NEUROTRANSMITTERS AND EMOTIONS

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PUBLISHED IN: Frontiers in Psychology

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88963-536-8
DOI 10.3389/978-2-88963-536-8

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NEUROTRANSMITTERS AND EMOTIONS

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Citation: Wang, F., Yang, J., Pan, F., Huang, J. H., Ho, R. C., eds. (2020). Neurotransmitters and Emotions. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88963-536-8

Table of Contents

frontiers in Psychology

EDITORIAL
published: 29 January 2020
doi: 10.3389/fpsyg.2020.00021

Editorial: Neurotransmitters and Emotions

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Keywords: neurotransmitters, emotions, dopamine, monoamine, norepinephrine

Editorial on the Research Topic

Neurotransmitters and Emotions

Despite the central importance of emotions for human existence (LeDoux, 1995, 2000), many debates are still had over the definition of emotion, the number of discrete basic emotions that exists, and whether different emotions have different physiological signatures (Gu et al., 2015). Although there has been no shortage of psychological research on these matters involving emotions, many core issues remain unaddressed (Gu et al., 2016). However, discoveries on representations of emotions in the brain may shed light on the nature of the complex emotional processes. In the efforts of elucidating the neural basis of emotions, the majority of the work focused on identifying neural structures responsible for the experience of particular emotions, which culminated in the limbic system theory of emotion in the mid-twentieth century. This approach to emotions has outlined the neural anatomical basis of emotions, and located important structures involved in basic emotion (LeDoux, 2000). This is consistent with the proposal of Basic Emotion Theory, which suggests that every basic emotion has a specific brain locus. This work was demonstrated by fMRI studies. However, many recent studies revealed that multiple neural structures could be implicated in one particular basic emotion, while a specific area could attribute to a number of basic emotions. For example, the amygdala has been recognized as the central site for all negative emotions, including fear and anger (Gu et al., 2019). In all, inconsistent findings have invoked numerous disputations on the neural basis approach to the study of basic emotions (Lindquist et al., 2012, 2013; Gu et al., 2019).

Here, we introduce an alternative approach—neuromodulators—to the study of emotions. Instead of isolated small brain areas, we hypothesize that basic emotions derive from the widely projected neuromodulators, such as dopamine (DA), serotonin (5-HT), and norepinephrine (NE). Darwin proposed that phylogenetically lower animals, such as insects, also have basic emotions, but they have distinctly different brain structures with similar monoamine neuromodulators. Ever since its discovery, monoamine has been deemed as the substrate for emotions. Antidepressants affecting monoamine neuromodulators have been used for almost all affective disorders (Lovheim, 2012; Lohoff et al., 2014). Even decades later, monoamine-targeted drugs are still the first-line of pharmacological treatment for affective disorders, such as anxiety, phobia, and depression (Gu et al., 2018b). Human eyes are able to perceive all colors thanks to the three types of cone cells, with each type sensitive to one of the three primary colors. Similar to the perception of color, we herein propose a new theory of emotion—"three primary color model" of basic emotions **(Figure 1)** (Gu et al., 2018a, 2019)"—that emotion is a product from the mixture of the three monoamines (Lovheim, 2012; Gu et al., 2018a). This theory of emotion states that central DA is a hedonic signal for salient stimuli, such as food, sex, and other needs; central 5-HT is related to disgust or

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Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *11 September 2019*
Accepted: *07 January 2020*
Published: *29 January 2020*

Citation:
Wang F, Yang J, Pan F, Ho RC and Huang JH (2020) Editorial: Neurotransmitters and Emotions. Front. Psychol. 11:21. doi: 10.3389/fpsyg.2020.00021

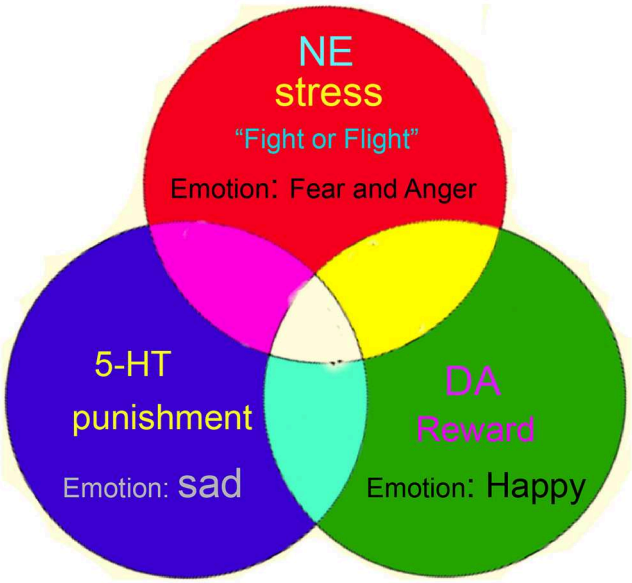

FIGURE 1 | Monoamine model of basic emotions. The number of basic emotions we humans have is still controversial. We propose a monoamine model of basic emotions, or called "three primary color model" of basic emotions. Norepinephrine (NE) is responsible for fear and anger emotions that trigger "fight or flight" response; fear and anger are classified as one core emotion—the stressful emotion—like two sides of the same coin. Joy is subsided by dopamine (DA), while punishment is subsided by serotonin (5-HT) (Adopted from our previous paper: Gu et al. Frontiers in Psychology, 2018, 9:1924).

punishment; and central NE is the substrate for emotions that trigger "fight or flight" response, such as fear and anger (Gu et al., 2019). Many recent emotion experimental and theoretical studies surging up recently support this emotion theory based on the three monoamines (Lovheim, 2012).

In addition to these monoamines, other hormones and neuromodulators may also be involved in a secondary pathway. For example, corticotropin-releasing hormone (CRH)—the stress hormone—might be involved in a pathway of central norepinephrine (NE) release (Simeon et al., 2007). The CRH induces the release of ACTH (adrenocorticotropic hormone), which can, in turn, alters physiological processes and behaviors (Chauhan et al., 2017). Ketamine, once known as a blocker to a particular glutamate receptor, has recently been used as an antidepressant (Yang et al., 2018). Oxytocin is believed to be related with love and attachment (Aguilar-Raab et al., 2019). Recent studies have proven that many neurotransmitters may play a pivotal role in emotions as substrates for emotions. Simply put, *emotions are nothing but neuromodulators*. We have solicited the most advanced studies evaluating the emotional functions of neurotransmitters, and accepted 11 peer-reviewed papers in this special collection.

Liu et al. introduced a literature review that probed into the relationship between the dysfunction of serotoninergic, noradrenergic, and dopaminergic systems with major depression disorders in multiple ways, such as animal studies and human imaging studies. They found that the altered monoamine neurotransmitter functions play a critical role in the mechanism of emotional disorders.

Gu et al. introduced three core affects: happiness, sadness, and stress, which are subsided respectively by three neuromodulators: dopamine, serotonin, and norepinephrine. Complex emotions are analogous to colors in the way that they are results of a proportional mix of the three core affects.

Ye et al. tried to relate the emotional neurotransmitters to the mechanism of Traditional Chinese Medicine.

Langenecker et al. presented genetic analysis with the serotonin transporter and monoamine oxidase A genes. Interestingly, they found that higher serotonin1A binding potentials were related to a substantial memory bias toward negative emotions.

Bian et al. identified several genes involved in post-traumatic stress disorders.

Shi et al. found that the dopaminergic reward system is impaired in bipolar depressive patients.

Yang et al. found that hydrogen sulfide in hippocampus might affect neurotransmitters to modulate sleep deprivation induced deficit of cognition.

Yu et al. investigated the neurophysiological characteristics of young people with depressive personality disorders.

Wei J. et al. reported their studies about the brain network involved in the development of blepharospasm, with a particular focus on inferior frontal gyrus, posterior cingulate cortex, and temporal gyrus.

Meng et al. reported a significant interaction effects of childhood maltreatment and emotion on executive attention scores in reaction times that reflect conflict resolution speed. They concluded that childhood maltreatment can induce brain dysfunctions, which are sensitive to negative emotional stimuli.

Wei S. et al. reported their studies on gene expressions in the hippocampus related with aggressive behaviors of premenstrual dysphoric disorders.

Collectively, these studies strengthen the association between neurotransmitters and basic emotions. Owing to the intricate nature of emotions, studies aiming at its connection with neurotransmitters are necessarily complex and multifocal. We sincerely hope that you will enjoy reading all the papers in this special edition.

AUTHOR CONTRIBUTIONS

FW, JY, FP, RH, and JH all helped in writing the editorial.

FUNDING

This work was partially supported by a grant from Foundation of Humanities and Social Science in Ministry of Education in China (FW, 19YJAZH083).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

ORIGINAL RESEARCH
published: 11 September 2018
doi: 10.3389/fpsyg.2018.01620

Voxel-Mirrored Homotopic Connectivity of Resting-State Functional Magnetic Resonance Imaging in Blepharospasm

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Objective: Several networks in human brain are involved in the development of blepharospasm. However, the underlying mechanisms for this disease are poorly understood. A voxel-mirrored homotopic connectivity (VMHC) method was used to quantify the changes in functional connectivity between two hemispheres of the brain in patients with blepharospasm.

Methods: Twenty-four patients with blepharospasm and 24 healthy controls matched by age, sex, and education were recruited. The VMHC method was employed to analyze the fMRI data. The support vector machine (SVM) method was utilized to examine whether these abnormalities could be applied to distinguish the patients from the controls.

Results: Compared with healthy controls, patients with blepharospasm showed significantly high VMHC in the inferior temporal gyrus, interior frontal gyrus, posterior cingulate cortex, and postcentral gyrus. No significant correlation was found between abnormal VMHC values and clinical variables. SVM analysis showed a combination of increased VMHC values in two brain areas with high sensitivities and specificities (83.33 and 91.67% in the combined inferior frontal gyrus and posterior cingulate cortex; and 83.33 and 87.50% in the combined inferior temporal gyrus and postcentral gyrus).

Conclusion: Enhanced homotopic coordination in the brain regions associated with sensory integration networks and default-mode network may be underlying the pathophysiology of blepharospasm. This phenomenon may serve as potential image markers to distinguish patients with blepharospasm from healthy controls.

Keywords: blepharospasm, resting state, functional magnetic resonance imaging, voxel-mirrored homotopic connectivity, support vector machine

OPEN ACCESS

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Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *02 June 2018*
Accepted: *13 August 2018*
Published: *11 September 2018*

Citation:
Wei J, Wei S, Yang R, Yang L, Yin Q, Li H, Qin Y, Lei Y, Qin C, Tang J, Luo S and Guo W (2018) Voxel-Mirrored Homotopic Connectivity of Resting-State Functional Magnetic Resonance Imaging in Blepharospasm. Front. Psychol. 9:1620. doi: 10.3389/fpsyg.2018.01620

INTRODUCTION

Blepharospasm (BSP) is a common clinical type of focal dystonia characterized by involuntary blinking and eyelid spasms (Hallett et al., 2008). BSP is a chronic disease with an incidence rate of 4.2/10 million in general population (Steeves et al., 2012). This disease occurs mostly in adults and manifested with an increased frequency of blinking in the early stage and consistent closed-eyes

or even functional blindness in the late stages. Furthermore, BSP will lead to poor quality of life, decline in employment, anxiety, and depression (Muller et al., 2002; Biuk et al., 2013; Valls-Sole and Defazio, 2016; Bedarf et al., 2017). However, the pathophysiology of BSP remains unclear.

Recent techniques, such as neuroimaging, facilitate the exploration of structural and functional abnormalities in patients with BSP. For example, Baker et al. reported regional abnormalities in the cortical and subcortical brain areas in BSP (Baker et al., 2003; Yang et al., 2013). A voxel-based morphometry study demonstrated increased gray matter in the putamen (Etgen et al., 2006) and primary sensorimotor cortex (Suzuki et al., 2011) in patients with BSP. Zhou et al. (Zhou et al., 2013) used the amplitude of a low-frequency fluctuation method and found abnormalities in the bilateral somatosensory regions in patients with BSP.

The above mentioned studies indicate that multiple abnormal nodes are involved in the pathophysiology of BSP. However, the reported findings are inconsistent in regard to specific networks. For example, a report with independent component analysis exhibited decreased connectivity within the sensorimotor network and the right fronto-parietal network but increased connectivity in the salience network in patients with BSP (Huang et al., 2017). By contrast, a functional connectivity study reported that patients with BSP showed altered functional connectivity at rest in widespread brain regions including basal ganglia, cerebellar, primary/secondary sensorimotor, and visual areas (Jochim et al., 2018). Therefore, BSP may be related to network abnormality, and dysfunction of these nodes is likely to be involved in the pathogenesis of BSP. However, whether or not these abnormalities result in simultaneous bilateral eyelid spasms in BSP remain ambiguous. Moreover, complex functional connectivities exist between two cerebral hemispheres, and their effects are complementary and coordinated. Both eyelids simultaneously experience spasms in BSP, and this event is related to abnormalities of functional interaction between two cerebral hemispheres. Thus, BSP might have also resulted from brain connection dysfunction. However, whether or not an abnormal functional interaction exists between two cerebral hemispheres in BSP should be confirmed.

Resting-state fMRI (rs-fMRI) has attracted increasing attention (Biswal et al., 1995), because it can detect patterns of coherent intrinsic activities of the brain and interactions between two hemispheres. Voxel-mirrored homotopic connectivity (VMHC) (Zuo et al., 2010) is a method of rs-fMRI used to analyze functional homotopy between two hemispheres. The synchrony in patterns of spontaneous activity between homotopic regions in each hemisphere is an important feature of the functional structure of the brain (Stark et al., 2008). The VMHC is designed to directly compare the interhemispheric resting-state functional connectivity. This process can also measure the correlations between blood oxygen level-dependent time series and reflect the communication pattern of information between two cerebral hemispheres. Thus, VMHC is extremely important for information integration of the brain. Recent studies used VMHC to show abnormal homotopic connection in patients with schizophrenia (Hoptman et al., 2012; Guo et al., 2014b, 2017a,b) and their unaffected siblings (Guo et al., 2014a), depression (Guo et al., 2013a,b), somatization disorder (Su et al., 2016), and Parkinson's disease (Hu et al., 2015). Moreover, Anderson et al. reported that homotopic resting-state functional connectivity was disrupted in individuals with autism (Anderson et al., 2011), and this finding indicated that homotopic function is an important part of the brain function. In addition, excision of corpus callosum could damage the sensory, motor, and cognitive functions of human brain. This phenomenon implies that the coordination between two hemispheres plays an important role for human behavior (Luo et al., 2015). The abovementioned studies suggest that abnormal interhemispheric connectivity may be an important factor in the occurrence of BSP. Based on the onset pattern of simultaneous effect on the bilateral eyelids of patients with BSP and the presence of different networks and loop injuries, we hypothesized that hemisphere connectivity might play a key role in the pathogenesis of BSP. Using VMHC to compare the fMRI data of patients with BSP with those of healthy controls, we evaluated spatial heterogeneity of interhemispheric functional connectivity and clarified the pathogenesis of BSP. To date, findings in brain networks of patients with BSP are limited. To our knowledge, the current study is the first work to employ VMHC to investigate the resting-state functional connectivity of the two hemispheres in patients with BSP.

Patients with BSP usually demonstrate psychiatric symptoms, such as anxiety and depression (Muller et al., 2002; Yang et al., 2017). Self-rating Anxiety Scale (SAS) and Self-rating Depression Scale (SDS) were used to evaluate the symptoms of anxiety and depression, respectively, to control the confounding impact from these conditions. Prior to the rating, all patients were screened to determine whether they were suffering from anxiety or depression. Moreover, Jankovic Rating Scale (JRS-S) was used to assess the severity of eyelid spasm. Then, The VMHC values of different brain areas were correlated with the degree of JRS-S, the course of disease, and the SAS and SDS scores to examine the relationship between VMHC values and clinical symptoms. Finally, SVM was employed to examine whether abnormal VMHC values could discriminate patients from controls with high accuracy and specificity.

MATERIALS AND METHODS

Subjects

We recruited 26 outpatients with BSP who visited the Department of Neurology in the First Affiliated Hospital of Guangxi Medical University between November 2012 and June 2014. The inclusion criteria for the BSP group were as follows: (1) met the criteria of BSP diagnosis according to the Clinical Guidelines of BSP (Defazio et al., 2013); (2) absence of structural changes with conventional MRI examination; (3) had not used botulinum toxin within 3 months prior to the study; (4) had not used medication for dystonia within 1 month prior to the study; and (5) right-handedness. Patients with the following conditions were excluded: (1) secondary BSP from other diseases, such as hepatolenticular degeneration and dry eye; and (2) history of neurological and psychiatric disorders.

The following information was collected from patients with BSP: sex, age, education level, course of disease, degree of illness severity, scores of SAS, and scores of SDS. JRS-S was used to assess the severity of BSP. SAS and SDS were used to examine the severity of anxiety and depression.

Twenty-four healthy volunteers from the community were recruited as age- sex-, and education level-matched controls. All individual controls were right-handed and had no history of severe neuropsychiatric diseases, medical illness, or family history of neurological or psychiatric disorders from the first-degree relatives.

All participants read and signed a consent form before the examination. The Ethics Committee of the First Affiliated Hospital, Guangxi Medical University approved this study.

Image Acquisition

Data were acquired using a German Siemens Trio Tim 3.0T scanner (Erlangen, Germany). Head fixers and earplugs were used for all subjects to reduce head movement and machine noise, respectively. The subjects were required to remain motionless, awake, and eye-closed during image acquisition. All patients underwent routine examination (T1W1 and T2W1) to exclude intracranial lesions. The following parameters were used for functional imaging: repetition time/echo time (TR/TE) = 2000/30 ms, 30 slices, 64 × 64 matrix, 90° flip angle, 24 cm FOV, 4 mm section thickness, 0.4 mm gap, and 250 volume (500 s).

Data Preprocessing

Images were preprocessed using the data assistant software (DPABI) (Yan et al., 2016). The first 10 time points were removed. All participants had less than 2 mm maximum displacement in the x, y, or z-axis and 2° of angular motion during data acquisition. The images were then normalized to the standard SPM8[1] echo planar imaging template with resampling to $3 \times 3 \times 3$ mm^3 voxels. The processed images were smoothed with an isotropic Gaussian kernel (full-width at half-maximum = 8 mm). Finally, the acquired data were subjected to temporal bandpass filtering (0.01–0.08 Hz) and linear detrending to reduce the effect of low-frequency drifts and physiological high-frequency noise. Spurious covariates and their temporal derivatives, including Friston-24 head motion parameters, white matter signals, and cerebrospinal fluid signals, were removed from the data using linear regression.

Interhemispheric Correlation and Statistical Analysis

The software REST[2] was used to analyze VMHC, and the details of VMHC computation has been expounded in a previous study (Zuo et al., 2010). Individual VMHC maps were generated for each participant by computing Pearson correlation (Fisher z-transformed) between a given voxel and a corresponding voxel in the contralateral hemisphere. Correlation values were then Fisher z-transformed to improve the normality. The resultant values were applied for group comparisons and generation of the VMHC maps.

Individual-level VMHC maps were analyzed using group-level voxel wise t-test to determine regional group differences in VMHC. The significance level was set to $p < 0.05$ for multiple comparisons corrected by the Gaussian Random Field theory (voxel significance: $p < 0.001$, cluster significance: $p < 0.05$). Given that resting-state functional connectivity could be influenced by micro motions (Power et al., 2012), the frame wise displacement values were computed as a covariate for each subject in the group comparisons. Pearson correlation was used to evaluate the relationships between VMHC values with significant group differences and degree of JRS-S, SAS scores, SDS scores, and the course of disease after the normality of these variables being assessed.

The demographic and clinical information was compared with the two-sample t-test and χ^2-test. The significance level was set to $P < 0.05$.

Classification Analysis Using SVM

SVM was conducted to evaluate the possibility of abnormal VMHC in these clusters to discriminate patients from healthy controls using the LIBSVM software package[3] in MATLAB (Chang and Lin, 2011). The LIBSVM classifier was trained by providing examples of the form <x, c>, where x represents the VMHC values of these abnormal clusters, and c is the class label ($c = +1$ for patients with BSP and $c = -1$ for healthy controls). A sample set was divided into a training set and a test set for SVM to evaluate the classification performance on the unobserved data. The leave-one-pair-out method was applied for the LIBSVM classifier algorithm. We constructed a random SVM cluster based on the brain fMRI data of the subjects for classification and feature selection. To acquire the optimal sensitivity and specificity, default functional kernels of Gaussian radial basis and the grid search method were applied to optimize the parameters with the "leave-one-subject-out" method.

RESULTS

Demographics and Clinical Characteristics of the Participants

A total of 24 patients were included in the further analysis (two patients were excluded due to excessive head movement). About 20.83 and 29.17% of patients with BSP had anxiety and depressive symptoms, respectively. Additionally, 3 (12.5%) patients with BSP had comorbidity of depression. Moreover, 19 patients with BSP (79.16%) had sensory tricks. These patients might temporarily improve eyelid spasms by wearing glasses, having material in the mouth, or touching the cheeks, forehead, or jaw. Half of the 24 patients (50.00%) experienced worsened eyelid spasms when talking. The patient and control groups did not differ significantly in sex, age, and education levels (**Table 1**).

[1] http://www.fil.ion.ucl.ac.uk/spm

[2] http://resting-fmri.sourceforge.net

[3] http://www.csie.ntu.edu.tw/~cjlin/libsvm

VMHC: Group Differences

Compared with the healthy control group, significantly high VMHC was found in the inferior temporal gyrus, interior frontal gyrus, posterior cingulate cortex and postcentral gyrus in the patient group (**Figure 1** and **Table 2**).

Correlation Analysis

No significant correlation was found between abnormal VMHC values and the severity of symptom, clinical course, and scores of SAS and SDS in the patients ($P > 0.05$).

SVM for Classification Analysis

SVM analysis was performed to determine whether or not abnormal VMHC values could satisfactorily discriminate patients with BSP from healthy controls. The results showed that the VMHC values in a single brain region could not discriminate patients with BSP from healthy controls with optimal sensitivity, specificity, and accuracy (**Table 3**), but the patients may be distinguished with high sensitivity, specificity, and accuracy (more than 80%) using a combination of two brain regions. Among the brain regions, the ability of discriminating patients with BSP from the healthy controls by the combination of the VMHC values in the inferior frontal gyrus and posterior cingulate cortex was optimal with an accuracy of 87.5% (42 of 48 in the 2 groups), a sensitivity of 83.33% (20 of 24 in the BSP group), and a specificity of 91.67% (22 of 24 in the control group). The combination of the VMHC values in the inferior temporal gyrus and postcentral gyrus showed an accuracy of 85.4% (41 of 48 in the 2 groups), a sensitivity of 83.33% (20 of 24 in the BSP group), and a specificity of 87.5% (21 of 24 in the control group; **Figures 2**, **3**). The other combinations such as inferior frontal gyrus/inferior temporal gyrus or posterior cingulate cortex/postcentral gyrus had unsatisfactory sensitivity and specificity.

DISCUSSION

In this study, higher VMHC values were found in some brain regions, including the inferior temporal gyrus, interior frontal gyrus, posterior cingulate cortex, and postcentral gyrus in patients with BSP than those in healthy controls ($P < 0.05$). Moreover, SVM analysis revealed that the VMHC values in a single brain region could not discriminate patients with BSP from healthy controls, contrary to the combination of two brain regions with high sensitivity, specificity, and accuracy. Moreover, no significant correlation was found between abnormal VMHC values and the severity of symptoms, clinical course, and scores of SAS and SDS in the patient group ($P > 0.05$).

