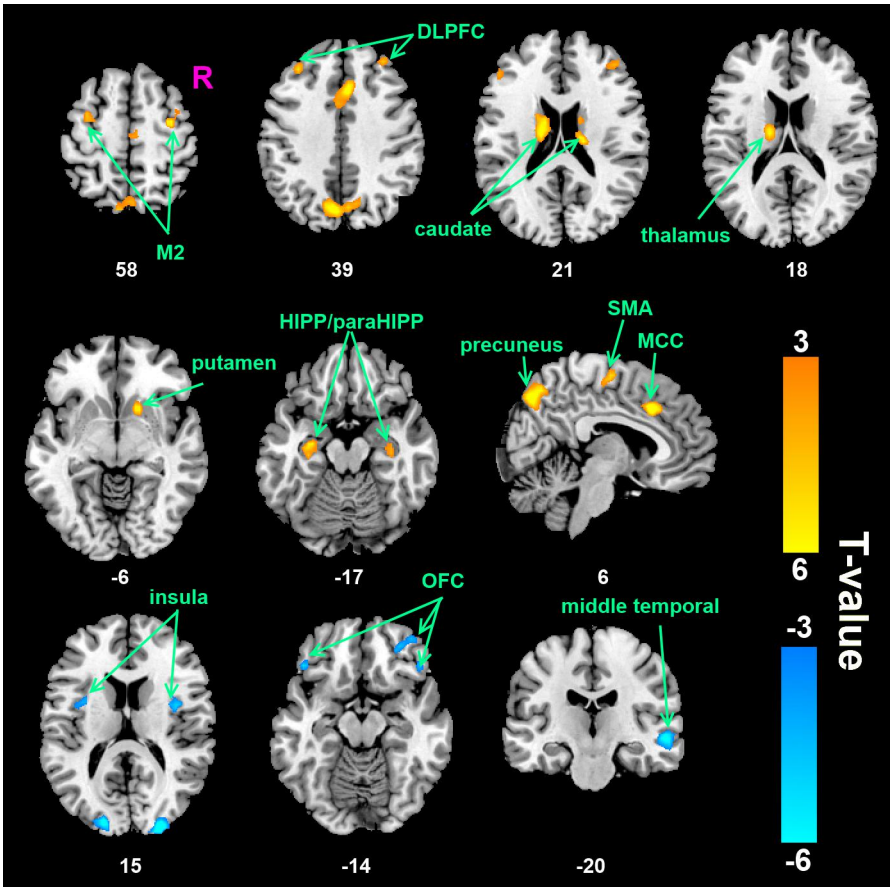
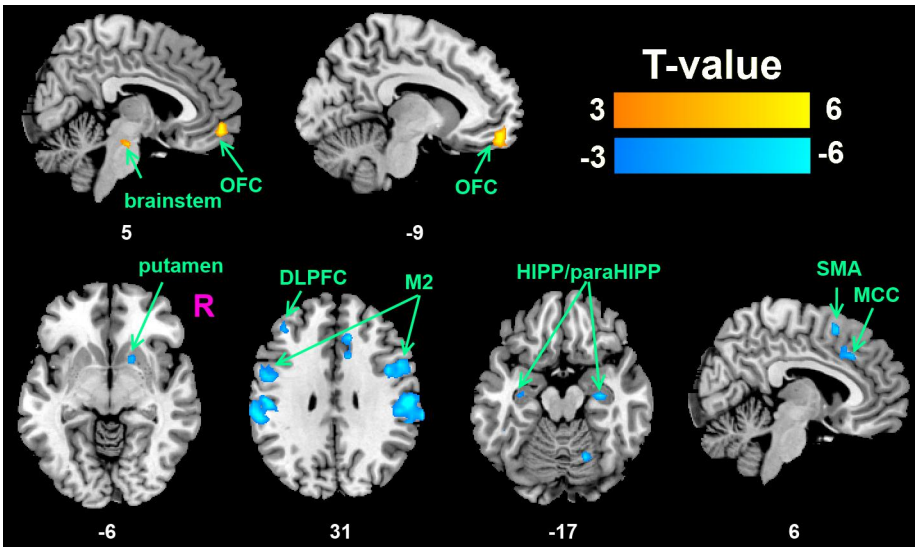


Figure.3 Regulating effect of EAS at GV20 in MDD patients.