Previous studies indicated that BSP was related to abnormal function of the basal ganglia (Perlmutter et al., 1997; Federico et al., 1998; Esmaeli-Gutstein et al., 1999). Meanwhile, other studies (Martino et al., 2011; Zhou et al., 2013) have reported the abnormalities in the central posterior and posterior cingulate gyrus in patients with BSP. Our report is consistent with those from previous studies (Martino et al., 2011; Zhou et al., 2013). Patients with BSP demonstrated various patterns of sensory tricks, such as motor, imaginary, forcible, and reverse sensory tricks (Hallett, 2002; Ramos et al., 2014), and more likely to suffer from significant prepulse inhibition hand stimulation when presented sensory tricks (Gomez-Wong et al., 1998). One report indicated increased sensory afferents in the eyelid activity control in patients with BSP (Hallett, 2002). Another report implied that the changes in sensory input played an important role in sensory integration abnormalities which might result from increased sensory afferents (Patel et al., 2014). Thus, balancing the movement output and sensory afferents might be a valuable strategy to improve symptoms of eyelid spasm. Furthermore, this report also implied that abnormalities in the sensory center and sensory integration were involved in the pathogenesis of dystonia. Wong et al. reported that 8 of 17 patients with BSP (47.06%) showed sensory tricks (Gomez-Wong et al., 1998). In our patient group, 19 of the 24 BSP patients (79.16%) presented sensory tricks. The eyelid spasms could be alleviated when the patient wears glasses and touches his cheek, forehead, jaw, or other parts of his body. The VMHC of the postcentral gyrus was significantly increased. This phenomenon suggested that patients with BSP had to process more complex information and programs to control eyelid movement. Thus, the integration of sensory-motor information might play an important role in the pathogenesis of BSP. Furthermore, 12 of the 24 BSP patients (50.00%) demonstrated reverse sensory tricks. These patients experienced worse symptoms when they talked (Greene and Bressman, 1998). Interestingly, a stimulus or action can alleviate the symptoms in some patients but not effective in other patients with BSP or even worsening their symptoms (Wider et al., 2004; Martino et al., 2010; Ramos et al., 2014). Thus, a sensory trick has a very unique individual nature. However, the complex input mechanism of sensory integration is unclear, and more studies are needed to explore the related neurophysiological factors in patients with BSP.

The patients in our study presented increased connection from bilateral inferior frontal gyrus, where Brodmann areas 44 and 45 of the inferior frontal gyrus are the main parts of Broca area. Previous study suggested that the frontal lobe plays an integrated role in sensory information processing, and this

TABLE 1 | Clinical profile of the participants.

	BSP patients (n = 24)	Healthy controls (n = 24)	p-value
Sex (male/female)	8/16	6/18	0.53[a]
Age (years)	49.58 ± 8.58	50.88 ± 8.13	0.59[b]
Years of education (years)	10.38 ± 2.34	10.63 ± 2.16	0.70[b]
Degree of JRS-S (1–4 degree)	2.63 ± 0.82		
SAS score	43.79 ± 8.11		
SDS score	47.95 ± 8.58		
Illness duration (months)	11.00 ± 3.82		

BSP, blepharospasm; SAS, Self-Rating Anxiety Scale; SDS, Self-Rating Depression Scale; JRS-S, Jankovic Rating Scale. [a]p-value for gender distribution was obtained by Chi-square test. [b]p-values were obtained by two-sample t-test.

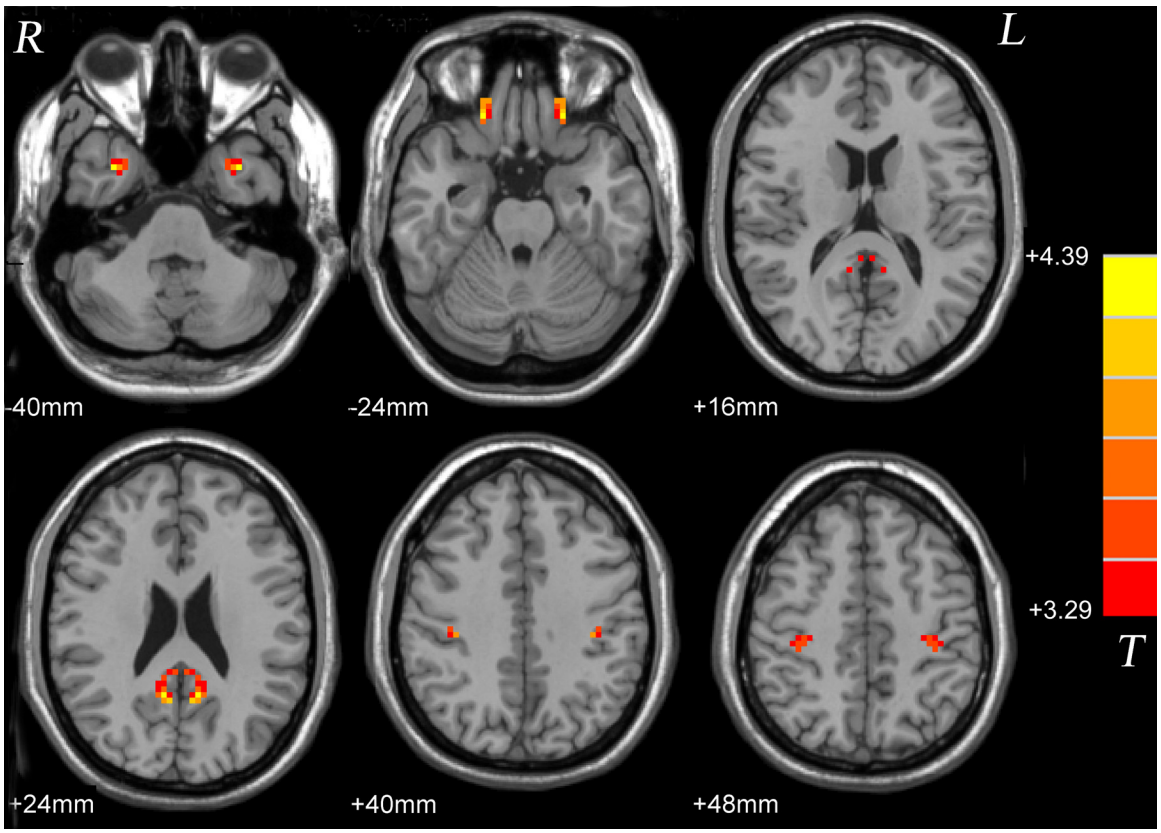

FIGURE 1 | Statistical maps showing VMHC differences between patients and controls. Red denotes increased VMHC, and color bars indicate *T*-values from *t*-tests between groups. VMHC, voxel-mirrored homotopic connectivity.

concept supports the hypothesis that the Broca area is part of the sensory network (Okada et al., 2016). Recent studies showed that the human hyperdirect low-frequency interactions between the prefrontal cortex and subthalamic nucleus (STN) support the regulation of several related brain functions (Kelley et al., 2018), and the STN is the key point of the motor network (Alexander and Crutcher, 1990). In Parkinson's disease, high frequencies of typical STN deep brain stimulation protocols are used to treat motor symptoms. Therefore, we can speculate that the STN can also become a therapeutic target in the future therapy of BSP.

In 2001, Raichle et al. (Raichle et al., 2001) first proposed default mode network (DMN). This network exhibits high levels of activity at rest. However, DMN becomes deactivated when specific goal-directed behavior is required. DMN consists of the posterior cingulate cortex, precuneus, medial prefrontal cortex, ventral anterior cingulate cortex, inferior parietal lobule, and several temporal lobes (Fransson, 2006). Zhou et al. found abnormalities in the medial prefrontal cortex and posterior cingulate cortex, and these abnormalities suggested significant differences in the DMN in patients with BSP (Zhou et al., 2013). The posterior cingulate cortex, which involves monitoring sensation, stereotactic positioning, and other functions, is the only region in the cingulate cortex that accepts thalamic

TABLE 2 | Significant group differences in VMHC.

Cluster location	Peak (MNI)			Cluster size	*T*-value
	x	*y*	*z*		
Patients > controls					
Inferior temporal gyrus	±33	6	−39	24	4.2707
Inferior frontal gyrus	±21	33	−24	44	4.2388
Posterior cingulate cortex	±9	−54	24	78	4.3927
Postcentral gyrus	±36	−27	42	76	4.1915

VMHC, voxel-mirrored homotopic connectivity; MNI, Montreal Neurological Institute.

TABLE 3 | Discriminate the patients from the controls by using the VMHC values of a single region with the SVM method.

Brain regions	Sensitivity	Specificity	Accuracy
Inferior frontal gyrus	79.17% (19/24)	75.00% (18/24)	77.08% (37/48)
Inferior temporal gyrus	62.50% (15/24)	87.50% (21/24)	75.00% (36/48)
Postcentral gyrus	58.33% (14/24)	91.67% (22/24)	75.00% (36/48)
Posterior cingulate cortex	70.83% (17/24)	83.33% (20/24)	77.08% (37/48)

VMHC, voxel-mirrored homotopic connectivity; SVM, support vector machines.

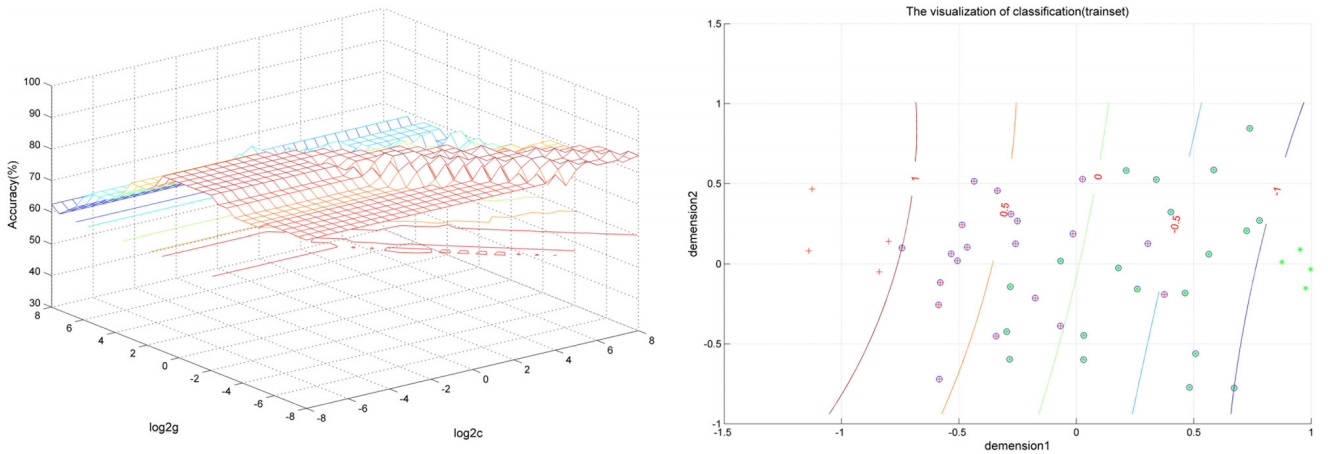

FIGURE 2 | Visualization of classification by support vector machine (SVM) using the combination of the VMHC values in the Inferior Frontal Gyrus and Posterior Cingulate Cortex. Left: SVM parameters selection result (3D visualization) [Grid Search Method]: Best c = 0.70711; Best g = 0.088388; Right: the visualization of classification with a combination of the VMHC values in the Inferior Frontal Gyrus and Posterior Cingulate Cortex.

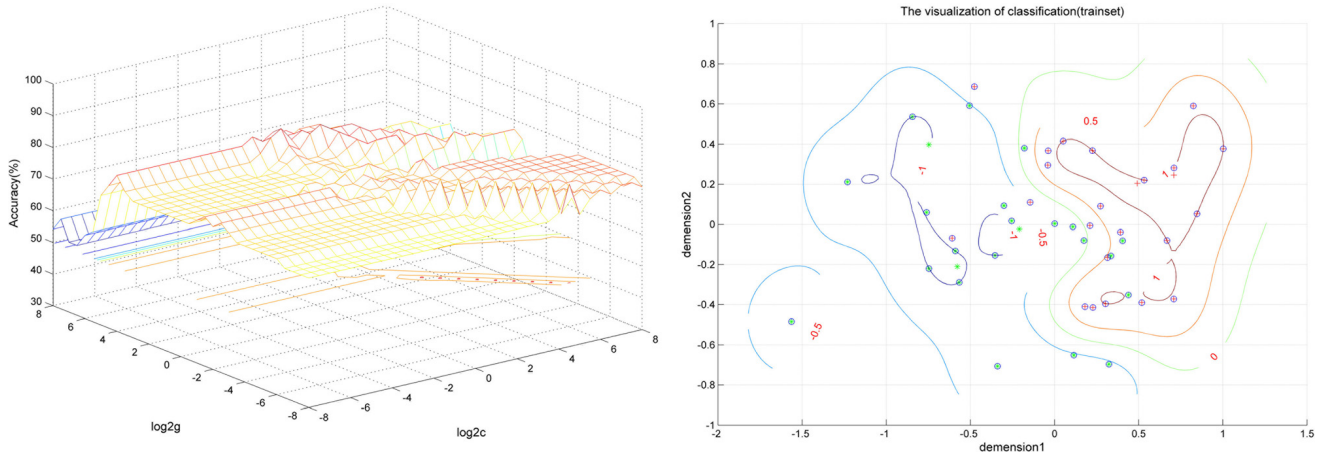

FIGURE 3 | Visualization of classification by support vector machine (SVM) using the combination of the VMHC values in the abnormal brain regions. Left: SVM parameters selection result (3D visualization) [Grid Search Method]: Best c = 0.70711; Best g = 11.3137; Right: the visualization of classification with a combination of the VMHC values in the Inferior Temporal Gyrus and Postcentral Gyrus.

occipital medial projection (Baleydier and Mauguiere, 1980). A bidirectional connection exists between the pulvinar thalamus and the secondary sensory cortex area (Brodmann areas 37, 39, and 40). The joint fibers connect to the ipsilateral occipital lobe, temporal lobe, frontal lobe, and contralateral brain region. An abnormal homozygous connection was found in the posterior cingulate cortex. This result suggested an abnormal loop between the posterior cingulate cortex and the thalamus and secondary sensory cortex. The posterior cingulate cortex is the intermediate connector between the two hemispheres. Moreover, the bilateral cerebral hemispheres are asymmetric. These findings suggest that the abovementioned changes may be compensatory reactions to the cortical center. Furthermore, the posterior cingulate cortex is an important node as a static state of DMN and might affect the functions and related connections of DMN. Damage in the bilateral temporal lobe of the monkey brain resulted in the degeneration of the fiber that connects the posterior cingulate cortex and the temporal lobe (Papez, 1995). In addition, the temporal gyrus is relevant to the information integration of vision. A study that experimented on color discrimination and visual contrast perception has demonstrated that the visual impairment of patients with BSP was not dependent on the severity of the disease or the course of the disease (Buttner et al., 1999). In the present study, increased VMHC values were found in the posterior cingulate cortex and inferior temporal gyrus, and these values suggested that patients with BSP might have abnormal visual-spatial sensory integration. However, whether or not the posterior cingulate cortex and inferior temporal gyrus are the exact key points of the visual spatial sensory integration remains unclear. The question needs to be confirmed by animal experiments. Our results provide

evidence for the establishment of animal models in the future.

No correlation was found between SAS or SDS scores and increased VMHC values in the patients. Three of the 24 patients with BSP were diagnosed with depression in this study. This finding indicated the higher morbidity of depression in patients with BSP than in the normal population (The morbidity of depression in the normal population was approximately 2–6%) (Blazer et al., 1994; Dunlop et al., 2003; Ohayon and Schatzberg, 2003; Gu et al., 2013). Moreover, several scholars inferred that patients with focal dystonia easily suffer from anxiety and depressive symptoms (Voon et al., 2010). SAS and SDS are self-rating scales which might be affected by some confounders, such as educational levels, intelligence, illness duration, and social environment. No correlation was found between abnormal VMHC and anxiety and depression of BSP, which is consistent with a previous study (Stamelou et al., 2012). Furthermore, no correlation was found between abnormal VMHC and the course of disease and severity degrees of BSP symptoms. In previous studies, no difference was found in the age and severity of dystonia between patients with BSP with or without mood disorders (Fabbrini et al., 2010). This phenomenon suggested that mood disorders were not a direct response to focal dystonia (Lencer et al., 2009; Horovitz et al., 2012). Thus, patients with BSP may suffer from anxiety and depression, but these conditions do not interfere with the evolution of the disease. Previous studies reported that changes in the gray matter volume in patients with BSP were not related to illness duration (Martino et al., 2011; Horovitz et al., 2012) and severity degree (Martino et al., 2011) of BSP symptoms. However, another study has shown that abnormal gray matter density in patients with BSP was related to the course of disease (Suzuki et al., 2011). The conflicting results may be due to abnormal sensory motor plasticity (Abbruzzese and Berardelli, 2011) and neuronal remodeling uncertainty (Opavsky et al., 2006).

SVM analyses showed that the VMHC values in one single brain region could not discriminate patients with BSP from healthy controls with optimal sensitivity, specificity, and accuracy, contrary to a combination of two brain regions with high sensitivity, specificity, and accuracy. Meanwhile, the specificity was particularly remarkable, because every healthy control was correctly classified. Therefore, the combination of high VMHC values in the inferior frontal gyrus and posterior cingulate cortex, as well as in the inferior temporal gyrus and postcentral gyrus, may serve as a potential image marker to distinguish the patients with BSP from healthy controls. Our report is consistent with those of fMRI studies in patients with schizophrenia (Li et al., 2018; Wang et al., 2018). Therefore, early detection and improved accuracy of the diagnosis in patients with BSP may be achieved based on the fMRI imaging using the combination of inferior frontal gyrus and posterior cingulate cortex or the inferior temporal gyrus and postcentral gyrus.

This study has several limitations. Patients with BSP demonstrate a wide spectrum of symptoms with different severity degrees and illness durations. However, stratified analysis was not performed because of the small sample size. More patients should be recruited to confirm the present report. We will use stratified analysis to study the relationship between abnormal VMHC values and the symptoms or course of disease. Then, whether or not connection abnormality is a fundamental change or compensatory performance of BSP will be confirmed. The VMHC method reflects the synchrony in patterns of spontaneous activity between homotopic regions in each hemisphere and does not fully reflect the functional state of the whole brain. Therefore, we can simultaneously employ the regional homogeneity method to analyze local consistency changes in the whole brain regions.

CONCLUSION

Abnormalities of BSP were found in the inferior temporal gyrus, interior frontal gyrus, posterior cingulate cortex, and post-central gyrus. The results suggested that those related brain areas may be good candidate regions to explore the nature of BSP and highlight the significance of sensory integration and DMN in the pathophysiology of the disorder. Moreover, a combination of high VMHC values in two brain areas can serve as a potential image marker to distinguish patients with BSP from healthy controls.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the First Affiliated Hospital of Guangxi Medical University. The protocol was approved by the the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

SL and WG designed the study. JW, RY, SW, LY, HL, QY, YL, YQ, JT, and CQ collected the original imaging data. WG, JW, and SW managed and analyzed the imaging data. JW and SW wrote the first draft of the manuscript.

FUNDING

This study was supported by grants from the National Key R&D Program of China (2016YFC1307100), the National Natural Science Foundation of China (Grant Nos. 81571310 and 81771447), and the Guangxi Appropriate Technology for Medical and Health Research and Development Project (Grant No. S201415-05).

ACKNOWLEDGMENTS

We thank all individuals who served as the study participants.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

ORIGINAL RESEARCH
published: 13 November 2018
doi: 10.3389/fpsyg.2018.02065

Gene Expression in the Hippocampus in a Rat Model of Premenstrual Dysphoric Disorder After Treatment With Baixiangdan Capsules

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *05 June 2018*
Accepted: *08 October 2018*
Published: *13 November 2018*

Citation:
Wei S, Sun P, Guo Y, Chen J, Wang J, Song C, Li Z, Xue L and Qiao M (2018) Gene Expression in the Hippocampus in a Rat Model of Premenstrual Dysphoric Disorder After Treatment With Baixiangdan Capsules. Front. Psychol. 9:2065. doi: 10.3389/fpsyg.2018.02065

Objective: To explore the targets, signal regulatory networks and mechanisms involved in Baixiangdan (BXD) capsule regulation of premenstrual dysphoric disorder (PMDD) at the gene transcription level, since the etiology and pathogenesis of PMDD are not well understood.

Methods: The PMDD rat model was prepared using the resident-intruder paradigm. The rats were tested for aggressive behavior, and those with scores in the lowest 30% were used as controls, while rats with scores in the highest 30% were divided into a PMDD model group, BXD administration group and fluoxetine administration group, which were evaluated with open-field tests and aggressive behavior tests. We also analyzed gene expression profiles in the hippocampus for each group, and verified differential expression of genes by real-time PCR.

Results: Before and after BXD or fluoxetine administration, scores in the open-field test exhibited no significant differences. The aggressive behavior of the PMDD model rats was improved to a degree after administration of both substances. Gene chip data indicated that 715 genes were differentially expressed in the control and BXD groups. Other group-to-group comparisons exhibited smaller numbers of differentially expressed genes. The effective targets of both drugs included the *Htr2c, Cdh3, Serpinb1a, Ace, Trpv4, Cacna1a, Mapk13, Mapk8, Cyp2c13, and Htr1a* genes. The results of real-time PCR tests were in accordance with the gene chip data. Based on the target genes and signaling pathway network analysis, we have elaborated the impact and likely mechanism of BXD in treating PMDD and premenstrual irritability.

Conclusion: Our work contributes to the understanding of PMDD pathogenesis and the mechanisms of BXD treatment. We speculate that the differentially expressed genes could participate in neuroactive ligand-receptor interactions, mitogen-activated protein kinase, calcium, and gamma-aminobutyric acid signal transduction.

Keywords: premenstrual dysphoric disorder, gene chip, Baixiangdan, differentially expressed genes, gene ontology, KEGG, signal pathways, traditional Chinese medicine

INTRODUCTION

Most women of childbearing age experience premenstrual syndrome (PMS), which is caused by an increase or decrease in ovarian steroids during ovulation (Rapkin and Winer, 2008). The symptoms include emotional problems such as irritability, depression, anxiety, emotional instability, anhedonia, and lassitude as well as physical problems such as breast tenderness, weight gain, distension, muscle and joint pain, headache, and limb edema (Baker and O'Brien, 2012). Recent research in China found that 15–20% of women of childbearing age experienced PMS, while 3–8% women in this age group showed the features of premenstrual dysphoric disorder (PMDD) (O'Brien et al., 2007). The ability to work or study, and the quality of life of women can be severely affected by the resulting emotional problems and physical discomfort, and hence, there is a need for fundamental research to clarify the etiology and pathogenesis of PMDD. In this study, we will not differentiate between PMS and PMDD, since PMDD is a serious form of PMS with emotional problems that can be severe and disabling.

The hippocampus is an important part of the limbic system (Tanaka et al., 1992), which participates in regulating physiological functions such as emotion (Guzman-Velez et al., 2016), learning and memory (Zhang et al., 2013; Ferbinteanu, 2016), hormonal responses, and immunity (Lin et al., 2016; Jo et al., 2017). The hippocampus is especially vulnerable to chronic stress, which can deleteriously affect its function and structure. As an important part of the system regulating autonomic nervous activity, the hippocampus probably plays a role in cognitive disorders caused by autonomic dysfunction (Garcia et al., 2010), but that role is not yet well understood. Magnetic resonance imaging studies have found that depression is associated with atrophy and functional impairment in the hippocampus (Czéh et al., 2001). The therapeutic effects of antidepressants are closely related to their effects on the part of the hippocampus called the dentate gyrus (Santarelli et al., 2003). Therefore, the hippocampus is a natural target for investigation of the neural mechanisms involved in PMS/PMDD.