Figures

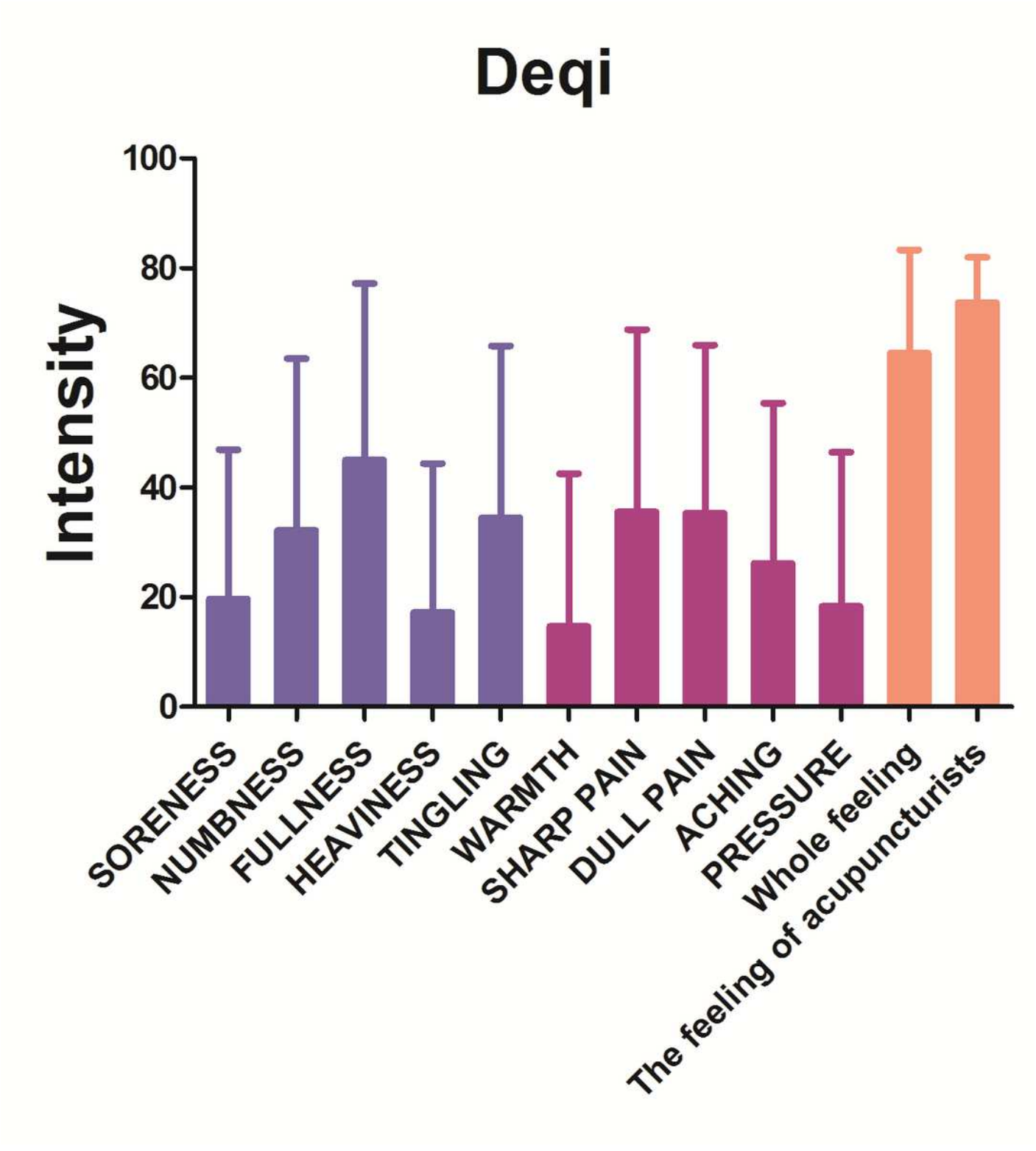


Figure 1

Results of acupuncture sensations in patients with MDD.

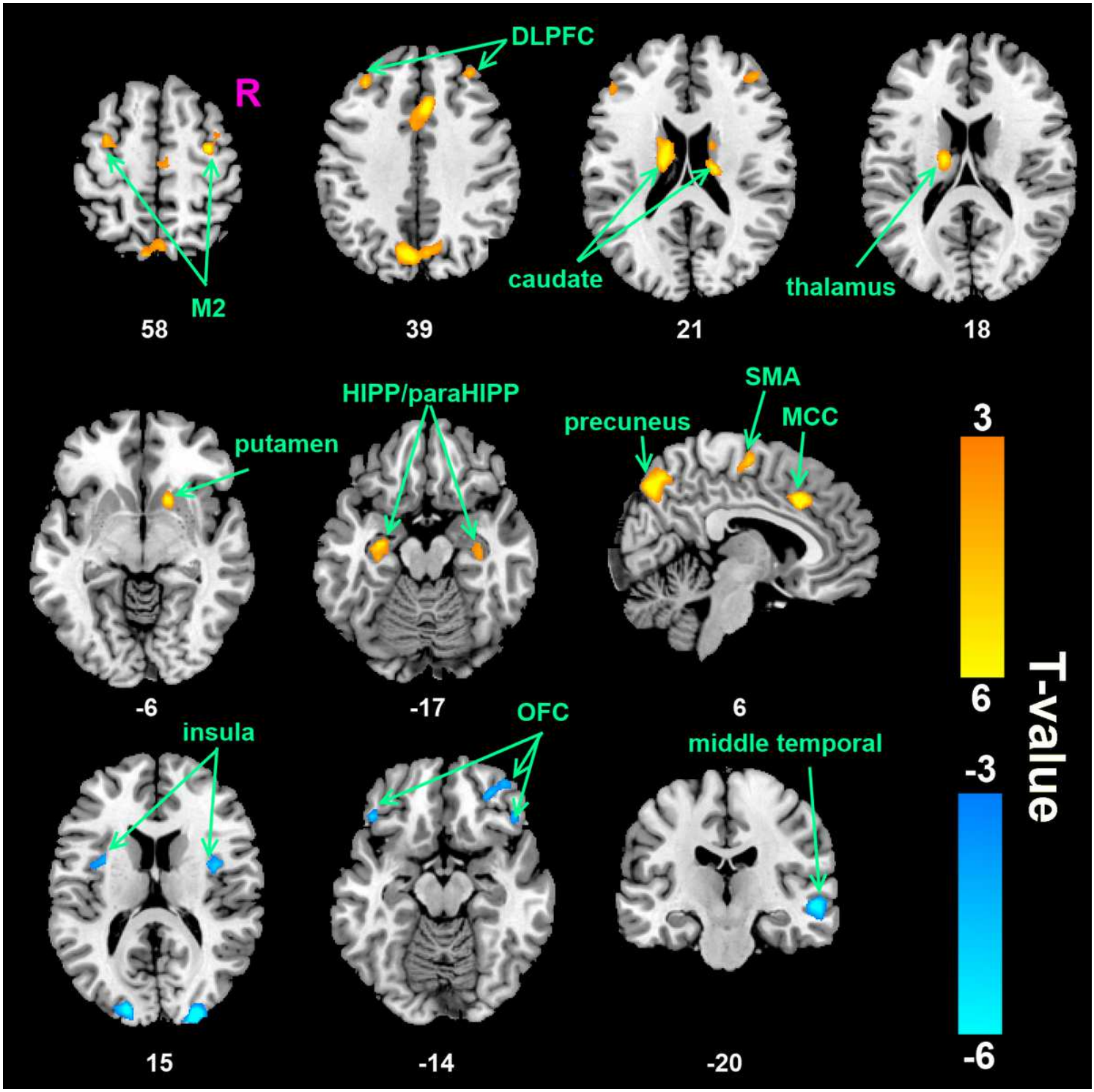


Figure 2

Altered DC values in MDD patients 559 compared with HC.