The anti-depressive effects of the widely used selective serotonin reuptake inhibitor, fluoxetine, rely on its ability to inhibit the 5-HT transporter and thereby reduce reuptake of 5-HT at the presynaptic membrane, consequently prolonging and enhancing the effects of 5-HT (Francois et al., 2003; Sarkisova and Folomkina, 2010). Baixiangdan (BXD) is a novel capsule formulation combining several plant extracts that have been used in traditional Chinese medicine to treat PMS/PMDD. Analytical studies have shown that the main active components of BXD are paeoniflorin, paeonol, and alpha-cyperone (Peng et al., 2010; Zhou et al., 2011; Xie et al., 2015), which may have antipyretic, anti-inflammatory, analgesic, and neuroprotective functions (Lee et al., 2008; Nizamutdinova et al., 2008). Both fluoxetine and BXD can stably and effectively treat PMS/PMDD, but the neural effects of BXD that underlie these actions are unclear. To clarify this question, this paper adopts gene chip technology to identify differentially expressed genes in a rat model of PMS/PMDD based on the widely recognized resident-intruder paradigm (Czéh et al., 2001; Schneider and Popik, 2007b). The goal was to identify relevant signal regulatory pathways and quantify the transcription level of differentially expressed genes, screened by real-time fluorescence quantitative polymerase chain reaction (RT-qPCR) technology.

MATERIALS AND METHODS

Animals

This study used 180 SPF female, healthy, non-pregnant Wistar rats, 6–8 weeks old and with a body mass of 120–140 g, supplied by Beijing Vital River Laboratory Animal Technology Co., Ltd., with production license number SCXK (Jing) 2012-0001. Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals, formulated by the National Institutes of Health, United States, and were approved by the Institutional Committee for Animal Care and Use of Shandong University of Traditional Chinese Medicine (Approval ID: DWSY201404013).

The living environment featured constant temperature and humidity (23 ± 3°C, 60 ± 5% relative humidity), 12 h/12 h light-dark cycle with day-night reversal (lights on at 20:00, lights off at 8:00). Except during the experimental period, they could freely consume food and water. All experimental operations were conducted under dim light (<28 lux) (Rygula et al., 2006).

Spayed female rats (intruders) were kept in another lab (with the same feeding conditions as the experimental rats), to ensure that the intruders and residents were unfamiliar with each other before the aggressive behavior test. In the aggressive behavior test, the intruders were temporarily placed into the cages of the residents.

Experimental Animal Screening

The estrous cycle of the rat is divided into proestrus, estrus, metestrus and diestrus phases. The proestrus and estrus phases constitute the receptive phases, while metestrus and diestrus constitute the non-receptive phases. The determination of the phase of the estrous cycle in this experiment was made according to the presence of keratinocytes, nuclear epithelial cells, and leucocytes as well as their proportions, examined in vaginal smears under a microscope (Marcondes et al., 2002). Except for the rats excluded from experiments during the feeding period, vaginal smearing was conducted every day from 13:00 to 14:00 to check whether the estrous cycle of the rats was regular.

Grouping

After 9 days of vaginal smear testing, rats with 4 days estrous cycles were selected as quasi-residents for the aggressive behavior test. Rats with other cycles were removed and spayed for later use as intruders. During the period in which the intruders recovered from the spaying surgery, daily vaginal smearing was conducted on the quasi-residents, and rats with irregular cycles were removed whenever they were detected. After 2 weeks, when the wounds of the intruders had healed, the aggressive behavior test and open field test (baseline phase data collection) were conducted on all residents in diestrus 1 (the 2nd day of non-receptivity). For model creation, the aggressive behavior

test scores of all residents were arranged in high-to-low order by analyzing the resident-intruder experiment videos. The rats with scores within the top 30% were divided randomly into three groups, the PMDD model group (PMDD), fluoxetine intervention group (FXT), and BXD intervention group (BXD), while those with scores within the bottom 30% were used as the control group (CTRL).

Drugs

Baixiangdan capsules were obtained from the Qingdao Haichuan Center for Innovative Biomedical Research (Qingdao, China, batch number: 20071020). Fluoxetine dispersible tablets were obtained from Eli Lilly and Company (Suzhou, China, batch number: H20050463).

Drug administration was performed during the diestrus 1 phase of the estrous cycle, for 5 days at a dosage of 1 mL of fluid per 100 g of body weight. The drugs were administered intragastrically once each afternoon at 14:00. For the FXT and BXD intervention group, fluoxetine capsules and BXD capsules were used with dosages of 2.7 and 0.2 g/kg/day, respectively, while for the PMDD model group and control group, pure water of the same volume was given.

Behavioral Experiments

Behavioral data were collected at baseline and after the drug administration interventions. Given that only rats with 4 days estrous cycle were selected for the experiment, behavioral data collection was always performed at diestrus 1.

The open field test (Katz et al., 1981) was conducted by placing the rats in an open field box (Xinruan XR-XZ301, Shanghai, China) with dimensions 50 cm × 50 cm × 50 cm. The animal behavior analysis system XR-Xmaze (Xinruan) was used for data collection. The specific steps were as follows: the operator held the front third part of the rat's tail and gently placed it in the central area of the open field box; then the observer began to observe and record the motion traces, break times, central grid stopping time, and number of fecal pellets dropped within 180 s. To minimize possible olfactory confounds, after each rat completed the test, the open field box was wiped clean with 75% ethyl alcohol. When the box was completely dry, data collection for the next rat commenced. In this experiment, the experimenters made an effort to grasp and hold the rats as gently as possible, to minimize the resulting stress.

The aggressive behavior test was conducted from 14:30 to 17:30 in the home environment of the residents. Because of the day-night reversal, this timeframe corresponded to the active part of the day for the rats. The specific steps were as follows: after removing the rats that were not to be tested from the cage, the rats and the cage were placed under the camera equipment; after a 15 min adaptation phase, intruders (spayed rats) were placed into the cage for 10 min. After the test, a blinded method was used for evaluation of aggressive behaviors. Three people who were trained together observed the video of each rat, and their observations were checked for consistency (Kappa > 0.95). Two types of aggressive behavior were recorded: frontal attack (springing when the intruder attempted to approach) and side attack (pushing the intruder away by springing from the side with arched back). The observers recorded scores codifying the reactions of the residents to the intruders, including the number of attacks, attack duration, number of bites, number of mounting events, mounting duration, and piloerection. A composite aggressive behavior score was calculated as follows:

$$\text{composite aggression (CA)} = (\text{number of attacks}) + 0.2 \times (\text{attack duration [s]}) + (\text{number of bites}) + 0.2 \times (\text{mounting duration [s]}) + (\text{piloerection})$$ (Albertet al., 1991).

Rat Brain Tissue and RNA Extraction

After decapitating each rat, the operators removed the hippocampus onto an ultra-clean work platform, then immediately placed it in liquid nitrogen for quick freezing, and after 30 min transferred it into a −70°C freezer for storage. In this experiment, all operating instruments, watch glasses, and EP tubes used were sterilized at high temperature after submerging them in diethylpyrocarbonate-treated water overnight. The ultra-clean work platform was scrubbed with diethylpyrocarbonate-treated water and 75% ethyl alcohol, and exposed to UV light to prevent contamination.

Suitably sized (50–100 mg) tissue samples were extracted to be frozen and minced with a biological grinding mill. Then 1 ml of TRIzol™ Reagent (Invitrogen, Thermo Fisher Scientific, Waltham, MA, United States) was added, and RNA was extracted after homogenizing with a bead mill homogenizer. The extracted RNA was stored after passing classical RNA integrity detection and purity detection tests (Vermeulen et al., 2011).

Gene Chip Experiment

Gene chip analysis was performed by Shanghai Kangcheng Biological Co. Ltd. (Shanghai, China). The rat whole genome oligonucleotide chip used in the experiment was synthesized at Agilent (Santa Clara, CA, United States). The genes covered by the rat whole genome expression chip produced by this company exceed 41,000. The probe design is described in various public databases, including Goldenpath, Ensembl, Unigene, Human Genome (Build 33), Refseq, GenBank, etc.

Under standard conditions, marker probes and the high-density genome chip were hybridized. They were cleaned thereafter, and after spin-drying the slide, the next step of scanning was initiated. Chips in different groups were scanned with an Agilent SureScan gene chip-microarray scanner, to obtain 16-bit tiff files. Agilent Feature Extraction (Version 10.7.3.1) image analysis software was used to analyze the chip images, and to convert the images into a digital format. Finally, GeneSpring GX11.5.1 (software) (Silicon Genetics) was used to conduct standardized processing of initial signal intensity, to obtain the standardized ratio (Cy3/Cy5). *T*-tests were used to compare levels of expression for each gene in each group. In this experiment, genes with $P < 0.05$ and ratio > 2.0 were considered to be differentially expressed.

Heat map and gene clustering analysis were performed using TIGR MultiExperiment Viewer (MeV) Version 4.1[1]. In this

[1]http://www.sigenae.org/index.php?id=88

experiment, genes with $P < 0.05$ and ratio > 2.0 were considered to be differentially expressed.

GO and KEGG Enrichment Analysis

Gene Ontology-TermFinder (Boyle et al., 2004) was used to identify Gene Ontology (GO) terms that annotate the list of enriched genes with significant *P*-values less than 0.05[2]. We used in-house scripts to locate information on differentially expressed genes in Kyoto Encyclopedia of Genes and Genomes (KEGG), a collection of databases dealing with genomes, biological pathways, diseases, drugs, and chemical substances[3].

Real-Time Fluorogenic Quantitative Polymerase Chain Reaction

The primers used in RT-PCR are listed in **Table 1**. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcription levels were used as an internal reference. A Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., Waltham, MA, United States) was used for reverse transcription, and 5 μg of RNA was used for each sample. RT-qPCR Master Mix (Toyobo Co., Ltd., Osaka, Japan) was used for RT-qPCR, which was run on an ABI 7500 fast real-time fluorogenic quantitative RT-PCR system (Life Technologies, Thermo Fisher Scientific).

The RT-qPCR experimental data was analyzed using ABI 7900 system SDS software (Life Technologies, Thermo Fisher Scientific), and relative quantitative analysis was conducted by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). An index quantifying expression difference (multiple) from the control was calculated by the following formulas.

$$\text{Relative quantity (RQ)} = 2^{-\Delta\Delta CT} \text{ and}$$
$$\Delta\Delta CT = (C_{T1} - C_{T2}) - (C_{T3} - C_{T4}).$$

C_{T1} and C_{T2} were, respectively, the threshold cycle numbers of target genes and reference genes of experimental samples; C_{T3} and C_{T4} were, respectively, the threshold cycle numbers of target genes and reference genes of control samples. We used samples from the control group (CTRL) as the control samples, with the gene expression level set to the control value 1. Three repeated experiments were conducted for every group of samples, and the average was taken as the result. All tested genes were selected considering gene chip data and previous studies.

Statistics

Graphpad Prism 5.0 software (GPW5-384305-RAG-5235, Graphpad software, Inc., La Jolla, CA, United States) was used to analyze the experimental data. Two-factor analysis of variance was used to compare the groups in the same period of the menstrual cycle before and after drug administration. Single-factor analysis of variance was used for comparing the groups in different periods of the menstrual cycle, with significance level $\alpha = 0.05$.

TABLE 1 | Primers used in RT-qPCR.

Gene name	Used as	Sequences	Tm
Htr2c	Forward	5′-TTCTTCATCCCGTTGACGATT-3′	54.3°C
Htr2c	Reverse	5′-TCGGTGTGACCTCGAAGTAAC-3′	57.8°C
Htr2c	Probe	5′-CGATCTACGTCCTGCGCCGTC-3′	63.3°C
Cdh3	Forward	5′-GACAGTGACCGATCTGGATTCC-3′	57.2°C
Cdh3	Reverse	5′-GGTGAAATGATCCCCATCGT-3′	54.9°C
Cdh3	Probe	5′-CCAACTCACCGGCATGGCGTG-3′	63.7°C
Serpinb1a	Forward	5′-TGGGTGTGGTGGACAGCAT-3′	59.5°C
Serpinb1a	Reverse	5′-CTCCCACATCCCCTTGAAGTAG-3′	56.9°C
Serpinb1a	Probe	5′-ACCAAACTTGTGCTGGTGAACGCCA-3′	63.5°C
Ace	Forward	5′-GGGAGAACATTTACGACATGGTAGT-3′	56.5°C
Ace	Reverse	5′-TCCAGCCCTTCTGTACCATTG-3′	58.3°C
Ace	Probe	5′-CCCGGACAAACCCAACCTCGATGT-3′	63.3°C
Trpv4	Forward	5′-ATCCGACGGGAGGTGACA-3′	58.4°C
Trpv4	Reverse	5′-CGTAGGCCCAGTCCTTGAAC-3′	57.8°C
Trpv4	Probe	5′-AGGACACACGGCACCTGTCTCGC-3′	65.5°C
Cacna1a	Forward	5′-GGCATGGTGTTCTCCATCTACTT-3′	56.8°C
Cacna1a	Reverse	5′-CCGCGATAGCTAAGAACACGT-3′	57.3°C
Cacna1a	Probe	5′-CGTCCTCACCCTCTTCGGGAACTACAC-3′	63.5°C
Mapk8	Forward	5′-CCGTACATCAACGTCTGGTATGAT-3′	56.5°C
Mapk8	Reverse	5′-CTCCCTTTCATCTAACTGCTTGTC-3′	56.7°C
Mapk8	Probe	5′-TCAGAAGCAGAGGCCCCACCACC-3′	65.4°C
Mapk13	Forward	5′- GGCGGCCAAATCCTACAT-3′	55.7°C
Mapk13	Reverse	5′-GGGAAAAGCTGTGTGAAATCCT-3′	55.6°C
Mapk13	Probe	5′-AGTCCCTGCCCCAGAGCCCCA-3′	67.9°C
Cyp2c13	Forward	5′-GACACCGCAGCCCCTCTAT-3′	59.5°C
Cyp2c13	Reverse	5′-TCTCTGAACCTCGTGGACCAT-3′	57.7°C
Cyp2c13	Probe	5′-AGGAGCCACATGCCCTACACAAATGC-3′	63.3°C
Htr1a	Forward	5′-TGTTGCTCATGCTGGTTCTCTAC-3′	57.1°C
Htr1a	Reverse	5′-CTGACAGTCTTGCGGATTCG-3′	55.9°C
Htr1a	Probe	5′-CGCATCTTCAGAGCCGCACGCT-3′	64.5°C
GAPDH	Forward	5′-ATCAACGGGAAACCCATCAC-3′	55.3°C
GAPDH	Reverse	5′-GACATACTCAGCACCAGCATCAC-3′	57.8°C
GAPDH	Probe	5′-TCCAGGAGCGAGATCCCGCTAACAT-3′	63.3°C

RESULTS

BXD and Fluoxetine Can Effectively Treat Rats in PMDD Irritability Model

At baseline, before drug administration, the open field test behavioral scores of rats in different groups did not differ significantly ($p > 0.05$) (**Figure 1A**). After drug administration, the open field test behavioral scores of rats in different groups again did not differ ($p > 0.05$) (**Figure 1A**), and moreover, the open field test behavioral scores of rats in different groups before and after the administration did not differ either ($p > 0.05$) (**Figure 1A**).

Before drug administration (baseline), in the aggressive behavior test, some rats showed very different responses to

[2]http://search.cpan.org/dist/GO-TermFinder/

[3]http://www.genome.jp/kegg/

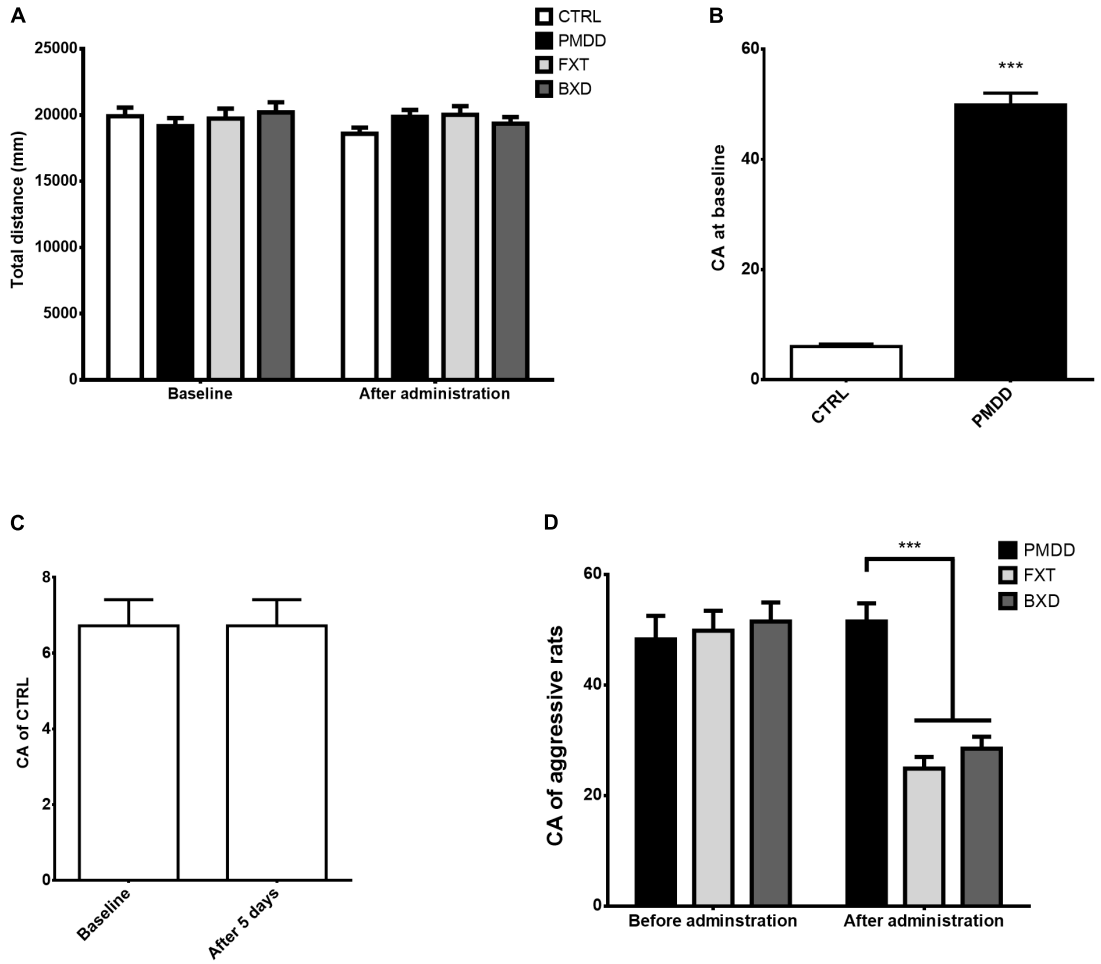

FIGURE 1 | Results of behavioral experiments. **(A)** Total distance (mm) traversed by control group (CTRL, $n = 8$), premenstrual dysphoric disorder model group (PMDD, $n = 9$), fluoxetine administration group (FXT, $n = 9$) and Baixiangdan capsule administration group (BXD, $n = 9$) in open field test at baseline and after administration. **(B)** Composite aggression (CA) scores of non-aggressive rats (control group, CTRL, $n = 8$) and aggressive rats (PMDD, $n = 27$) in aggressive behavior test at baseline. Aggressive rats included those from the PMDD model group ($n = 9$), fluoxetine administration group ($n = 9$) and Baixiangdan capsule administration group ($n = 9$). None of the aggressive rats were given drugs at baseline, and therefore could be treated as equivalent to PMDD model rats for statistical purposes. $^{***}P < 0.001$ vs. control group. **(C)** Composite aggression (CA) scores of the control group (CTRL, $n = 8$) in aggressive behavior test at baseline and after 5 days. As the control group was not given drugs, the time points labeled "after administration" were changed as "after 5 days." **(D)** Composite aggression (CA) scores of PMDD model group (PMDD, $n = 9$), fluoxetine administration group (FXT, $n = 9$) and Baixiangdan capsule administration group (BXD, $n = 9$) in aggressive behavior test before and after drug administration. As there was no baseline testing of aggressive behavior test if rats were given drugs or tap water, the time points labeled "baseline" were changed to "Before administration" $^{***}P < 0.001$ vs. PMDD model group.

intruders from other rats, that is, some were aggressive and some were not ($p < 0.001$) (**Figure 1B**). After the non-aggressive rats were placed into the control group and fed with pure water for 5 days, the composite aggression scores before and after did not differ significantly ($p > 0.05$) (**Figure 1C**). As described in the Methods section, the aggressive rats were then divided into the PMDD model group, fluoxetine group, and BXD group, and administered pure water, fluoxetine, and BXD, respectively, for 5 days. The composite aggression scores of the PMDD model group before and after administration did not differ significantly ($p > 0.05$) (**Figure 1D**), but the composite aggression scores of the fluoxetine group and BXD group both showed significant declines after administration ($p < 0.001$ and $p < 0.001$, respectively) (**Figure 1D**). After administration, when the fluoxetine group and BXD group were compared with the PMDD model group, there were again significant declines in the composite aggression scores for both ($p < 0.001$ and $p < 0.001$, respectively) (**Figure 1D**).

Results for Differentially Expressed Genes

When the PMDD model group was compared with the control group, the number of differentially expressed genes was 137. When the BXD group was compared with the control group, the number of differentially expressed genes was 715. When the fluoxetine group was compared with the BXD group, the number of differentially expressed genes was 199 (**Table 2**

TABLE 2 | Numbers of differentially expressed genes in each group.

Groups	Acronyms	Up-regulated genes	Down-regulated genes	Comments
Control group	CTRL	N.A.	N.A.	N.A.
PMDD model group	PMDD	132	5	$P < 0.05$ and ratio > 2.0
Fluoxetine administration group	FXT	112	87	
BXD administration group	BXD	284	431	

and **Supplementary Materials**). The gap in the number of differentially expressed genes between the two drug treatment groups was very large, suggesting that BXD's mechanism of action against PMS/PMDD is more complex and targets more systems than that of fluoxetine. Besides, the heat maps exhibited a similar tendency, that is, the number of differentially expressed genes between the PMDD model group and fluoxetine group was less than the number of differentially expressed genes between the PMDD model group and BXD group (**Figures 2A,B**). We also found a significant difference in the number of differentially expressed genes between both drug treatment groups (**Figure 2C**).

The GO analysis indicated that the differentially expressed genes might have participated in several molecular, biological, and cellular processes. As a result, the number of differential genes divided according to functional distribution was larger than that of initial differential genes screened. Since the functions of many of the differentially expressed genes are not yet clear, it may also lead to an opposite situation (**Table 3** and **Supplementary Materials**).

The KEGG database was used to conduct an analysis of the signal regulatory pathways that involve the differentially expressed genes. When compared with the BXD group, the signal pathways altered in the fluoxetine group were obviously fewer. In addition, more PMDD irritability-related pathways were affected in the BXD group than in the fluoxetine group, indicating that compared with fluoxetine, the BXD capsules acted on more targets against PMDD. Some genes that were differentially expressed jointly participated in the same signal pathways. For example, when comparing the BXD group and control group, 16 differentially expressed genes were involved in neuroactive ligand-receptor interaction pathways. In addition, 12 differentially expressed genes were involved in the endoplasmic reticulum protein processing pathway and the mitogen activated protein kinase (MAPK) signal pathway. There were also cases of the same differentially expressed gene participating in several signal regulatory pathways. For example, when the fluoxetine group was compared with the control group, the differentially expressed gene *Camk2a* (calcium/calmodulin dependent protein kinase II) participated in a total of 13 pathways. Comparison of the BXD group and the model group showed that the differentially expressed genes were related to 34 signal pathways, including 9 differentially expressed genes involved in neuroactive ligand-receptor interaction pathways and 4 genes involved in cytokine-cytokine receptor interaction pathways. The *Rt1-Bb* (RT1 class II, locus Bb) gene was the gene that was involved in the largest number (15) of signal pathways (**Table 4** and **Supplementary Materials**).

Through analysis of the effects of different drugs in the gene chip results, 20 representative differentially expressed genes with obvious intervention effects related to PMS/PMDD irritability were listed (**Table 5**). Analysis of the signal regulatory pathways affected in the different groups indicated that the pathways related to PMS/PMDD irritability involve olfactory transduction, calcium signaling, cell adhesion, PI3K-Akt signaling, ovarian steroidogenesis, Jak-STAT signaling, neuroactive ligand-receptor interactions, prolactin signaling, extracellular matrix (ECM)-receptor interaction, vitamin digestion and absorption, etc. By linking the differentially expressed genes with obvious intervention effects with the listed signal regulatory pathways, differentially expressed genes for signal regulatory pathways can be identified, namely *Htr2c*, *Cdh3, Serpinb1a, Ace, Trpv4, Cacna1a, Mapk13, Mapk8, Cyp2c13,* and *Htr1a*.

Comparison of Selected Gene Expression of Hippocampal Tissues in Different Groups

To lower the errors in the RT-PCR experiment, three repetitions were performed for every sample, and in data processing the average CT value for the three amplification curves for every sample was used to calculate the relative quantity. Compared with the control group, the genes *Htr2c, Cdh3, Serpinb1a, Ace,* and *Trpv4* in the hippocampus of rats in the treatment groups showed up-regulated expression; the genes *Cacna1a, Mapk8, Mapk13, Ctp2c13,* and *Htr1a* showed down-regulated expression (**Table 6**). Among the up-regulated genes, when comparing the PMDD model group with the fluoxetine group and BXD group, the expression levels declined to some degree, indicating that the two drugs had treatment effects on PMS/PMDD irritability.

DISCUSSION

PMDD Rat Model

Previously used rat models of PMDD include marble burial (Schneider and Popik, 2007a, 2009), progesterone withdrawal (Andréen et al., 2009), emotional stimulation dominated multi-factor continuous modeling induction (Qiao et al., 2017), resident-intruder (Czéh et al., 2001; Schneider and Popik, 2007b), etc. However, according to clinical diagnostic standards for human PMDD patients (American Psychiatric Association, 2000), the phase in which some of these symptoms are manifested corresponds to the luteal phase (non-receptive phase of rats). The resident-intruder method adopted in this study as a PMDD rat model has faced much less controversy, because the

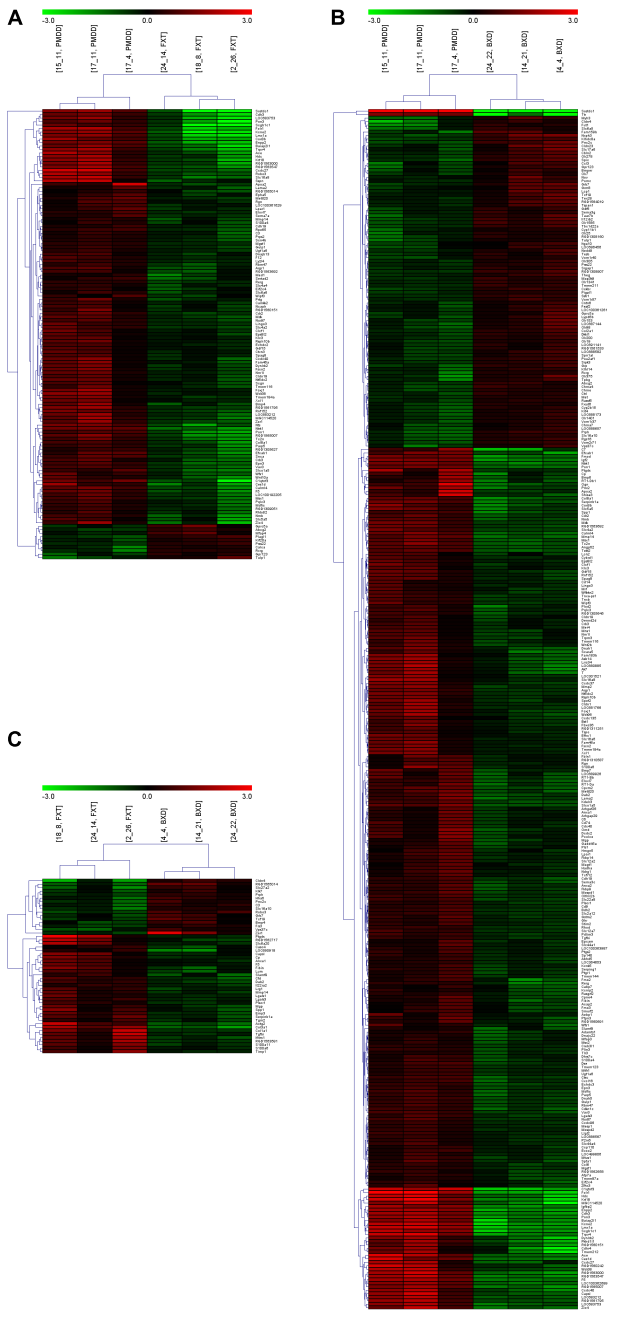

FIGURE 2 | Heat maps and clustering analyses of multiple comparisons. **(A)** Comparison between the PMDD model group (PMDD, samples including 15_11, 17_11 and 17_4) and fluoxetine administration group (FXT, samples including 24_14, 18_8 and 2_26). **(B)** Comparison between the PMDD model group (PMDD, samples including 15_11, 17_11 and 17_4) and Baixiangdan administration group (BXD, samples including 24_22, 14_21 and 4_4). **(C)** Comparison between the fluoxetine administration group (FXT, samples including 24_14, 18_8 and 2_26) and Baixiangdan administration group (BXD, samples including 24_22, 14_21 and 4_4). $P < 0.05$ and ratio > 2.0.

expression of animal aggression occurs at the same phase as symptom expression in human PMDD patients. Compared with metestrus and diestrus 2, diestrus 1 yields the most obvious aggressive behaviors of rats. Therefore, in experiments with fluoxetine and BXD intervention, behavioral data during the baseline phase and after drug intervention were collected in diestrus 1.

The estrous cycle of 60–70% of rats is 4–5 days (Marcondes et al., 2002). The selected rats in this experiment were those with 4 days cycles; rats with other cycles were removed. It has been reported that proestrus and estrus last for around 24 h in total, and metestrus, diestrus 1 and diestrus 2 each last for around 24 h (Ho et al., 2001). However, Hubscher et al. (2005) found that proestrus lasted for 12–14 h, estrus lasted for 25–27 h, metestrus lasted for 6–8 h, and diestrus lasted for 55–57 h. For the receptive phase, these two cycles have a gap of almost 12 h. Therefore, in this experiment, rats whose vaginal smear results for leucocytes were (−, +, +, +) or (−, −, +, +) were considered as rats with regular estrous cycles (the ideal status was −, +, +, +). According to research by Ho et al. (2001), about 30–60% of normal injury-free female rats show no aggressive behaviors during any phase of the estrous cycle. These rats were the non-aggressive ones in this experiment, also referred to as the control group in the following drug intervention experiments. After the aggressive behavior test, rats within the top 30% of aggression scores were equally divided into the PMDD model group, fluoxetine group and BXD group (Schneider and Popik, 2007b). Spaying could reduce aggressive behaviors in female rats, and estrogen and progestin could be given with specific therapies to restore cyclic hormone secretion (Melchior et al., 2004), thus recovering cyclic aggressive behavior. Therefore, spaying was conducted in rats with irregular estrous cycle to erase the regularity and enhance stability, and these rats were then used as intruders in resident-intruder experiments.

Possible Action Targets and Mechanism of BXD Capsule Against PMDD Irritability

Research on the anti-depressive effects of fluoxetine has been thorough, and the drug has also been widely applied clinically for PMDD depression (Francois et al., 2003; Sarkisova and Folomkina, 2010). This paper used fluoxetine as a positive control for the effects of BXD capsules in behavioral experiments and the following chip experiment. On the other hand, because there have been few reports on the use of BXD in PMS/PMDD, this paper focuses on the possible targets of BXD and the mechanism by which BXD capsules affect PMDD irritability. Though our behavioral experiments revealed that fluoxetine and BXD had a similar effect on the PMDD model, some studies have shown different effects. Some studies have shown that the active compounds in BXD could ease most of symptoms of PMDD (Lee et al., 2008; Nizamutdinova et al., 2008; Peng et al., 2010; Zhou et al., 2011; Xie et al., 2015), while other studies showed that fluoxetine was mainly to treat PMDD depression (Francois et al., 2003; Sarkisova and Folomkina, 2010; Li et al., 2016), which is only one subtype of PMDD; these results suggest that BXD could be active on multiple targets. Through analysis of the 6 signal regulatory pathways influenced by BXD capsules, we found that BXD can alter PMDD irritability by influencing various intercellular

TABLE 3 | Functions of differentially expressed genes in each group.

Groups	Acronyms	Molecular function	Biological process	Cellular component	Comments
Control group	CTRL	N.A.	N.A.	N.A.	N.A.
PMDD model group	PMDD	15	111	8	Ratio > 2.0
Fluoxetine administration group	FXT	26	146	5	
BXD administration group	BXD	34	186	25	

TABLE 4 | Regulated and associated signaling pathways in groups.

Groups	Associated signaling pathways
PMDD vs. CTRL	Olfactory transduction; Calcium signaling pathway; Cell adhesion molecules; PI3K-Akt signaling pathway; Ovarian steroidogenesis; Jak-STAT signaling pathway; Neuroactive ligand-receptor interaction; Prolactin signaling; ECM-receptor interaction; Vitamin digestion and absorption
FXT vs. CTRL	Steroid hormone biosynthesis; Calcium signaling pathway; Serotonergic synapse; Vitamin digestion and absorption; Dopaminergic synapse; Neuroactive ligand-receptor interaction; GABAergic synapse; Gap junction; p53 signaling pathway; Cholinergic synapse
BXD vs. CTRL	Calcium signaling pathway; Serotonergic synapse; Glutamatergic synapse; Steroid hormone biosynthesis; Vascular smooth muscle contractio; Axon guidance; Neuroactive ligand-receptor interaction; Long-term potentiation; Adrenergic signaling in cardiomyocytes; Dopaminergic synapse

PMDD, premenstrual dysphoria disorder; FXT, fluoxetine; BXD, Baixiangdan; CTRL, control.

TABLE 5 | Differentially expressed genes obviously affected by fluoxetine or Baixiangdan.

Gene symbol	Gene ID	MvsC (FC)	FvsC (FC)	BvsC (FC)
Msx1	NM_031059	3.54	1.03	1.23
Cldn1	NM_031699	4.67	1.64	1.49
Pon3	NM_001004086	4.73	1.54	2.24
Crb3	NM_001025661	4.08	1.28	2.04
Slc5a5	NM_052983	5.35	1.69	1.29
Htr2c	NM_012765	4.72	1.68	2.31
Cdh3	NM_053938	5.66	1.47	1.30
Serpinb1a	NM_001031642	3.71	2.23	1.44
Ace	NM-012544	5.32	1.59	1.41
Trpv4	NM_023970	5.74	1.29	2.29
Cyp2c23	NM_031839	−8.76	−2.87	−3.35
Hp	NM_012582	−18.45	−3.36	−4.55
C5	XM_345342	−7.23	−1.78	−1.54
Serpinc1	NM_001012027	−12.54	−3.27	−4.36
Aldob	NM_012496	−4.79	−2.23	−1.97
Cacna1a	NM_012918	−2.63	−1.76	−1.71
Mapk13	NM_019231	−2.08	−1.35	−1.24
Mapk8	XM_341399	−1.52	−1.38	−1.33
Cyp2c13	NM_138514	−3.45	−1.57	−1.41
Htr1a	NM_012585	−2.33	−1.17	−1.75

TABLE 6 | Relative quantity (RQ) values of selected genes in each group.

Gene name	Relative quantity (RQ)			
	CTRL	PMDD	FXT	BXD
Htr2c	1	4.72	3.16	3.11
Cdh3	1	5.66	2.29	2.08
Serpinb1a	1	3.71	2.16	1.95
Ace	1	5.32	2.68	2.09
Trpv4	1	5.74	1.88	1.78
Cacna1a	1	0.45	0.85	0.75
Mapk8	1	0.66	0.7	0.9
Mapk13	1	0.48	0.78	0.73
Cyp2c13	1	0.29	0.57	0.63
Htr1a	1	0.43	0.89	0.72

PMDD, premenstrual dysphoria disorder; FXT, fluoxetine; BXD, Baixiangdan; CTRL, control.

and intracellular signal transduction pathways. The important targets include 5-Htr (5-Htr1a, 5-Htr2c, 5-Htr3a), mitogen-activated protein kinase (Mapk8, Mapk13), Ca2+ channel proteins (Cacna1a, Cacn2d3, Cacn1i), Drd2, Glul, Gabarapl2, etc.

5-hydroxytryptamine (5-HT), acting mostly on G-protein coupled receptors, serves as a key signal molecule for neuroactive ligand-receptor interaction, and as an important central neurotransmitter, it is closely related to PMDD (Yonkers et al., 2008). 5-Htr1a participates in the stress response involving the hypothalamus-pituitary-adrenal axis system, and is closely associated with negative emotions such as human anxiety and depression, changes in cognitive ability and dietary behaviors, as well as mental disorders such as schizophrenia and Alzheimer's disease (Nichols and Sanders-Bush, 2001; Müller et al., 2007). 5-Htr2c has regulatory effects on emotion, anxiety, dietary behavior, and reproductive behavior (Heisler et al., 2007). According to our qPCR results, when compared with the control group, the mRNA protein expression level of 5-Htr2c in the hippocampus of rats in the PMDD model group was significantly increased (FCA = 5.64), and the level of 5-Htr1a was significantly decreased. After drug intervention, the mRNA expression levels of the two differential genes basically returned to normal. This is consistent with the chip results, demonstrating the reality and reliability of the chip results as well. Besides, another traditional Chinese medicine, Shuyu capsule, which

was found to reduce 5-HT_{3A}R and 5-HT_{3B}R expression (Li et al., 2016) and has some of the same herbal ingredients as BXD, was seen to effectively treat PMDD depression.

Mitogen activated protein kinase, one of the most important cellular signal transduction pathways, is closely associated with physiological and pathological processes such as cell growth, development, division, apoptosis and intercellular function synchronization (Kumar et al., 2004; Zhang et al., 2015), and also participates in nervous system impairment and restoration (Riddick et al., 2017). There are four main MAPK signal pathways, including ERK, JNK (c-Jun terminal kinase / stress-activated protein kinase), P38, and ERK5 (Large mitogen activated protein kinase 1). MAPK8 (JNK1) and MAPK13 (mitogen-activated protein kinase 1) serve, respectively, as key genes in the JNK pathway and P38 pathway, and JNK and P38 pathway activation are both closely related to cell apoptosis (Xia et al., 1995). JNK pathway activation can promote the apoptosis of various types of cells (Xia et al., 1995). Research has shown that JNK pathway activation can lead to neuronal atrophy or death, and is closely related to neurodegenerative diseases such as Parkinson's disease (Chen et al., 2012) and Alzheimer's disease (Yao et al., 2017). This study revealed that when the PMDD model group was compared with the control group, MAPK8 and MAPK13 saw down-regulated expression, but after BXD intervention, the two genes did not see significant up-regulation or down-regulation, indicating that in regulation of the JNK and P38 pathways, the two genes might not have a role in the mRNA expression level, but work through regulation of phosphorylation levels.

Ca^{2+} channels have two common forms, namely, voltage-dependent calcium channels and ligand-gated calcium channels. The types and their physiological functions differ in different tissue cells and different parts of the same cell (Spedding and Paoletti, 1992). The physiological functions include calcium homeostasis maintenance (Nayler and Sturrock, 1986), control of muscle contraction (Held et al., 2007), release of neurotransmitters (Miki et al., 2013), promotion of cell growth and proliferation, regulation of hormone secretion (Sosial and Nussinovitch, 2015), and influences on gene expression (Doran et al., 2007). Cacna1a, a P/Q type of voltage-dependent calcium channel located in nerve cell membranes, is widely distributed in the neuromuscular junction, and mainly participates in mediating the release of certain neurotransmitters (Catterall, 1998). Our chip results showed that when the PMDD model group was compared with the control group, the expression of several calcium ion pathway protein genes (Cacna1a, Cacn2d3, Cacn1i, etc.) was down-regulated, but returned to normal level after BXD intervention. Cacna1a also serves as a key gene in the GABA synapse pathway and dopamine synapse pathway, so it can be deduced that BXD can influence various signal pathways in the central nervous system by acting on genes for calcium ion pathway protein, thus realizing the treatment of PMDD irritability. The signaling pathways through Ca^{2+} channels was described in our previous report, in which we found that paeoniflorin, one of the active compounds of BXD, could inhibit one of the subtypes of the Ca^{2+} channels (Song et al., 2017).

Based on these considerations, it can be predicted that BXD participates in the regulation of pathways involved in the activation of upstream nerve receptor-ligand interaction by regulating the expression or phosphorylation level of important regulatory factors represented by 5-HT and MAPK, thereby realizing the signal transduction of downstream Ca2+ and MAPK and the activation of or inhibition of pathways such as GABAergic synapse and dopaminergic synapses, etc., under the influence of numerous relevant neurotransmitters, hormones, and growth factors. Ultimately, it reduces excitation of the hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary-gonadal axis of the PMDD rat models, thus resulting in restoration of a normal nerve-internal secretion-immune network functional state.

DATA AVAILABILITY STATEMENT

LX and MQ are responsible for providing the data supporting the results reported in the current study when required. The initial data obtained by gene chip have been uploaded as **Supplementary Materials** (in three.zip files).

AUTHOR CONTRIBUTIONS

SW contributed to the PMDD modeling. PS, YG, and JC were responsible for data of gene chip and RT-qPCR. JW, CS, and ZL provided essential assistance. LX and MQ directed this project, designed the experiments, and provided key advice.

FUNDING

This study was supported by National Natural Science Foundation of China (No. 81573854), Ministry of Science and Technology (No. 2017ZX09301064001), and China Postdoctoral Science Foundation (2017M622222).

ACKNOWLEDGMENTS

We thank Adam & Stone Bio-Medicals Ltd. Co. (Soochow, China) for language polishing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2018.02065/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

MINI REVIEW
published: 21 November 2018
doi: 10.3389/fpsyg.2018.02201

Emotional Roles of Mono-Aminergic Neurotransmitters in Major Depressive Disorder and Anxiety Disorders

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A growing body of researches support a role for dysfunction of serotoninergic, noradrenergic, and dopaminergic systems in the neurobiological processes involved in major depression disorder (MDD) and anxiety disorders (ADs). The physiological changes underlying abnormal signaling of 5-HT, NE, and DA may be due to either reduced presynaptic release of these neurotransmitters or aberrant signal transductions, and thus contributing to the alterations in regulation or function of receptors and/or impaired intracellular signal processing. Animal models demonstrate crucial responsiveness to disturbance of 5-HT, NE, and DA neurotransmissions. Postmortem and biochemical studies have shown altered concentrations of 5-HT, NE, and DA metabolites in brain regions that contribute importantly to regulation of mood and motivation in patients with MDD or ADs. Neuroimaging studies have found abnormal 5-HT, NE, and DA receptors binding and regulation in regard to receptor numbers. Medications that act on 5-HT, NE, and DA neurons or receptors, such as SSRIs and SNRIs, show efficacy in both MDD and ADs. The overlapping treatment response presumably suggests a common mechanism underlying the interaction of these disorders. In this paper, we reviewed studies from multiple disciplines to interpret the role of altered 5-HT, NE and DA mono-amine neurotransmitter functions in both MDD and ADs.

Keywords: major depressive disorder, anxiety disorders, mono-aminergic neurotransmitters, serotonin, norepinephrine, dopamine

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *03 July 2018*
Accepted: *24 October 2018*
Published: *21 November 2018*

Citation:
Liu Y, Zhao J and Guo W (2018) Emotional Roles of Mono-Aminergic Neurotransmitters in Major Depressive Disorder and Anxiety Disorders. Front. Psychol. 9:2201. doi: 10.3389/fpsyg.2018.02201

INTRODUCTION

Major depressive disorder (MDD) is a debilitating disease characterized by depressed mood or lack of interest. Anxiety disorders (ADs) are characterized by excessive fear and anxiety. Epidemiological, cross-sectional, and prospective studies converge on the general notion that MDD and ADs are mutually interacting, and each increases the risk for the emergence and/or exacerbation of the other. MDD shares many overlapping symptoms with ADs, including attention deficit, sleep disturbance, fatigue, arousal, and psychomotor abnormality. Epidemiological, clinical, and basic studies found that 60–90% of patients with MDD simultaneously suffered from ADs (Tsuang et al., 2004). Furthermore, 50% of patients with MDD meet the diagnostic criteria for ADs at the same time, suggesting high comorbidity rate between them (Nutt, 1997; Kessler et al., 2003).

Their comorbidity shows more severe symptoms and social function deficits, high suicide rate, poor prognosis, and chronic disease condition compared with those without comorbid anxiety or depressive symptoms (Devane et al., 2005; Bystritsky et al., 2008). The biological etiology of MDD and ADs is closely associated with mono-amine neurotransmitter deficiency and the abnormal function of neurotransmitter receptors. The disturbance of the three mono-amine neurotransmitter systems, serotonin (5-HT), norepinephrine (NE), and dopamine (DA) system may be present in diverse neural circuits in different brain regions (Castren, 2005; Hamon and Blier, 2013). Furthermore, the disruption of these monoamine neurotransmitters may even affect the function of their receptors and the downstream receptors. This article aimed to review the emotional roles of mono-aminergic neurotransmitters in MDD and ADs.

THE ROLE OF 5-HT SYSTEM IN MDD AND ADs

Disturbances in the synthesis, release, transport, and retake of 5-HT may aggravate depression. The reduction and excess of 5-HT in the brain may play an important role in the regulation of the emotional condition of the disease.

It is noted that reserpine interferes with synaptic vesicular of serotonin, depletes brain stores of 5-HT, and increases the urine concentrations of 5-HT major metabolite 5-hydroxyindoleacetic acid (5-HIAA), thereby producing depressive symptoms in human (Shore et al., 1955). These reserpine-induced depressive symptoms can be reversed by monoamine precursors (Hirschfeld, 2000). Taken together, these findings support that 5-HT systems are a biochemical basis for MDD. Coppen and associates found that lack of 5-HT in the brain may cause depressive symptoms, including depressive mood, self-accusation, and criticism (Coppen, 1967; Hogenelst et al., 2016). 5-HT deficiency in the brain may enhance negative emotions in MDD, including depressive mood, self-accusation and criticism, disgust, fear, anxiety, hostility, irritability, and loneliness (Coppen, 1967). Previous studies found that serum 5-HT concentrations in patients with MDD were significantly lower than those in healthy controls, suggesting 5-HT deficiency in patients with MDD (Bot et al., 2015; Phillips, 2017). Similarly, postmortem studies showed reduced concentrations of 5-HT and its main metabolite 5-HIAA in the brain tissue of depressed and suicidal patients (Asberg et al., 1976; Roy et al., 1989). Long-term reduction of serotonin synthesis may contribute to high susceptibility of MDD. Growing experimental and clinical evidence links the effects of antidepressants with brain 5-HT systems, and indicates that central 5-HT systems perturbance is a key factor in the pathophysiology of MDD. The serotonergic dysfunction involved in MDD development mainly includes low neuronal 5-HT synthesis and abnormal function of 5-HT receptors (Artigas, 2013). Serotonin reuptake inhibitors (SSRIs), such as sertraline, fluvoxamine, and fluoxetine, can increase extracellular 5-HT concentrations of 5-HT neurons in the midbrain dorsal raphe nucleus (Aberg-Wistedt, 1989; Artigas, 1993), and are effective in improving depressive symptoms (Fabbri et al., 2014; Castellano et al., 2016). 5-HT produces its physiological functions through the binding and interaction with multiple 5-HT receptors. Respectively, different subtypes of 5-HT receptors and 5-HT pathway can modulate different neural substrates (Wetzler et al., 1991).