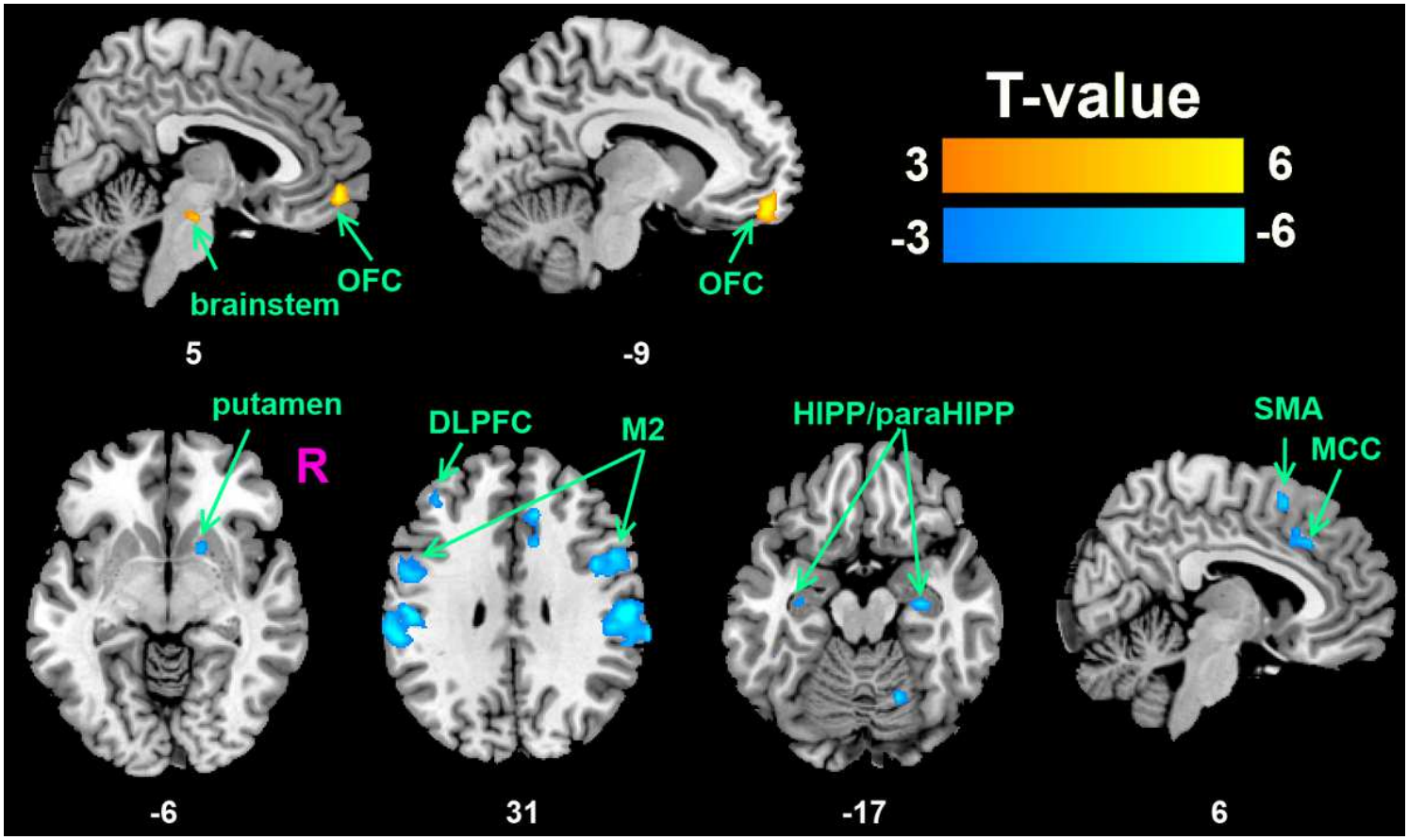


Figure 3

Regulating effect of EAS 573 at GV20 in MDD patients.