Seven families of 5-HT receptors, including 5-HT1 to 5-HT7 receptors with their subtypes, have been identified (Hoyer et al., 1994; Barnes and Sharp, 1999). Numerous studies have systematically conceptualized that abnormalities of 5-HT1 (5-HT1A, 5-HT1D) and 5-HT2 (5-HT2A, 5-HT2C) receptors in the central nerve system may account for the manifestation of MDD (Hamon and Blier, 2013; Nautiyal and Hen, 2017). Postmortem and neuroimaging studies suggested that patients with MDD exhibited elevated density and/or activity of 5-HT1A autoreceptors compared with healthy controls (Parsey et al., 2006; Boldrini et al., 2008; Andrade et al., 2015), which were not sensitive to treatment of antidepressants (Lemonde et al., 2003). Autoreceptors can indirectly modulate the uptake and release of neurotransmitters, and are of importance in response to treatment (Pineyro and Blier, 1999; Ferres-Coy et al., 2013). Activation of 5-HT1A autoreceptors can lead to negative modulation of firing of serotonin system and decrease of serotonin release. However, studies show a general reduction in the density of postsynaptic 5-HT1A receptors in depressed patients, which may result in poor response to antidepressant treatment (Bhagwagar et al., 2004). Further, ligands with 5-HT1A agonist activity can produce both antidepressant (Choi et al., 2012) and anxiolytic (Vianna and Carrive, 2009) properties. Studies have also shown the aberrant sensitivity of postsynaptic 5-HT1D receptors and a distinctly higher distribution of 5-HT1D receptors in the globus pallidus in patients with MDD and/or suicide victims (Lowther et al., 1997; Whale et al., 2001; Murrough et al., 2011). Evidence indicates that 5-HT2A receptors play roles in MDD. The blockade of 5-HT2A receptors may enhance antidepressant-like profiles mediated by 5-HT1A receptors in the cortical and limbic brain regions (Artigas, 2013). Also, the antagonism of 5-HT2A receptors can induce a decreased regulation of 5-HT2A receptors that is considered to be beneficial in the treatment of MDD (Gray and Roth, 2001). Further, 5-HT2C receptor protein dysfunction has been observed in the prefrontal cortex region in suicidal patients as well (Gray and Roth, 2001). Taken together, presynaptic 5-HT1A autoreceptors show prejudicial action in antidepressant treatments, whereas the activation of postsynaptic 5-HT1A receptors in the corticolimbic networks is possibly of benefit in the antidepressant treatments. In addition, blockade of 5-HT2A receptors and 5-HT2C receptors abnormalities also play crucial roles in improving depressive symptoms.

Several important brain regions such as the prefrontal cortex, amygdala, and hippocampus are involved in the pathophysiology of MDD. The insula is also considered crucial in explaining affective deficits in depression. In a positron emission tomography study performed by Biver et al. (2018) 5-HT2 receptor specific radiotracer [I8F] altanserin was used to investigate *in vivo* distribution of 5-HT2 receptor in patients with MDD. The finding revealed that uptake of [I8F] altanserin was significantly decreased in brain regions

including the right anterior insula and right posterolateral orbitofrontal cortex, indicating the diminished 5-HT2 receptor binding in these brain regions (Vicario et al., 2017; Biver et al., 2018). A meta-analysis reported reduced 5-HT1A receptor binding in the insula, raphe nuclei, hippocampus, occipital cortex, and anterior cingulate cortex in patients with MDD (Wang et al., 2016). Functional connectivity analyses revealed that individuals with MDD exhibited abnormal activity in the dorsal insula during the anticipation of painful stimuli relative to healthy controls (Strigo et al., 2013). These results provide evidence that the pathophysiology of MDD may involve serotonergic neurotransmission perturbance in various brain regions. Otherwise, serotonergic activity can affect brain activities in certain regions, which can be used to predict the severity of MDD. For example, functional neuroimaging studies observed that abnormal activities within brain regions including the insula and anterior cingulate cortex could be used to predict the severity of depressed mood (Ryan et al., 2012).

Differentially expressed multiple 5-HT receptor subtypes on multiple cell types result in 5-HT neurotransmission possessing both antianxiety and anxiogenic effects (Albert et al., 2014). The activation of 5-HT1A receptor in the hippocampal tissue can produce anxiolytic effects. Likewise, Paul's and associates integrated evidence of anxiety from mouse genetic models involving knockout, mutation, over-expression, or suppression of genes of 5-HT1A receptors, suggesting that both pre- and post-synaptic 5-HT1A receptors contributed to anxiety phenotypes (Albert et al., 2014). High 5-HT1A autoreceptors, suppression of postsynaptic receptors or low 5-HT neurotransmission will lead to anxiety phenotype, whereas downregulation of 5-HT1A enhances anti-anxiety effects (Albert et al., 2014).

The agonism of 5-HT2A receptor in the amygdala may cause anxiety symptoms and insomnia (Bystritsky et al., 2008). 5-HT2A antagonism has been indicated to have an anxiolytic effect in subjects with generalized anxiety disorder in preclinical studies (Bressa et al., 1987). Furthermore, acute stimulation of 5-HT2A and 5-HT2C receptors projecting from the raphe nucleus to the amygdala and marginal limbic cortex may cause acute mental agitation, anxiety, and panic attack. Stimulation of 5-HT2A receptors in sleep centers of the brainstem may cause slow-wave sleep disturbances, resulting in night-time sleep arousals (Muntner, 2010). However, studies with 5-HT2C receptors agonists in elevated-plus maze model of anxiety generate conflicting results (Charney et al., 1987; Rodgers et al., 1992; Durand et al., 2003). Although 5-HT2C receptors stimulation is majorly anxiogenic, it is also anxiolytic sometimes. In the elevated-plus maze model studies, anxiolytic effects were observed though 5-HT2C receptors activation within the periaqueductal gray (Graeff, 2004). Conversely, 5-HT2C receptors agonism induced anxiogenic-like effects in the ventral part of the hippocampus and basolateral nucleus of amygdala (Alves et al., 2004; Vicente and Zangrossi, 2012). Additionally, upregulation of 5-HT2C receptors in the basolateral nucleus of amygdala could induce a similar anxiogenic effect (Li et al., 2012). Specially, the dual anxiolytic-anxiogenic effect of 5-HT2C receptors agonists may be of importance in antianxiety treatment. Clinically, in the initial treatment of SSRIs, the therapeutic effects are often delayed by the aggravation of anxiogenic-like profiles, including jitteriness or agitation (Pohl et al., 1988; Nutt and Glue, 1989). The increased anxiety induced by acute administration of SSRIs is possibly due to their predominant action on 5-HT1A autoreceptors, which may result in the reduction of 5-HT release and 5-HT neuron firing (Blier et al., 1987). Intriguingly, these acute adverse effects have been shown to be modulated by desensitization or blockade of 5-HT1A autoreceptors, as well as activation of 5-HT2C receptors (Bristow et al., 2000; Bagdy et al., 2001). Collectively, low 5-HT in central nerve system is also high risk for ADs. However, animal model of anxiety considers that high 5-HT in early postnatal phase may contribute to anxiety phenotype. 5-HT1A receptors activation is anxiolytic in chronic or long-term SSRIs treatment, whereas may produce anxiogenic-like effects due to the stimulation of presynaptic 5-HT receptors in acute SSRIs administration. 5-HT2A antagonism exerts anxiolysis, whereas 5-HT2C receptors may show region specific and dual anxiolytic-anxiogenic roles in treatment of ADs.

Several important brain regions such as the amygdala, cingulate cortex, and raphe nucleus are involved in the pathophysiology of ADs. The insular role is also crucial in the pathophysiology of ADs. In particular, SPECT and positron emission tomography studies have reported decreased 5-HT1A receptor binding in the insula, amygdala, anterior cingulate cortex, medial prefrontal cortex, and raphe nucleus in panic disorder (Neumeister et al., 2004; Nash et al., 2008). One study has reported reduced 5-HT1A receptor binding in the insula, amygdala, and anterior cingulate cortex in social anxiety disorder (Lanzenberger et al., 2007). Functional neuroimaging data provide evidence that exaggerated activation in the insular and cingulate cortices may be predictive of anxious traits (Shin and Liberzon, 2010; Vicario et al., 2017). Alvarez et al. (2015) found that anxiety-prone participants exhibited elevated activation in the bilateral dorsal anterior insula in response to anticipation of noxious event. These results suggest that the serotonergic activity has an impact on brain activity and provide evidence of altered serotonin neurotransmission in multiple brain regions or circuits in pathophysiology of ADs (Boshuisen et al., 2002).

Antidepressant drugs enhance 5-HT neurotransmission in patients with MDD.It can also improve many types of ADs, including panic disorder and generalized anxiety disorder (GAD) (Kahn et al., 1988).The decline of 5-HT neurotransmission function can not only affect the development of emotional disorders such as MDD and ADs, but can also induce emotional disturbance through activities relative to other neurotransmitter systems (Sumner et al., 2014). In general, low 5-HT has been shown to be associated with the presence of depressive and anxiety symptoms. Additionally, both 5-HT1A and 5-HT2A/2-HT2C receptors are majorly involved in depression and anxiety profiles. These observations clearly support that anxiety and depressive symptoms can be treated simultaneously.

THE ROLE OF NE IN MDD AND ADs

NE is ingested into the noradrenergic nerve endings by tyrosine transporter through a precursor formation of tyrosine and NE

and is converted to NE by a series of transformations. In 1979, Zis and associates proposed the NE hypothesis of MDD that depressive symptoms were caused by the decrease of NE in the central nervous system (Zis and Goodwin, 1979). Postmortem studies of depressed patients have found increased conformation of central α2-adrenergic autoreceptors and decreased NE transporter binding affinity in the locus ceruleus (Klimek et al., 1997) with no significant alteration in density of α2-adrenergic receptors in raphe nuclei (Escriba et al., 2004). Studies have observed increased binding of agonist ligands at α2-adrenergic autoreceptors on the NE neurons cell body, indicating higher functions of these NE autoreceptors and thus suggesting a lower noradrenergic neurotransmission in MDD (Hamon and Blier, 2013). The α2-adrenergic autoreceptors occur presynaptically on noradrenergic and serotonergic neuronal terminals and exert suppression effects in the release of neurotransmitters upon stimulation. In addition, postmortem studies have demonstrated elevations in mRNA levels for α2-adrenergic autoreceptors in the frontal cortex from suicide subjects, and the majority of the subjects had MDD diagnosis before death (Vicente and Zangrossi, 2012). Possible interpretation of these detections is that hypersensitive presynaptic α2-adrenergic autoreceptors can contribute to reduction in NE and serotonin release. NE reduction in the central nervous system is associated with depletion of positively affective resources in patients with MDD, including decrease in pleasure, interest, happiness, alertness, energy, and passion and loss of confidence. Patients with MDD had lower NE function in lobar NE, causing anhedonia, loss of energy and passion, and other relative depressive symptoms (Bystritsky et al., 2008). Conversely, the symptoms of anxiety were assumed to be caused by hyperactivity of NE in the central nervous system. In stress conditions, corticotropin-releasing factor can activate the NE energy pathway in the locus coeruleus-temporal hippocampal, which releases NE and inducing wakefulness and anxiety symptoms (Muntner, 2010). Further, researchers observed elevated serum catecholamine concentrations in patients with GAD, indicating the excess of NE (Homan et al., 2015; Reader et al., 2015). Preliminary evidence suggests that single-nucleotide polymorphism involved in the function of adrenergic receptors is a susceptibility factor for general anxiety disorder (Zhang et al., 2017). Animal model studies found that antagonism of β-adrenergic receptors within central nerve system could attenuate the anxiogenic effects of cocaine (Wenzel et al., 2014) and disrupted anxiety-like phenotypes including aversive, fear and stress-related behaviors (Kindt et al., 2014; McCall et al., 2017).

THE ROLE OF DA IN MDD AND ADs

DA is a neurotransmitter in the hypothalamus and pituitary that is a key neurobiological substrate for reward, concentration, motivation, psychomotor speed, and the ability to experience pleasure, which may play a role in the modulation of human emotions (Coppen, 1967). Dopaminergic activity has been demonstrated to be involved in depressive (Ryan et al., 2012) or anxious (Vicario et al., 2017) processing. A strong evidence links reward-related, hedonic, and motivated behaviors with the mesolimbic DA system (Cabib and Puglisi-Allegra, 1996). Impairment of these functions are all prominent characteristics of MDD. Moreover, immediate bidirectional control (inhibition or excitation) of specified midbrain DA neurons modulates multiple independent depressive symptoms caused by chronic stress, suggesting that processes affecting depressive symptoms alter the DA neural encoding of action in the limbic circuitry (Tye et al., 2013). Further, poor functioning of DA neurons may cause depressive symptoms, including hopelessness and loss of interest (Kasch et al., 2002; Dunlop and Nemeroff, 2007). Patients with MDD showed lower level of DA metabolites in the cerebrospinal fluid compared with healthy controls (Jokinen et al., 2007). Deficiency in DA receptor function may lead to the failure in inhibition from the prefrontal cortex to the amygdala, and induce the over excitability of the amygdala, resulting in the emergence of fear and pathological anxiety.

DA receptors have two subtypes, the D1 and D2 receptor. Studies have demonstrated reduced dopamine transporters density and D2 receptor binding in the striatum in patients with social anxiety disorder compared with healthy controls (Schneier et al., 2000; Shin and Liberzon, 2010).DA blockers can increase the severity of social fear symptoms (Clausius et al., 2009). Furthermore, the level of homovanillic acid, a DA metabolite in the cerebrospinal fluid, had a lower level in patients with depressive comorbid with social fear (Jokinen et al., 2007). Thus, these conclusions may suggest DA or DA receptor dysfunction in patients with MDD, ADs, and with comorbidities.

Furthermore, the activity of dopaminergic neurons in the ventral striatum could affect insular activity, which could be used to predict the symptom severity of MDD and ADs (Black et al., 2002). There was evidence that reduction in expression of dopamine receptors in the striatal pathways and enhanced functioning of the insula and adjacent operculum were involved in mood alterations and associated behaviors such as eating disorder (Stice et al., 2009; Frank et al., 2012). Animal model studies and clinical case reports also suggested dopaminergic circuit could contribute to mood regulation through the insula. The agonist of DA receptor, particularly the D3 receptor, could reduce cerebral blood flow in the insula of the baboons, which was further supported by the treatment of MDD in several clinical trials (Goldberg et al., 2004; Zarate et al., 2004).

INTERACTIONS BETWEEN 5-HT, NE AND DA

The NE and 5-HT neurotransmitter systems are mutually interacted in the central nervous system (Quesseveur et al., 2013). NE plays a role in the regulation of the release of 5-HT. Stimulating the α2 receptor on the axon terminals can inhibit the release of 5-HT, and stimulating the α1 receptors on neuronal cell bodies or dendrites may cause positive feedback

on the release of 5-HT. At the same time, 5-HT systems can exert negative influence on NE systems through the 5-HT2A and 5-HT2C receptor-mediated mechanisms (Hamon and Blier, 2013). Evidence suggests that 5-HT2A receptors can enhance the release of NE under the SSRIs treatment (Sullivan et al., 2005). Both NE and 5-HT nerve fiber project signaling in the frontal cortex and hippocampus play an important regulatory role in cognition and behavior, especially in mental and emotional regulation in the central nervous system. Further, the two neurotransmitters signaling in the hippocampus and frontal cortex has been the target of a large proportion of research on MDD, ADs, and their treatments (Graeff et al., 1996).

There is multiple interaction between the serotonergic and dopaminergic systems. Increased or reduced neurotransmission of serotonergic or noradrenergic systems can affect dopamine function and induce similar changes in dopaminergic signaling. The antidepressant and anxiolytic efficacy of clinical therapeutics may partly result from the alterations in DA neurotransmission of the DA reward-learning circuit signaling. In patients with MDD or ADs, dopamine-related disturbances can be presumably improved through this mechanism. Several positron emission tomography and SPECT studies found that increase in striatal dopamine receptor binding and dopamine transporter availability correlated with improvement in Hamilton Depression Rating Scale scores (Mischoulon et al., 2002; Yang et al., 2008). In addition, substantial interaction exists between serotonergic cells of the midbrain raphe and target dopaminergic cell bodies in the ventral tegmental area in central nervous system. Findings of Mataix et al. indicated that stimulation of 5-HT1A receptors in the medial prefrontal cortex could enhance activity of the ventral tegmental area DA neurons, along with meso-cortical DA release. In particular, 5-HT systems can also exert a negative influence on DA systems through the 5-HT2A and 5-HT2C receptor-mediated mechanisms (Clausius et al., 2009). Both of them play important roles in the regulation of mood and mental movement in the central nervous system. Acute stimulation of the basal 5-HT2A receptor may inhibit DA function in the basal area and causes acute motor changes, including psychomotor retardation and dystonia. Stimulating the 5-HT2A receptor in the midbrain may inhibit DA activity, causing apathy and sex reduction. Additionally, 5-HT2C receptors play a role in the tonic regulation of ascending dopaminergic activity, which may be a potential effect of antidepressant drugs.

CONCLUSION

In general, the three mono-aminergic neurotransmitter systems are mutually interacting, each playing roles in the regulation of diverse human emotions (see the mechanism in **Figure 1**). Depression and anxiety may be directly caused by dysfunction in brain areas including hippocampus, amygdala, and the prefrontal cortex (Mayberg et al., 1997; Steffens and Krishnan, 1998; Bremner, 2002)or by the neural systems modulated by mono-amine neurotransmitter systems in these brain regions (Delgado and Moreno, 2000). The diffuse nerve fibers of 5-HT, DA, and NE in the hypothalamus, thalamus, basal forebrain, and the prefrontal cortex could play roles in the regulation of dysmnesia. However, lack of energy and fatigue could associate with hypofunction of NE and DA in the prefrontal cortex (Muntner, 2010). In other words, part of the depressive and anxiety symptoms may be related to a certain

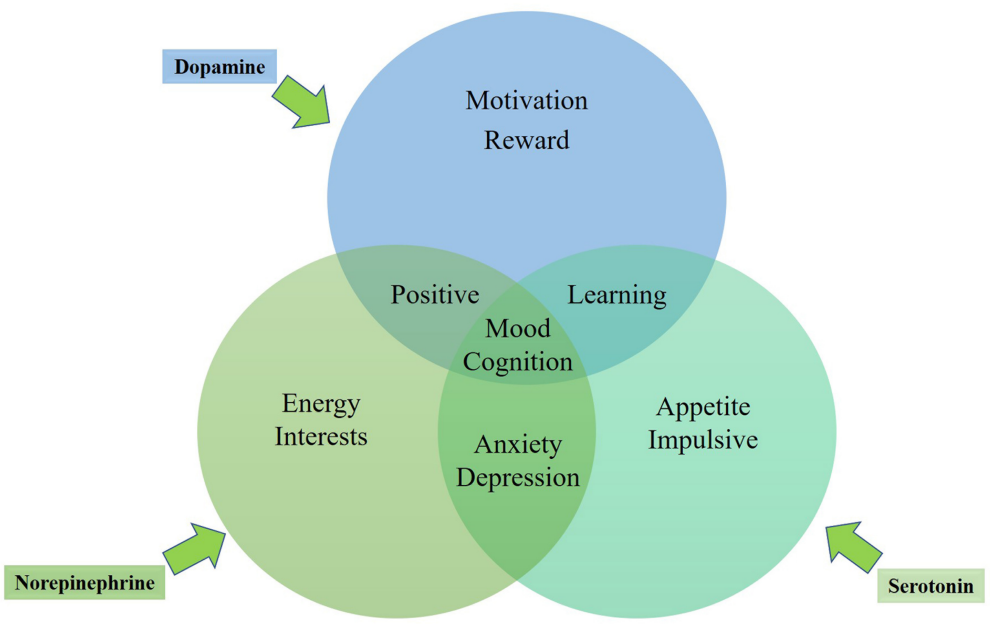

FIGURE 1 | Emotional role of 5-HT, NE and DA in the regulation of depression and anxiety.

neurotransmitter system, while other symptoms may be related to a variety of other neurotransmitter systems. No single drug can fully improve any psychiatric disorder. However, a certain drug can possibly improve depressive mood by enhancing the information-processing function in a brain region, while another drug with a different mechanism can ease other symptoms such as insomnia, anxiety or concentration deficiency by improving the information-processing function in other brain regions. The treatment with the same antidepressant drug is effective for both depressive and anxiety symptoms, also supporting the possibility of the same neurobiological neurotransmitter dysfunction mechanism underlying the symptoms of MDD and ADs.

AUTHOR CONTRIBUTIONS

YL wrote the protocol and first draft of the manuscript. YL and JZ contributed to concept and design. YL and WG revised critically the manuscript for important intellectual content.

FUNDING

This study was supported by grants from the National Key R&D Program of China (2016YFC1307100 and 2016YFC1306900) and the National Natural Science Foundation of China (Grant Nos. 81571310, 81630033, 81771447, and 81471363).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

ORIGINAL RESEARCH
published: 21 December 2018
doi: 10.3389/fpsyg.2018.02586

Differentiation of Transformed Bipolar Disorder From Unipolar Depression by Resting-State Functional Connectivity Within Reward Circuit

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Previous studies have found that neural functional abnormalities detected by functional magnetic resonance imaging (fMRI) in brain regions implicated in reward processing during reward tasks show promise to distinguish bipolar from unipolar depression (UD), but little is known regarding resting-state functional connectivity (rsFC) within the reward circuit. In this study, we investigated neurobiomarkers for early recognition of bipolar disorder (BD) by retrospectively comparing rsFC within the reward circuit between UD and depressed BD. Sixty-six depressed patients were enrolled, none of whom had ever experienced any manic/hypomanic episodes before baseline. Simultaneously, 40 matched healthy controls (HC) were also recruited. Neuroimaging data of each participant were obtained from resting-state fMRI scans. Some patients began to manifest bipolar disorder (tBD) during the follow-up period. All patients were retrospectively divided into two groups (33 tBD and 33 UD) according to the presence or absence of mania/hypomania in the follow-up. rsFC between key regions of the reward circuit was calculated and compared among groups. Results showed decreased rsFC between the left ventral tegmental area (VTA) and left ventral striatum (VS) in the tBD group compared with the UD group, which showed good accuracy in predicting diagnosis (tBD vs. UD) according to receiver operating characteristic (ROC) analysis. No significant different rsFC was found within the reward circuit between any patient group and HC. Our preliminary findings indicated that bipolar disorder, in early depressive stages before onset of mania/hypomania attacks, already differs from UD in the reward circuit of VTA-VS functional synchronicity at the resting state.

Keywords: bipolar disorder, depression, reward circuit, functional connectivity, resting-state, functional magnetic resonance imaging

OPEN ACCESS

Edited by:
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Shandong University, China

Reviewed by:
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Carnegie Mellon University, United States
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Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *12 September 2018*
Accepted: *03 December 2018*
Published: *21 December 2018*

Citation:
Shi J, Geng J, Yan R, Liu X, Chen Y, Zhu R, Wang X, Shao J, Bi K, Xiao M, Yao Z and Lu Q (2018) Differentiation of Transformed Bipolar Disorder From Unipolar Depression by Resting-State Functional Connectivity Within Reward Circuit. Front. Psychol. 9:2586. doi: 10.3389/fpsyg.2018.02586

INTRODUCTION

Bipolar disorder (BD) and unipolar depression (UD) are two of the most debilitating illnesses worldwide (Murray and Lopez, 1997). BD mainly differs from UD in the presence of mania/hypomania. Clinical manifestations of BD are more complex than those of UD. However, approximately half of bipolar individuals present with a major depressive episode as their first

mood episode (Tondo et al., 2010; Etain et al., 2012). When in a depressive episode, symptoms are similar in BD and UD, which heavily obstructs the accurate diagnosis of BD. Up to 60% of BD patients seeking treatment for depression are initially diagnosed with UD. Only 20% BD who are experiencing a depressive episode are precisely diagnosed within the first year of treatment (Hirschfeld et al., 2003). Moreover, treatments for BD and UD are very different, with stabilizers for BD and antidepressants for UD being prescribed. Inappropriate medication might lead to poor prognosis, such as increased suicidal behavior, switching to mania, and higher health care costs (Bowden, 2010; Goodwin, 2012; Baldessarini et al., 2013). Therefore, it is of great importance to distinguish BD from UD as early as possible.

Numerous clinical characteristics have been recognized as risk factors for developing BD, including (1) family history of BD or affective disorder, (2) early age of onset (less than 25 year-old), (3) recurrence (more than 4 episodes), (4) substance abuse, (5) psychotic symptoms, and (6) refractory (Ostergaard et al., 2014; Tondo et al., 2014; Woo et al., 2015; Bukh et al., 2016; Ratheesh et al., 2017). In addition, several clinical rating scales may help to detect subthreshold manic/hypomanic symptoms in depression, such as the Hypomania Checklist (Angst et al., 2005), the Screening Assessment of Depression Polarity (Solomon et al., 2006), and the Bipolar Inventory Symptoms Scale (Bowden et al., 2007). Although helpful, the aforementioned strategies are based on phenomenological observation and depend heavily on the professionalism of clinicians. For early identification of BD from UD, objective methods are needed.

Neuroimaging techniques, especially magnetic resonance imaging (MRI) can objectively reflect the structural and functional condition of the neural system. Numerous MRI studies have provided evidence that individuals with BD could be differentiated from those with UD by abnormal gray matter volumes in several brain regions. For example, in a cross-sectional MRI study, Rive et al. (2016) found that depressed subjects with BD and UD could be classified based on the gray mater volumes of the middle frontal gyrus, parahippocampal gyrus, and the orbital part of the superior frontal gyrus. Other studies showed reduced ventral diencephalon volumes in euthymic BD vs. UD (Sacchet et al., 2015), reduced gray matter volumes in the hippocampus and the amygdala (Amy), but increased gray matter volumes in the anterior cingulate cortex (ACC) in individuals with BD relative to individuals with UD (Redlich et al., 2014). The white matter connectivity may also be useful in differentiating BD from UD. Damme et al. (2017) found that white matter connectivity between the nucleus accumbens (NAcc) and both the medial orbitofrontal cortex (mOFC) and Amy were associated with elevated mania/hypomania proneness. Regarding functional neuroimaging, substantial evidence indicates that abnormalities in brain regions implicated in reward processing during reward tasks show promise to distinguish bipolar from UD. Compared with healthy controls, UD showed reduced caudate and NAcc responses to rewards (Pizzagalli et al., 2009), and less ventral striatal activation during reward anticipation (Stoy et al., 2012). On the contrary, BD patients showed elevated striatal reactivity (Dutra et al., 2015), and increased functional connectivity between the ventral striatum (VS) and OFC (Dutra et al., 2017) across monetary and social rewards compared to the healthy controls.

The reward circuit mediates goal-directed behaviors, including emotions, motivation, and cognition. Key brain regions in the reward circuit are the ACC, the orbital prefrontal cortex (OFC), the VS, the ventral tegmental area (VTA) and the amygdala (Haber and Knutson, 2010). These brain reward regions have been assigned specific functions: the VTA–VS is the center of reward, the ACC and OFC are responsible for working memory and executive control, and the amygdala is crucial for associative fear- and reward-related memories (Russo and Nestler, 2013). In addition, other structures, including the dorsal prefrontal cortex, hippocampus, thalamus, and lateral habenular nucleus, are also important components in regulating the reward circuit. Connectivity between these areas forms a complex neural network that mediates different aspects of reward processing. Activation of the reward circuit leads to increased motivation, behavior directed toward attaining rewards, and positive emotions, or to anger when goal-striving is frustrated. Downregulation or deactivation of the reward circuit leads to decreased motivation, increased withdrawal, and emotions such as sadness and anhedonia. Reward hypersensitivity is suggested to underlie risk for manic/hypomanic symptoms (Alloy et al., 2016). Collectively, BD seems to be characterized with reward hyperactivation, while reward hypoactivation is involved in UD (Alloy et al., 2016).

However, there are some different findings in tasks based functional magnetic resonance (fMRI) studies. Foti et al. (2014) reported that reward processing during a laboratory gambling task was heterogeneous within MDD, indicating that not all MDD were characterized by reward dysfunction. In a study using a card-guessing task, BD patients showed decreased, not increased, activation of reward regions including the NAcc, caudate nucleus, and prefrontal areas compared with UD (Redlich et al., 2015). In another task study (Sharma et al., 2016), reduced activation in the bilateral VS and left OFC to social reward was found to be correlated with greater depression severity in the BD patients, but not the unipolar ones. These inconsistent findings may be explained by the variety of task paradigms. Accepting this, such different reward processing in BD and UD still suggests the role of the reward circuit in distinguishing the two disorders. Whether this different functioning of the reward circuit between BD and UD is state-dependent or has a trait-like profile is unclear. If recognized as a task-independent trait, differences of reward functioning should also exist at resting state, which may help to identify BD and UD earlier.

A resting-state fMRI study of the reward circuit can provide much benefit in the absence of specific tasks. During rs-fMRI scanning, participants are not required to perform a specific task. This avoids limitations due to the interference of different task paradigms and ensures a high degree of cooperation. Consequently, rs-fMRI may improve the relative consistency of findings across multiple studies. Previous studies suggested that resting-state functional connectivity (rsFC)

between large-scale brain networks (Goya-Maldonado et al., 2016), and between region of interest (ROI) and other brain regions (Ambrosi et al., 2017), can differentiate unipolar and bipolar depression. One study exists which directly compared reward circuit rsFC between BD and UD (Satterthwaite et al., 2015). Moreover, aberrant reward circuit rsFC has already been identified in major depressive disorder (Felger et al., 2016; Gong et al., 2017) and many other medical conditions, including sleep disturbance (Avinun et al., 2017), attention deficit hyperactivity disorder (ADHD) (Dias et al., 2013; Tomasi and Volkow, 2014), and schizophrenia and cannabis use disorder (Fischer et al., 2014). This evidence verifies the dysfunction of the reward circuit not only during tasks but also at resting state.

In the present study, we aimed to explore neurobiomarkers for early recognition of BD by retrospectively comparing rsFC within the reward circuit between UD and depressed BD. Generally, the measure of rsFC can represent the functional synchronicity of spontaneous activity between a given region and any other regions in the whole brain. In this study, rsFC between key regions of the reward circuit (the OFC, the ACC, the VS, the VTA and the amygdala) was used to define functional synchronicity within the reward circuit. Interestingly, BD patients enrolled in our study were in depressive episodes at the baseline and had never experienced any manic/hypomanic episodes before. These depressed patients then began to manifest bipolar disorder (transformed bipolar disorder, tBD) during the follow-up period. We hypothesize that rsFC between key brain regions of the reward circuit differs between tBD and UD, which may contribute to the early distinction of BD from UD.

MATERIALS AND METHODS

Participants

Seventy-seven patients with a preliminary diagnosis of MDD were enrolled at the Department of Psychiatry of the Affiliated Nanjing Brain Hospital of Nanjing Medical University from September 2011 to May 2017. The diagnosis of MDD was established according to the Diagnostic and Statistical Manual of Mental Disorders, fourth version (DSM-IV-TR). The 17-item Hamilton Rating Scale for Depression (HAMD-17) (Hamilton, 1960) was applied to assess depression severity. The Mini-International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998) was used to ensure the diagnosis of MDD and the absence of any other psychiatric disorders. The 32-item hypomania checklist (Hirschfeld et al., 2000; Angst et al., 2005) was used to screen out any lifetime manic/hypomanic episodes, with all patients scoring lower than 12. Participants with current or past history of other mental disorders were excluded.

The present study was a longitudinal observational follow-up study. During the follow-up period, patients were observed for at least 3 years unless they developed mania/hypomania. Patients who began to display mania/hypomania were defined as tBD. Thirty-seven patients were classified into the tBD group at the end of the observation in December 2017. The remaining 40 patients did not suffer from a manic/hypomanic episode after more than 3 years' follow-up. It was considerable to refer to these patients as less likely to develop BD in the future, and they were defined as the UD group.

Forty healthy controls (HC) were recruited from local community. M.I.N.I. was also applied to confirm the absence of a psychosis history. HC were excluded if they reported family history of any mental disorders in first degree relatives.

All participants were Han Chinese, right handed, 18 to 55 years old, with a minimum education of 6 years. Additional exclusion criteria for all participants included nervous system disease, serious physical illness, substance abuse/dependence and any MRI contraindications.

This study was approved by the Research Ethics Review Board of Affiliated Nanjing Brain Hospital of Nanjing Medical University. All participants were informed of the study and provided written informed consent.

MRI Data Acquisition

At the baseline after addition, all participants underwent MRI scan on a 3.0T Siemens Verio scanner with an 8-channel radio frequency coil at the Radiology Department of the Affiliated Nanjing Brain Hospital of Nanjing Medical University. Before the scan, subjects were instructed to lie still with their eyes closed, to relax but not fall asleep, and not to think of anything specific. Each subject was positioned comfortably in the coil and fitted with soft ear plugs to reduce scanner noise. Firstly, 3D T1-weighted images were acquired with the following parameters: repetition time (TR) = 1900 ms, echo time (TE) = 2.48 ms, flip angle = 9°, field of view (FOV) = 250 mm × 250 mm, matrix size = 256 × 256, 176 axial slices of 1 mm thickness, in-plane voxel resolution = 1 mm × 1 mm, acquisition time = 4 min 18 s. Further, a total number of 133 volumes of resting-state functional images (TR = 3000 ms, TE = 40 ms, flip angle = 15°, FOV = 240 mm × 240 mm, matrix size = 64 × 64, 32 axial slices of 4 mm thickness, acquisition time = 6 min 45 s) were acquired using gradient-recalled echo-planar imaging.

Resting-State Functional Image Preprocessing

The image format was transferred using the MRIcroN[1]. Preprocessing was conducted by the Data Processing Assistant for Resting-State fMRI (DPARSF) toolbox[2]. First, the first 6 volumes were removed for stable magnetization and to adapt the participants to the scan. Then, the remaining 127 volumes were slice-time corrected, head-motion realigned, spatially normalized using a T1-weighted image by DARTEL segmentation, and saved with a spatial resolution of 2 mm × 2 mm × 2 mm. Smoothing was done with a 4-mm full-width at half maximum (FWHM) isotropic Gaussian kernel, temporal band pass filtering (0.01–0.08 Hz) was done to reduce low frequency drift and physiological high-frequency noise, and detrending was done to reduce the influence of the rising temperature of the MRI equipment. Subsequently, nuisance signals including Friston 24 head motion parameters as well as white matter and cerebrospinal signals were regressed out. In the present study, six patients (three UD and

[1]http://www.mricro.com

[2]http://www.restfmri.net/forum/DPARSF

three tBD) and two HC were excluded due to head motion of more than 2.0 mm maximum displacement in any dimension or 2.0 degrees of angular motion. One HC was excluded for abnormal anatomic signals. Six participants (four UD, one tBD and one HC) were excluded because of bad normalization. Finally, 66 patients (33 UD and 33 tBD) and 36 HC went into further functional connectivity analysis in DPARSF.

ROI-to-ROI Functional Connectivity Analysis

Based on the hypothesis mentioned above, we created 8 ROIs: the mOFC (MNI: 2, 46, −8), the ACC (MNI: −2, 28, 28), the left VS (MNI: −12, 12, −7) and the right VS (MNI: 12, 10, −6), the left Amy (MNI: −20, −2, −16) and the right Amy (MNI: 20, −2, −20), the left VTA (MNI: −4, −16, −14) and the right VTA (MNI: 4, −18, −14) at Montreal Neurological Institute (MNI) space. The coordinates of the mOFC, the ACC and the bilateral VS were derived from a meta-analysis of Bartra et al. (2013), which had been widely applied in fMRI studies (Satterthwaite et al., 2015; Pan et al., 2017). Concerning that Bartra's meta-analysis didn't provide precise coordinates of the amygdala, and that the sphere of the VTA in this meta-analysis may contain other structures of the brainstem, the coordinates of the bilateral Amy and the bilateral VTA were derived from an fMRI study that investigated the effects of city living on the reward system (Kramer et al., 2017). The WFU pickatlas[3] was used to create ROIs with 4-mm-radius spheres for the bilateral VTA and 5-mm-radius spheres for the rest, centered according to previous studies (Kahn and Shohamy, 2013; Satterthwaite et al., 2015; Kramer et al., 2017; Pan et al., 2017). ROI-to-ROI functional connectivity was performed using the DPARSF toolbox. A time series of each ROI was extracted and averaged across all voxels within the ROI. Individual images were normalized into a standard template to get rid of individual location variance. Then, Pearson's correlation coefficients between each pair of ROI regions were regarded as the strength of the functional connectivity. The correlation coefficients were transformed into Fisher's z-score to improve normality and allow for further analysis. Thus, a z-score matrix of each individual functional connectivity was separately obtained. Since functional connectivity was directionless, we extracted the upper triangular matrix values (28 connections per subject) for statistical analysis.

Statistical Analysis

One-way analysis of variance (ANOVA) and a chi-square test (only for gender) were performed in SPSS 19.0 software (SPSS Inc., Chicago, IL, United States) to compare demographic data. Clinical data which could be recorded as continuous variables including age at onset, total illness duration, current episode duration, and number of depressive episodes and HAMD-17 score between UD and tBD groups were analyzed using two-sample t-tests. Other clinical categorical variables were analyzed by chi-square tests between the two patient groups, such as family history of affective disorder, chronicity (defined as one single depressive episode that lasts for at least 2 years without significant remission) (Ratheesh et al., 2017), refractory (no improvement after sufficient treatment of two or more antidepressants), suicide attempt and diurnal depression variance, as well as treatment (type of antidepressant, stabilizer, rTMS, and MECT). Concerning that the treatment during the follow-up period might impact the prognosis of depression (i.e., whether they remain unipolar or develop into bipolar), binary logistic regression analysis was conducted. In the logistic regression analysis, the group (tBD or UD) was held as the dependent variable, and treatment was held as the independent variable. Significance was set at $P < 0.05$ two-tailed alternatives.

To examine the baseline rsFC differences between tBD and UD patients, a randomized permutation test with 5000 times was used. A permutation test is a type of statistical significance test in which the distribution of the test statistic under the null hypothesis is obtained by calculating all possible values of the test statistic under rearrangements of the labels on the observed data points (Nichols and Holmes, 2002). The detailed steps of the permutation test in this present study are as follows: (1) The averaged functional connectivity was computed for the original group labeling; (2) for each resampling, the group labels were randomly rearranged, and the averaged functional connectivity for the permuted data were computed; (3) step 2 was repeated until a predefined number of resamplings had been performed; and (4) the hypothesis was accepted or rejected based on the proportion of permuted averaged functional connectivity equal to or greater than the original. The rsFC differences in the baseline may serve as neurobiomarkers for early differentiation of BD from UD. As mentioned before, several clinical and demographic data were suggested to be risk factors for the transition from depression to BD, including family history of BD or affective disorder, early age of onset (less than 25 years-old), recurrence (more than four episodes), and refractory status (Dudek et al., 2013; Woo et al., 2015; Ratheesh et al., 2017). On the other hand, demographic factors such as age and education also might influence brain function. In order to test whether the between-group rsFC differences at baseline were due to these potential confounding factors, a general linear model (GLM) was performed. In the GLM, rsFC values of significant difference between UD and tBD were held as the dependent variables, groups were held as independent variables, and potential confounding factors (age, education years, onset age, number of episodes, family history, and refractory) were held as covariates. A significant difference was set at a threshold $P < 0.05$ FDR-corrected.

Additionally, to evaluate the accuracy of the rsFC values in predicting diagnosis (tBD vs. UD), we also carried out receiver operating characteristic (ROC) analysis, which could obtain the area under the curve (AUC) using SPSS software. Meanwhile, three statistics including sensitivity (SN), specificity (SP), and odds ratio (OR) were calculated to assess the diagnostic efficiency.

Our primary hypothesis concerned differences between tBD and UD patients. In order to provide information regarding the extent to which observed tBD and UD differences represent abnormal neural functioning, we also performed exploratory comparisons by including a group of HC and conducted

[3]http://fmri.wfubmc.edu/software/pickatlas

permutation testing between HC and each patient group, respectively.

RESULTS

Demographic and Clinical Characteristics

No significant differences in age, gender, and education level were found among the three groups. All clinical characteristics compared did not significantly differ between tBD and UD, including onset age, number of episodes, total illness duration, current episode duration, and total score of HAMD-17, family history of affective disorder, chronicity, refractory, suicide attempt, diurnal depression variance, and treatment (please see details in **Table 1**). Results of binary logistic regression analysis showed that group (tBD or UD) was not related to treatment (**Supplementary Table 1**), indicating that transition to BD was not due to differences in treatment.

Resting-State Functional Connectivity

Among the 28 connections within the reward circuit, rsFC between the left VTA and the left VS (rsFC value: tBD: 0.057 ± 0.223, UD: 0.234 ± 0.236; $P = 0.001$, $P < 0.05$ with FDR correction), between the left VTA and the right VS (rsFC value: tBD: 0.082 ± 0.223, UD: 0.234 ± 0.236; $P = 0.008$, uncorrected), and between the right VTA and right VS (rsFC value: tBD: 0.108 ± 0.229, UD: 0.227 ± 0.250; $P = 0.049$, uncorrected) were lower in the tBD group compared with the UD group. Only the rsFC between the left VTA and left VS was significantly different between tBD and UD (**Figures 1**, **2**).

General linear model analysis showed that the rsFC differences between the two patient groups survived even after several possible confounding factors (family history of affective disorder, number of episodes, refractory, age of onset, education and age) were taken into account (**Supplementary Table 2**). Moreover, ROC analysis showed good accuracy of the left VTA-left VS rsFC (AUC = 70%, SN = 87.9%, SP = 51.5%, OR = 7.703, $P = 0.005$, **Figure 3**).

Exploratory comparisons showed that, relative to HC, UD showed higher rsFC between the left VTA and the left VS (rsFC value: UD: 0.234 ± 0.236, HC: 0.101 ± 0.137; $P = 0.003$, uncorrected), between the left VTA and the left Amy (rsFC value: UD: 0.271 ± 0.288, HC: 0.146 ± 0.267; $P = 0.033$, uncorrected), between the right VTA and the left VS (rsFC value: UD: 0.171 ± 0.228, HC: 0.024 ± 0.234; $P = 0.005$, uncorrected), and between the right VTA and the right VS (rsFC value: UD: 0.205 ± 0.243, HC: 0.077 ± 0.230; $P = 0.015$, uncorrected). Conversely, relative to HC, the tBD group showed higher rsFC between the bilateral VTA and the left Amy (left VTA-left Amy rsFC value: tBD: 0.270 ± 0.315, HC: 0.146 ± 0.267; $P = 0.040$, uncorrected. right VTA-left Amy rsFC value: tBD: 0.290 ± 0.301, HC: 0.126 ± 0.286; $P = 0.012$, uncorrected), and lower rsFC between the right VS and the left Amy (rsFC value: tBD: 0.227 ± 0.261, HC: 0.331 ± 0.226; $P = 0.042$, uncorrected).

TABLE 1 | Demographic and clinical characteristics among three groups.

Variables	UD (n = 33)	tBD (n = 33)	HC (n = 36)	P-value
Sex (M/F)	17/16	17/16	18/18	0.941[a]
Age, y	30.91 ± 8.28	31.39 ± 8.30	32.41 ± 8.86	0.684[b]
Education, y	13.85 ± 3.02	13.88 ± 2.79	15.02 ± 2.26	0.053[b]
Onset age, y	28.03 ± 8.97	28.48 ± 9.29		0.840[c]
Total illness duration, mo	33.64 ± 53.31	40.50 ± 62.20		0.632[c]
Current episode duration, mo	7.55 ± 12.81	4.92 ± 4.97		0.279[c]
Number of episode	1.82 ± 1.26	2.09 ± 1.36		0.400[c]
Total score of HAMD-17	20.76 ± 9.26	22.27 ± 7.27		0.462[c]
Family history of AD	1 (3.0%)	4 (12.1%)		0.355[a]
Chronicity	5 (15.2%)	7 (21.2%)		0.751[a]
Refractory	3 (9.1%)	3 (9.1%)		1.000[a]
Suicide attempt	5 (15.2%)	11 (33.3%)		0.150[a]
Diurnal depression variance	15 (45.5%)	14 (42.4%)		1.000[a]
Treatment				
SSRI/SNRI	20/13	18/15		0.618[a]
Stabilizer	5 (15.2%)	9 (27.3%)		0.228[a]
rTMS	4 (12.1%)	4 (12.1%)		1.000[a]
MECT	4 (12.1%)	6 (18.2%)		0.733[a]

UD, unipolar depression; tBD, transformed bipolar depression; HC, health control; HAMD, Hamilton Depression Rating Scale; AD, affective disorder; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin and norepinephrine reuptake inhibitor; rTMS, repetitive transcranial magnetic stimulation; MECT, modified electroconvulsive therapy.

[a] *The P-value was obtained by two-tailed Pearson chi-square t-test.*

[b] *Data presented as the range of minimum-maximum (mean ± SD). The P-value was obtained by one-way analysis of variance.*

[c] *Data presented as the range of minimum-maximum (mean ± SD). The P-value was obtained by two-sample two-tailed t-test.*

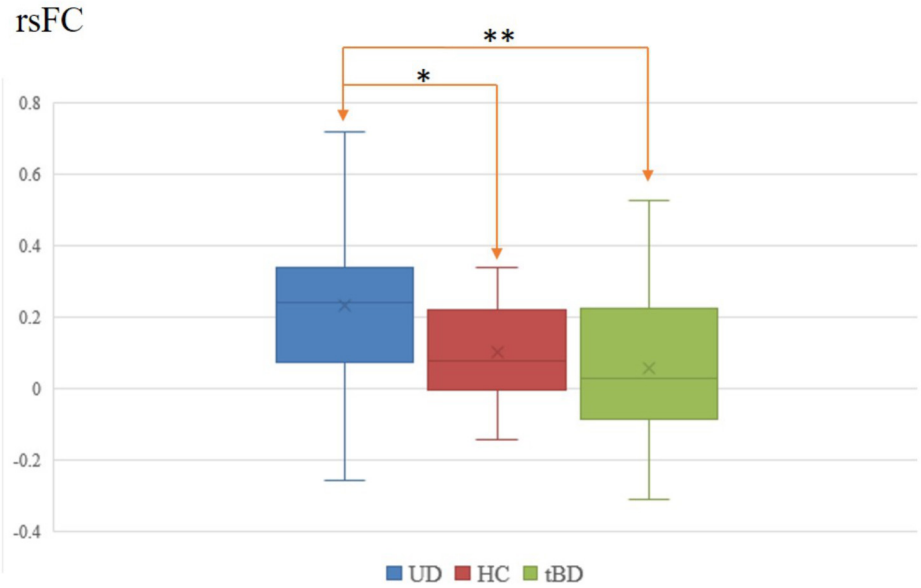

FIGURE 1 | Left VTA and left VS rsFC difference between tBD, UD, and HC. *$P < 0.05$, uncorrected; **$P < 0.05$, FDR corrected.

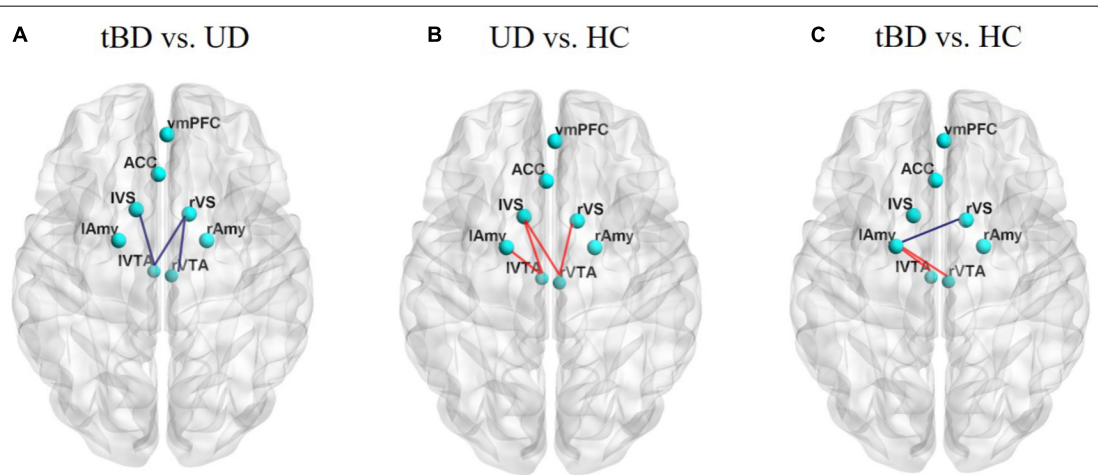

FIGURE 2 | (A) Showed rsFC differences between tBD and UD. **(B)** Showed rsFC differences between UD and HC. **(C)** Showed rsFC differences between tBD and HC. The tBD showed significant lower rsFC between the left VTA and left VS ($P = 0.001$, $P < 0.05$ with FDR correction). Other rsFC differences were not significant ($P < 0.05$, uncorrected). rsFC presenting lower (blue) or higher (red). Superior view of a 3D brain. tBD, transformed bipolar disorder; UD, unipolar depression; mOFC, medial orbitofrontal cortex; ACC, anterior cingulate cortex; VS, ventral striatum; VTA, ventral tegmental area; Amy, amygdala.

Unfortunately, these rsFC difference did not survive after FDR correction (**Figures 1**, **2**).

DISCUSSION

This longitudinal study directly compared baseline rsFC within the reward circuit between tBD and UD. Consistent with our hypothesis, results showed that BD (in the depressive state before suffering from any mania/hypomania episodes) differed from UD in rsFC between the left VTA and the left VS. This result was not confounded by soft bipolar indications encompassing family history of affective disorder, refractory bipolar, or suicide attempt. The rsFC difference accounted for accurate differentiation between bipolar and UD in ROC analysis, which may contribute to the early distinction between the two affective disorders in depressive states.

Our main finding was that tBD showed lower VTA-VS rsFC compared with UD. The VTA and NAcc (main part of the VS) are key mesolimbic nodes in the reward circuit (Haber and Knutson, 2010), which are connected by the medial forebrain bundle (Keller et al., 2013). Dopamine projections from the VTA (site of dopamine neurons) to the NAcc represent the primary pathway in the reward circuit (Padoa-Schioppa and Cai, 2011;

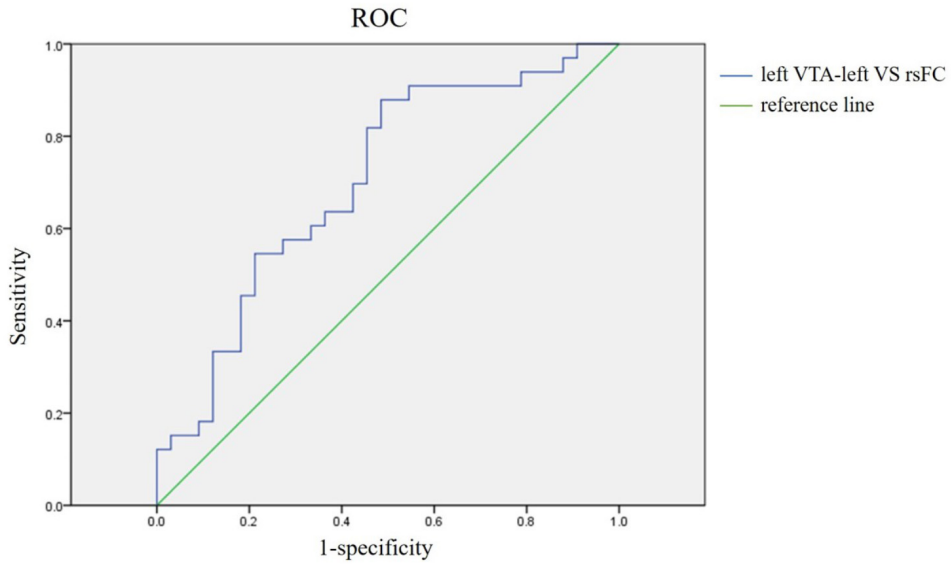

FIGURE 3 | Receiver operating characteristic analysis of the left VTA-left VS rsFC. Area under curve (AUC) representing the sensitivity to the discriminant tBD and UD, AUC = 70%. Sensitivity = 87.9%, Specificity = 51.5%, odds ratio = 7.703, $P = 0.005$.

Russo and Nestler, 2013). Important aspects of reward processing are coded by dopaminergic neurons arising from the VTA and projecting to the ventral striatum (VS) via the mesolimbic pathway. The VTA–VS dopamine system has been found to be of eminent importance in a variety of motivated behaviors and cognition (Camara et al., 2009). VTA dopamine signals are suggested to modulate blood oxygenation level dependent (BOLD) signaling in the NAcc (Knutson and Gibbs, 2007), and is known to be crucial for reward processing (Padoa-Schioppa and Cai, 2011). Additionally, the VTA and the VS (NAcc) receive a multitude of afferents from cortical areas (medial prefrontal cortex, mOFC, dorsal ACC), limbic regions (Amy, hippocampus) and other brain regions implicated in reward processing (Camara et al., 2009; Russo and Nestler, 2013; Yetnikoff et al., 2014). Aberrant VS rsFC was suggested to reflect distributed striatal integration of coalescing signals from an impaired reward circuit (Pan et al., 2017). In our present study, resting-state functional synchronicity was different between patients in the prodromal phase of BD (tBD) and UD, but this difference was limited to the left VTA and left VS (center of the reward circuit). Meanwhile, no functional synchronicity differences were found in the rest of the reward circuit. This could explain why symptoms in depressive episode of tBD and UD were similar. On the other hand, reduced rsFC of the VTA–VS could be reflective of an impaired dopamine signaling system. Therefore, lower rsFC of VTA-VS may indicate that depression related to BD may be more severe than that of UD. The rsFC differences between the left VTA and the left VS in our study possibly indicate divergent dysfunction in the reward circuit.

Numerous task-related fMRI studies have verified reward circuit dysfunction of hyperactivation (or hyperconnectivity) in BD (Nusslock et al., 2012; Schreiter et al., 2016) and hypoactivation (or hypoconnectivity) in UD (Ubl et al., 2015; Gong et al., 2017), respectively. The only study directly investigating rsFC within the reward circuit between BD and UD, to our knowledge, reported higher functional connectivity at resting state within the reward system, including the VS, VTA, anterior insula and thalamus in BD compared with UD (Satterthwaite et al., 2015). These are contrary to our present results of lower VTA-VS rsFC. Such a discrepancy could due to the fact that patients labeled as BD in Satterthwaite's study already experienced mania/hypomania before fMRI scanning, unlike our prodromal "bipolar" depressive ones. Similarly, the abovementioned study failed to find any significant differences of rsFC within the reward circuit between BD and HC as we did in the present study. Although speculative, it is possible that functional synchronicity of the reward circuit at rest is normal in depressive episodes, but already different between patients in the prodromal phase of BD and those with UD. As diagnosis of BD is precisely established at the onset of mania, the reward circuit is impaired severely enough and overreacts to reward stimuli during tasks (Alloy et al., 2015). There is lower functional synchronicity within the reward circuit at rest before mania but higher during tasks in BD vs. UD; such low-to-high fluctuation prompts more serious impairments in BD, which could be supported by the evidence of reward hypersensitivity in BD and hyposensitivity in UD (Alloy et al., 2016).

Notably, differences in rsFC within the reward circuit were only demonstrated between the VTA and the VS, which could be explained by several reasons. On one hand, the VTA-VS system, as an essential pathway in the reward circuit (Russo and Nestler, 2013), might be the first or optimal feature for the early distinction of bipolar from unipolar. On the other hand, the current methodology of fMRI rsFC is useful but maybe not powerful enough to detect other identification, which will be achieved by future developments in neuroimaging.

Limitation

Some limitations should be considered. First, UD patients enrolled in this study still have the possibility to manifest BD in the future (Ratheesh et al., 2017). In light of this point, grouping is not absolutely correct. To reduce this potential impact, the diagnoses of MDD in the UD group were confirmed by a follow-up lasting of no less than 3 years. Such a follow-up design and the transition rate (8.6∼25%) (Holma et al., 2008; Gilman et al., 2012; Woo et al., 2015; Bukh et al., 2016; Holmskov et al., 2017; Ratheesh et al., 2017) limited the sample size of UD and tBD, respectively. Secondly, an additional resting-state fMRI scan at the end of the follow-up, especially for tBD, may replicate previously consistent findings (reward hyperactivity in BD and hypoactivity in UD) (Alloy et al., 2016), which would make our conclusion more reliable. Although in the absence of such additional data due to retrospective design, our findings did expand the knowledge of BD in the prodromal stage. Lastly, the measure of functional connectivity fails to illustrate the direction of abnormal brain interaction due to the relatively low time resolution of rs-fMRI data (common in most other rs-fMRI studies). Developing more advanced neuroimaging techniques may help overcome this disadvantage in future.

CONCLUSION

In conclusion, the present study verified the hypothesis that bipolar disorder, in its prodromal stage of mania/hypomania, differs from UD in the reward circuit of VTA-VS functional synchronicity during resting-state, which exhibited good accuracy for early distinction between the two mood disorders. Our findings, together with the previous reward hypersensitivity theory, might indirectly indicate more severe impairment of the reward circuit in bipolar disorder.

AUTHOR CONTRIBUTIONS

ZY and QL designed the experiments. JS and JG performed the experiments. JS wrote the manuscript. JG, RY, XL, and RZ contributed to clinical data collection and assessment. JG, XW, JS, MX, and KB analyzed the results. ZY, QL, and JS approved the final manuscript. All authors assisted with carrying out the experiments.

FUNDING

This work was supported by the National Natural Science Foundation of China (Grants Nos. 81571639, 61372032, and 81871066), Jiangsu Provincial Medical Innovation Team of the Project of Invigorating Health Care through Science, Technology and Education (CXTDC2016004), Jiangsu Provincial key research and development program (BE2018609), the National High Technology Research and Development Program (863 Program) of China (Program No. 2015AA020509), the Young Medical Talents Foundation of Jiangsu Province, China (Program No. QNRC2016050), and Nanjing Science and Technology Development Project, Jiangsu Province, China (Program No. YKK15110). The funding organizations played no role in the study design, data collection and analysis, or manuscript approval.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2018.02586/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

ORIGINAL RESEARCH
published: 10 January 2019
doi: 10.3389/fpsyg.2018.02711

Electrophysiological Characteristics in Depressive Personality Disorder: An Event-Related Potential Study

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This study aimed to investigate the neurophysiological characteristics of young people with depressive personality disorder using event-related potentials (ERP). To explore the effects of visual-emotional words on ERP, mainly N350, we recruited 19 individuals with a depressive personality disorder and 10 healthy controls. ERP were recorded while the subjects took decisions on target words that were classified into three categories: emotionally positive, negative, and neutral. The ERP signals were then separately averaged according to the subjects' classifications. Data analysis showed that the amplitude of N350 was larger in response to positive and negative words than to neutral words. The latency of N350 was longer in negative words, in contrast with positive and neutral words. However, no difference was found between the two groups. These results suggest that neurophysiological characteristics of young people with a depressive personality disorder in visual-emotional word processing have not yet been influenced by their personality traits. To some extent, N350 reflected semantic processes and was not sensitive to participants' mood state.

Keywords: depressive personality disorder, event related potential, N350, emotion, word classification

OPEN ACCESS

Edited by:
Fang Pan,
Shandong University, China

Reviewed by:
Wenhai Zhang,
Hengyang Normal University, China
Daxing Wu,
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Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *08 July 2018*
Accepted: *17 December 2018*
Published: *10 January 2019*

Citation:
Yu H-H, Gu S-m, Yao F-M, Wang Z-R and Fu W-Q (2019) Electrophysiological Characteristics in Depressive Personality Disorder: An Event-Related Potential Study. Front. Psychol. 9:2711. doi: 10.3389/fpsyg.2018.02711

INTRODUCTION

A great deal of evidence has accumulated in clinical neurophysiology concerning cognitive functions in depressive patients (Mao et al., 2005; Krompinger and Simons, 2011; Dai and Feng, 2012; Zhao et al., 2015; Kiang et al., 2017; Xie et al., 2018). However, only a few studies have focused on the depressive personality disorder (DPS) in healthy young populations (Shimizu et al., 2006). College is an important period for individuals in their lifelong psychological development. Childhood negative or traumatic experiences, the pressure of adapting to new environments, together with the uncertainties of the future, exert many negative effects on the personality composition of college students, some of whom develop depressive personality. Therefore, the purpose of the present study is to explore the neurophysiological characteristics of young people with depressive personality disorder.

Similar to clinically depressed subjects, individuals with depressive personality traits are usually somber, restrained, and socially regressive (Noordhof et al., 2018). Since the application of event-related potentials (ERP) technology has been undertaken to explore neural physiological aspects of depression (MacNamara et al., 2016; Kiang et al., 2017), many ERP abnormalities have been reported to be related to depression. For example, it was found that depressive patients had smaller N400s than controls, specifically for negative adjectives, suggesting that depression is

associated with stronger-than-normal functional neural links between self-concept and negative characteristics (Kiang et al., 2017). A study aimed to investigate the intensity of evaluation of social stimuli in depression and showed that participants with depression had higher intensity scores for sad faces compared with the normal control group, longer reaction times for all faces compared with other groups, and higher P1 and P2 amplitude for sad faces compared with other faces. This finding suggested that the participants with depression were more receptive to negative facial expressions (Dai and Feng, 2012). MacNamara examined emotional processing abnormalities among 97 outpatients with generalized anxiety disorder (GAD) or major depressive disorder (MDD) using the late positive potential (LPP) and found that both diagnoses were associated with increased LPP. Both MDD and GAD were associated with an increased reaction time to targets that followed emotional pictures (MacNamara et al., 2016). Hui Xie examined the intentional forgetting of negative and neutral material in individuals with depressive tendencies. The results indicated that individuals with depressive tendencies had difficulties suppressing the memory encoding with negative words, while the suppression of memory encoding of neutral words was relatively intact. Furthermore, compared to individuals without depressive tendencies, depressive individuals had larger word-evoked P2 and late positive potential for negative items, as well as enhanced cue-evoked P1 and N2 for the negative items which were required to be forgotten (Xie et al., 2018).

According to the previous studies, N350 was identified as a phonological/lexical component (Bentin et al., 1999; Spironelli and Angrilli, 2009; Spironelli and Angrilli, 2015); moreover, other researchers found that negative components during 300–400 ms activated by emotional words significantly differed from those by neutral words (Kissler et al., 2006; Kiefer et al., 2007; Herbert et al., 2008; Fan et al., 2016). These results put forward a question concerning why there were differences in these ERP components between emotional and neutral words if they only manifested phonological/lexical processing. As we know, phonological units of words are associated with meanings; yet we can recognize phonological units as words without knowing exactly what they mean. Therefore, these 300–400 ms negative components should participate in at least part of the semantic task in visual word recognition. There was evidence for N400 to be sensitive to semantic deviations for stimuli with a semantic context (Kiefer, 2002; Briesemeister et al., 2014). However, to the best of our knowledge, studies exploring whether N350 will be affected by emotional content of words are rare. One of the goals of the present study was to bridge this gap.

Another reason why we examined N350 was that a number of studies suggested that both a word's emotional content and the participants' emotional state may affect the N400 ERP response (Chung et al., 1996; Federmeier et al., 2001; Kiefer, 2002). Yet, for the time being, very few studies have investigated the impact of the subjects' emotional state on the N350 component.

In the present study, 19 undergraduate students who met the diagnostic criteria of depressive personality disorder (DPS) and 10 healthy undergraduate students participated. Our goals included examining how visual-emotional words affected word recognition processing in a mild depressive state of young persons through recorded ERP, mainly the N350 component, and whether the emotional state of participants would have any effect on the ERP or N350.

MATERIALS AND METHODS

Subjects

Participants were drawn from an initial sample of 1999 undergraduate students from Grade One and Grade Two of Suzhou University. Fifty-three students with depressive personality traits screened by Personality Disorder Diagnosis Questionnaire, Fourth Edition (PDQ^{4+}) were further diagnosed by the Personality Disorder Interview (PDI-IV) semi-structured interview. Nineteen individuals finally diagnosed with depressive personality disorder took park in the study as the DPS group: (9 female, mean age 20.21 years). All 19 subjects did not take any antidepressants or other psychotropic drugs. Ten healthy undergraduates participated as the normal control group (6 female, mean age 19.70 years). With respect to exclusion criteria, participants in both groups should not have been diagnosed with psychiatric disorders including schizophrenia, affective disorder, bipolar disorder, intellectual disability, substance abuse, and dementia, as well as chronic medical disorders including endocrine disorders, cardiovascular disorders, and diseases related to the central nervous system. This study protocol conformed to the ethical guidelines of Suzhou University and was approved by the institutional ethics committee (the Ethic Committee of Suzhou University). Written informed consent was obtained from each participant.

Questionnaires

Personality Disorder Diagnosis Questionnaire, Fourth Edition (PDQ^{+4})

The PDQ^{+4}, designed by Dr. Hyler according to the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) diagnostic criteria for personality disorders, was used to assess whether an individual has personality disorders traits. Dr. Jian Yang translated it into Chinese in 1996. The validity and reliability of the questionnaire were tested by Yun-ping Yang in 2002. Research showed that the PDQ^{+4} is high in sensitivity and low in specificity and could be used as a personality disorder screening questionnaire (Yang et al., 2002).

Personality Disorder Interview (PDI-IV)

The PDI-IV, a semi-structured interview instrument, developed by Dr. Thomas A. Widiger according to the DSM-IV, was employed to diagnose the 12 personality disorders. The PDI-IV provided a set of 3–5 consistent questions for each diagnostic criterion of the 12 personality disorders. The interviewer can give more detailed explanations for each question to ensure that the subjects understand what the questions mean. The PDI-IV evaluation is in the form of a 3-point scale, that is, from 0 to 2, where "0" means that there is no corresponding symptom, "1"

is equal to the existence of the corresponding symptom, "2" is equal to the existence of a more serious or more corresponding symptoms. The original scale of the positive demarcation was 4–5 points, and the test takes about 20–30 min. The reliability between different interviewers was 0.34–0.89, with the mean being 0.74 (Yang et al., 2000).

Experimental Instruments and Stimuli

Electroencephalographs (EEGs) were recorded from 30 scalp locations over both hemispheres by a 64-channel electroencephalograph using 64 electrodes. The general experimental procedure, selection of stimuli, and stimulus presentation closely followed that of former studies (Blackburn et al., 1990; Dietrich et al., 2000; Yao et al., 2004). Two hundred Chinese mood words (each word containing two Chinese characters) were selected from the standardized Chinese mood words system, 40 of which were positive adjectives (i.e., industrious, modest, and dignified) as target stimuli accounting for 20% of the stimuli; 40 were negative adjectives (i.e., sad, gloomy, and angry) as target stimuli accounting for 20% of the stimuli; and 120 were neutral nouns (i.e., river, wood, and glass) as non-target stimuli accounting for 60% of the stimuli.

Procedure

Research Team Members Training

The team consisted of two clinical psychologists and two graduate students majoring in medical psychology. The one-week training included studying the instruments, and practice for the interview was held before the use of the PDQ^{+4} and PDI-IV to ensure consistency in comprehension and practice between the team members. The PDQ^{+4} was distributed among the undergraduate students and the positively screen individuals were interviewed using the PDI-IV to diagnose the personality disorders.

Stimuli and Tasks

The subjects were 19 undergraduate students, who met the depressive personality disorder diagnostic criteria and 10 normal students. Both groups were instructed to perform a target words classification task. The experimental procedure was programmed using E-prime. The 200 Chinese words were presented randomly on the computer screen. Each word was presented for 500 ms, with the SOA (stimulus onset asynchrony) being 1800 ms. The whole experiment consisted of 6 blocks. Block 1 was presented as a sample trial; Blocks 2–6 were then presented subsequently, each of which lasted 4 min. The subjects were instructed to stay awake with their eyes-opened and were tested in a quiet room (sound was attenuated and the temperature was controlled at 25°C). They were instructed to respond by pressing keys when they saw mood adjectives. In this experiment, odd-numbered subjects were requested to press the F key rapidly with the left forefinger as soon as they saw positive mood words, to press the J key with the right forefinger as soon as they saw negative words, and not to press any key when they saw neutral words. Even-numbered subjects were requested to press the opposite pattern of keys. The reaction time and accuracy of the subjects' words classification were recorded.

ERP Recording and Quantification

Electroencephalograms were recorded using bilateral mastoids as reference. Horizontal electro-oculograms (EOGs) were recorded differentially from the outer canthi of each eye and vertical EOGs from supra and infraorbital sites. Electrode cream was applied between the electrodes and scalp to maintain the impedances at 5k℧ or less. EEGs were recorded continuously and processed off-line overlaid. EOGs were corrected automatically using Neuroscan. Other artifacts were also fully rejected. Recorded EEGs were classified into three kinds of ERPs as positive, negative, and neutral words, and overlaid separately; the amplitude and peak latency were measured automatically. N350 was investigated in a time-window of 300–400 ms and was calculated as the mean amplitude (Bentin et al., 1999; Schendan and Kutas, 2007). We defined interest regions of Fz, F3, F4, Cz, and Pz to test the N350 component (Shen et al., 2013), and the average of the regions was calculated. The scalp distribution was presented in topography.

Statistics

A statistical analysis was conducted with *t*-test and chi-square for behavioral data. EEG data were analyzed with repeated measurement ANOVA, which included between-subjects factor of group (two levels: DPS and normal control) and two within-subjects factors: word types (three levels: positive, negative, and neutral) and electrode site (five levels: Fz, F3, F4, Cz, and Pz).

RESULTS

Comparison of the Two Groups in Word Classification

A *t*-test showed that the average response time in the DPS group (727.70 ± 327.63) was significantly prolonged as compared to that in the normal control group (681.53 ± 360.33) ($t = 3.077$, $p < 0.01$). A chi-square test showed that the average accuracy in the two groups was not significantly different ($\chi 2 = 0.514$, $p > 0.05$).

ERPs Evoked by Mood Words in DPS and Control Groups

Negative ERPs evoked by mood words in the two groups were named as N100 and N350 by latency period and components' serial number (**Figure 1**).

N350 Amplitude Analysis

Repeated measurement ANOVA computed on N350 amplitude showed a significant main effect of word type: $F(2, 26) = 29.599$, $p < 0.001$. Pairwise *t*-tests revealed larger N350 amplitude (i.e., more negative) for positive and negative words as compared to neutral words ($p < 0.001$). There were smaller N350 amplitudes for positive words as compared to negative words, but the difference was not significant. Concerning interactions, the three-way group by word type by electrode site interaction revealed no interactions between group, electrode site, and word type: $F(8, 20) = 0.604$, $p > 0.05$. The result is illustrated in **Figure 2**.

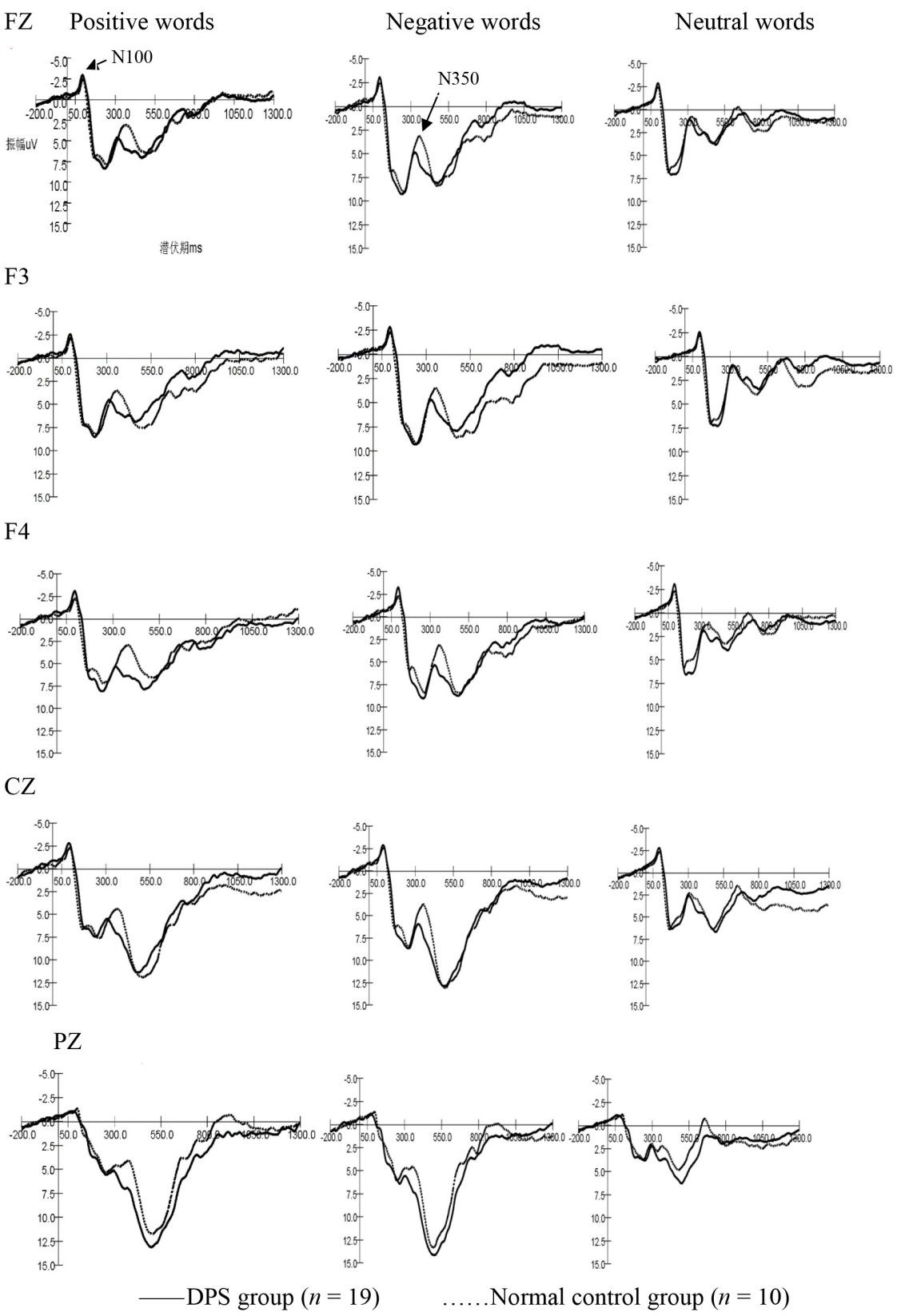

FIGURE 1 | The ERP waveforms evoked by three kinds of words in normal controls and the DPS group.

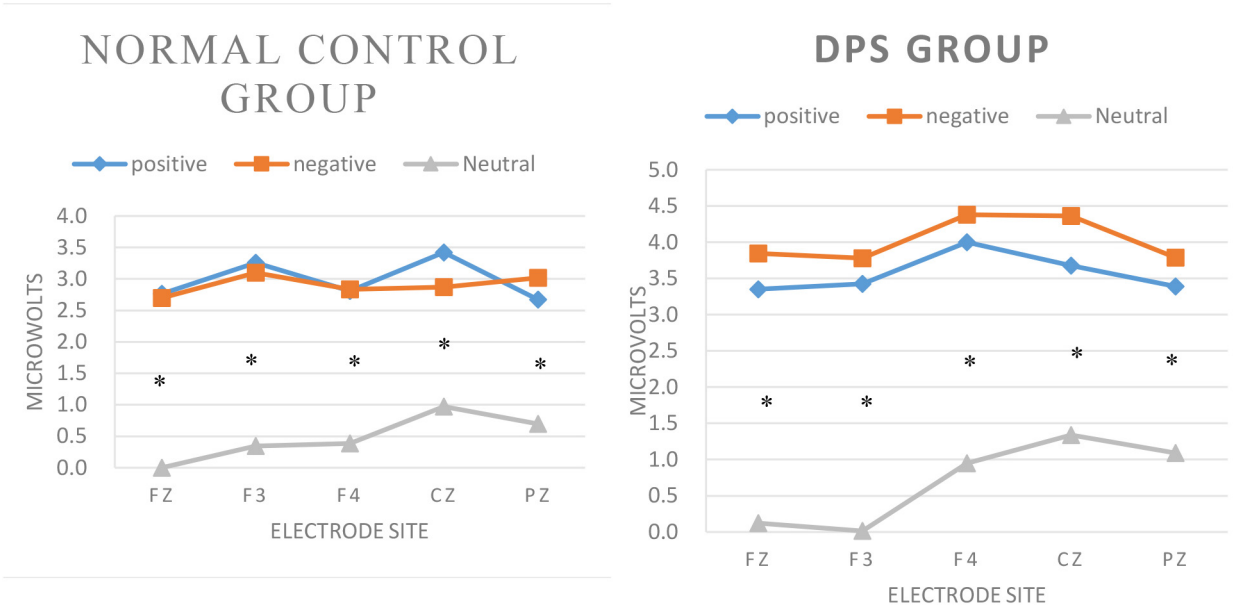

FIGURE 2 | Amplitude analysis: Significant three-way group by word type by electrode site interaction during word classification tasks. Asterisks: significant pairwise *t*-tests.

N350 Latency Analysis

Repeated measurement ANOVA computed on N350 latency showed significant main effects electrode site: $F(2, 26) = 4.485$, $p < 0.01$ and word type: $F = 3.603$, $p < 0.05$. The follow up pairwise *t*-tests revealed that Pz had shorter N350 latency than any other electrodes ($p < 0.01$). Negative words had longer latency than positive words ($p < 0.05$). Positive and neutral words, as well as negative and neutral words, did not differ. There were no interactions between the three factors: $F(8, 20) = 0.735$, $p > 0.05$. The pairwise comparisons are illustrated in **Figure 3**.

Brain Topography of ERP Components

The most obvious amplitude changes of N350 evoked by positive and negative words in normal controls were located in the frontal region (Fz), while those evoked by neutral words had no prominent changes (**Figure 4**). The obvious amplitude changes of N350 evoked by positive and negative words in the DPS group were mainly distributed in the right parietal region (P4) and the right occipital region (O3), and that evoked by neutral words had a similar distribution, but less in extent compared to normal controls.

DISCUSSION

The aim of this study was to investigate the influence of the emotional content of words on brain word recognition processing in non-medicated college students with a depressive personality disorder ($n = 19$) compared with a control group ($n = 10$) utilizing event-related brain potentials (ERPs). In a continuous word recognition paradigm, subjects were instructed to discriminate words that were classified into three different categories of emotional content (positive, negative, and neutral).

Behavioral data showed that the response time was longer in the DPS group than in the normal control group. This result was consistent with several earlier reports (Mao et al., 2005; Shimizu et al., 2006; MacNamara et al., 2016). There was evidence that even a mild depressive state might lead to cognitive impairment such as the inability to concentrate on a task, having difficulties in comprehensive reading, and slow response to stimuli (Rosenberg et al., 2013; Chan et al., 2018). Hence, the inefficient response performance might be due to cognitive impairment in depressive individuals.

The negative deflection around 400 ms after word presentation has been known to contribute to semantic processing in healthy individuals (Kiefer et al., 2007; Herbert et al., 2008). Previous studies reported that pathological responses to words occur at a similar latency after word presentation in depressive subjects. Hideki Shimizu investigated the relationship between the ERP response of emotive words and the depression score in 35 healthy subjects with both high and low Beck Depression Inventory scores and found that the high score group had a more enhanced N400 than that in the low score group. Moreover, the peak latency of the high-scoring group was significantly longer compared to the low-scoring group at P3, Cz, and Pz sites, while there was no difference in amplitude between positive and negative words (Shimizu et al., 2006). Another study found that patients with depression had smaller N400s than controls, specifically for negative adjectives (Kiang et al., 2017). In the present study, there was no difference in N350 between the DPS and normal control groups. As we know, several researchers found that N400 was sensitive to the participants' mood state in visual word processing (Chung et al., 1996;

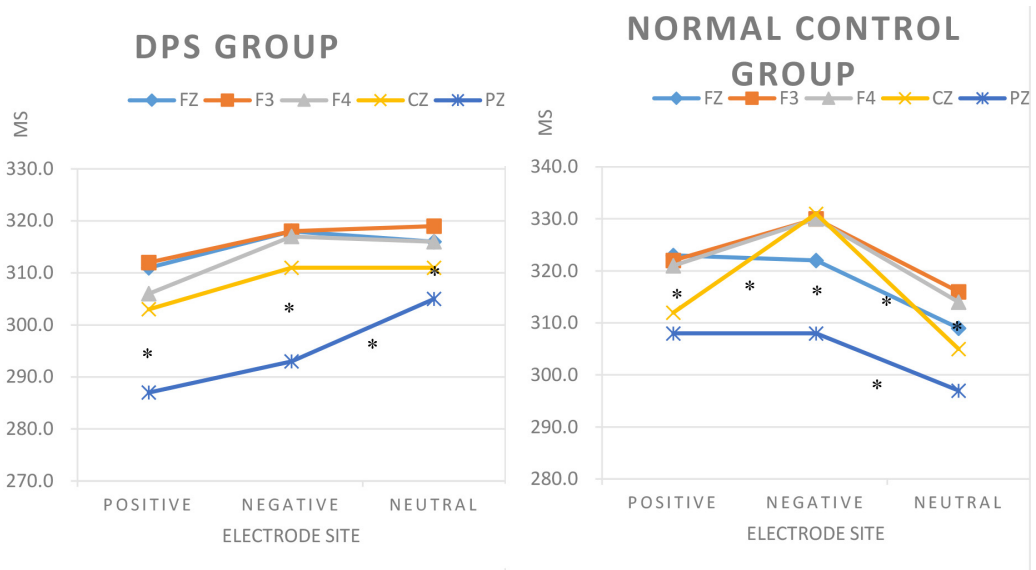

FIGURE 3 | Latency analysis: Significant three-way group by word type by electrode site interaction during word classification tasks. Asterisks: significant pairwise *t*-tests.

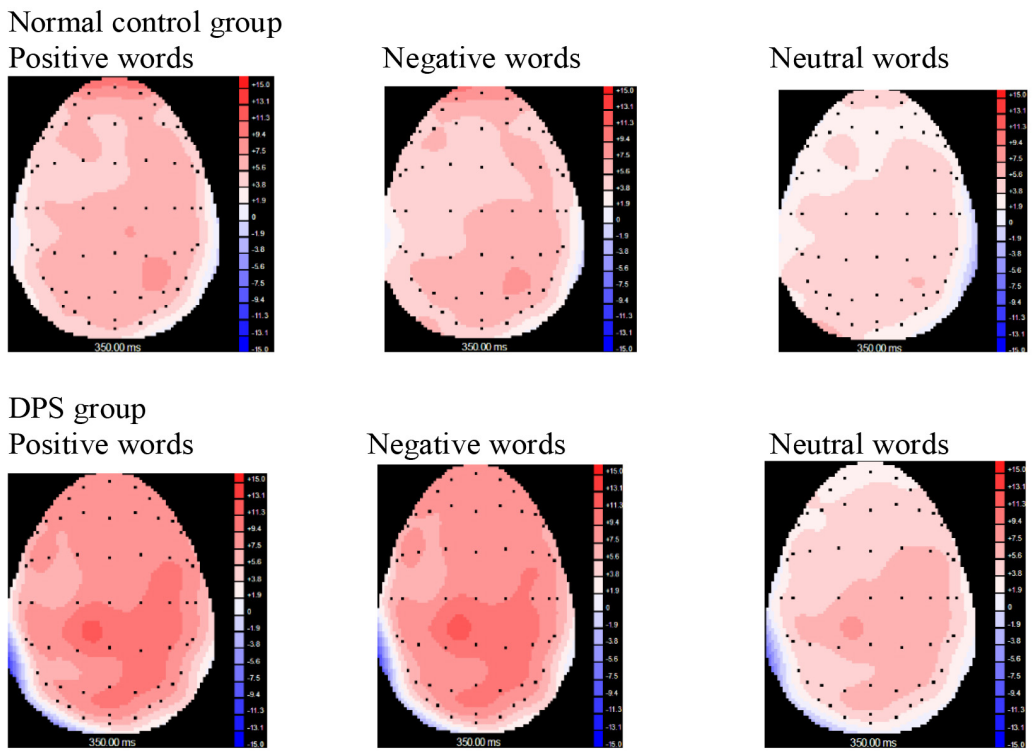

FIGURE 4 | Brain topography of ERPs (N350) evoked by word processing of the two groups.

Federmeier et al., 2001; Kiefer, 2002). Our findings might suggest that the individual's mood state cannot be reflected as early as 350 ms after the stimuli onset.

Although there was no difference between the two groups, the amplitude of N350 activated by emotional words was significantly larger than neutral words, while there was no significant difference between positive and negative words. According to the previous studies, N350 was identified as a phonological/lexical component (Bentin et al., 1999; Spironelli and Angrilli, 2009, 2015). However, the contradiction was that if

N350 only reflected the phonological process, minor differences should exist between emotional and neutral words because we can recognize phonological units as words without knowing exactly what they mean. Furthermore, different from letter-words in Latin languages, Chinese characters are pictographs, which imply little phonology. Therefore, this result implied that the neural system had already started the semantic processing at the peak latency of about 350 ms after stimulus onset.

This hypothesis was supported by other researchers. Johanna Kissler concluded in a review article concerning visual-emotional word processing with ERP that emotional word content can activate word processing at all stages from access to word meaning (around 200 ms), to contextual integration (around 400 ms), evaluation, and memory encoding (around 600 ms). Of importance is the fact that the interpretation of N400 has changed from an index of semantic access to a signature of the interaction between single-word semantics and context. Accordingly, an abundance of evidence demonstrates that some aspects of word meaning must be active before N400 is elicited (Kissler et al., 2006). The present study adds new evidence to this supposition.

Regarding how the amplitude of N350 is larger for emotional words than neutral words, one of the most plausible explanations is that a larger amplitude reflects more neurons activated in emotional word processing. Subcortical structures, most prominently the amygdala, have been implied in all stages of the emotional content-driven amplification process (Naccache et al., 2005; Almeida et al., 2014). An fMRI study found that both negative and positive words activated the amygdala, and negative word processing revealed a positive correlation between amygdala activity and scores of trait anxiety and subclinical depression. During negative versus neutral word reading, subjects with high trait anxiety also showed a stronger functional coupling between the left amygdala and left dorsolateral prefrontal cortex (DLPFC) (Laeger et al., 2012). Therefore, subcortical, primarily amygdala, activity may be a source of cortical-amplifying mechanisms in response to emotional stimuli visible in ERPs.

An N350 latency analysis showed that negative words had longer latency than positive words. Again, no significant difference was found between the two groups. So far, we have not found the N350 component reported in depressive subjects during word recognition. In Hideki Shimizu's study, for the N400 component in the positive/negative series, the peak latency of the high-scoring group was significantly longer compared to the low-scoring group at P3, P4, Cz, and Pz sites. Hideki considered that the large and delayed N400 component was due to the enhancement and continuation of semantic processing of emotive words in high-scoring subjects, relative to that in the low-scoring group. However, there were contrary results regarding the latency of ERP. Jiu Chen used a visual-emotional oddball paradigm to manipulate the processing of emotional information, while ERP was recorded in patients with major depression, and found that patients with recurrent major depression had longer N170 latencies when identifying happy and neutral faces, but shorter N170 latencies when identifying sad faces. With respect to this result, Jiu Chen suggested it might due to a negative bias of patients with recurrent major depression who were more aroused by sad faces than other emotions(Chen et al., 2014). The presumption for these controversial results was that participants in this and Hideki Shimizu's studies were all relatively healthy young people who had depressive personality traits instead of being patients with major depression. This might suggest that the persons with a depressive personality had not yet developed a negative bias, which plays an important role in the maintenance of depressive symptoms. Their longer latency to negative words might reflect the natural bias toward positive information in normal persons. There is evidence supporting this explanation. Herbert investigated the extent to which emotional connotation influences cortical potentials during reading and revealed that healthy subjects may have a natural bias toward pleasant information, facilitating late ERPs (N400, LPP) to pleasant adjectives as well as their superior recall (Herbert et al., 2008).

The lack of group differences in the current study probably lay on two reasons. First, the sample size of normal controls was relatively small, which could affect the explanation of our results. Second, all participants were college students whose social function was normal, though the DPS subjects met the diagnostic criteria of depressive personality disorder.

CONCLUSION

In sum, the current study demonstrated the effects of emotional word content on N350 cortical indices during a continuous word recognition paradigm in DPS and normal control groups. For the N350 component, our analysis was exploratory because, as yet, very few studies have investigated the impact of emotional content on this component. However, the effects of facilitated cortical processing in emotional words, reflected by larger N350 amplitudes for positive and negative words as opposed to neutral words and longer N350 latency for negative words compared to positive and neutral words, provide strong evidence for the notion that N350 also reflected semantic processes and was not sensitive to participants' mood state. No difference between the two groups might imply that neurophysiological characteristics of young people with a depressive personality disorder have not yet been influenced by their personality traits.

AUTHOR CONTRIBUTIONS

W-QF and Z-RW designed the experiments. H-HY, F-MY, and S-mG performed the experiments. H-HY and S-mG wrote the manuscript.

FUNDING

This work was supported by Beijing Municipal Science and Technology Project (Z181100001518005).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
published: 24 January 2019
doi: 10.3389/fpsyg.2019.00053

Inhibited Endogenous H_2S Generation and Excessive Autophagy in Hippocampus Contribute to Sleep Deprivation-Induced Cognitive Impairment

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Specialty section:
This article was submitted to Cognitive Science, a section of the journal Frontiers in Psychology

Received: 02 September 2018
Accepted: 09 January 2019
Published: 24 January 2019

Citation:
Yang S-Q, Jiang L, Lan F, Wei H-j, Xie M, Zou W, Zhang P, Wang C-Y, Xie Y-R and Tang X-Q (2019) Inhibited Endogenous H_2S Generation and Excessive Autophagy in Hippocampus Contribute to Sleep Deprivation-Induced Cognitive Impairment. Front. Psychol. 10:53. doi: 10.3389/fpsyg.2019.00053

Background and Aim: Sleep deprivation (SD) causes deficit of cognition, but the mechanisms remain to be fully established. Hydrogen sulfide (H_2S) plays an important role in the formation of cognition, while excessive and prolonged autophagy in hippocampus triggers cognitive disorder. In this work, we proposed that disturbances in hippocampal endogenous H_2S generation and autophagy might be involved in SD-induced cognitive impairment.

Methods: After treatment of adult male wistar rats with 72-h SD, the Y-maze test, object location test (OLT), novel object recognition test (NORT) and the Morris water maze (MWM) test were performed to determine the cognitive function. The autophagosome formation was observed with electron microscope. Generation of endogenous H_2S in the hippocampus of rats was detected using unisense H_2S microsensor method. The expressions of cystathionine-β-synthase (CBS), 3-mercaptopyruvate sulfurtransferase (3-MST), beclin-1, light chain LC3 II/LC3 I, and p62 in the hippocampus were assessed by western blotting.

Results: The Y-maze, OLT, NORT, and MWM test demonstrated that SD-exposed rats exhibited cognitive dysfunction. SD triggered the elevation of hippocampal autophagy as evidenced by enhancement of autophagosome, up-regulations of beclin-1 and LC3 II/LC3 I, and down-regulation of p62. Meanwhile, the generation of endogenous H_2S and the expressions of CBS and 3-MST (H_2S producing enzyme) in the hippocampus of SD-treated rats were reduced.

Conclusion: These results suggested that inhibition of endogenous H_2S generation and excessiveness of autophagy in hippocampus are involved in SD-induced cognitive impairment.

Keywords: autophagy, cognitive impairment, hydrogen sulfide, sleep deprivation, hippocampus

INTRODUCTION

Sleep plays a key role in human life and work, but sleep deprivation (SD), namely irregular and inadequate sleep, shows a rising trend in today's society (Feng et al., 2016; Honn et al., 2018). Therefore, the association between SD and cognitive impairment has been paid close attention (Zhang and Liu, 2008; Jin et al., 2017; Li S. et al., 2017). It is well known that SD affects human health and work efficiency (Honn et al., 2018) and that SD is a common state leading to a global cognitive decline for individuals (Jackson et al., 2013). Meanwhile, extensive studies confirmed that rats deprived of sleep is embodied in various morphological and neurobiological changes in the brain and a decline of cognitive behavior (Zhang L. et al., 2013; Zhao et al., 2014; Hajali et al., 2015; Kreutzmann et al., 2015). Recent studies showed that the oxidative stress and the disorder of neurotransmitters play important roles in the cognitive impairment induced by SD (Lu et al., 2018; Nabaee et al., 2018; Siddique et al., 2018). In addition, increasing evidence showed that SD impacts the epigenome that plays an important role in regulating learning and memory (Duan et al., 2016; Gaine et al., 2018). Although the research of cognitive impairment induced by SD has been reported, further exploring the mechanism underlying SD-induced cognitive impairment is necessary for a greater understanding of the pathophysiology about SD and its effects on cognition.

Hydrogen sulfide (H_2S) is a colorless gas with the smell of rotten eggs and was considered toxic in the past (Reiffenstein, 1992). In recent years, it has been confirmed that H_2S is an important neuroprotective and neuromodulatory agent (Bae et al., 2013; Zhang and Bian, 2014; Jiang et al., 2016). Endogenous H_2S is largely synthesized in mammalian tissues by cystathionine-β-synthase (CBS) in the brain and 3-mercaptopyruvate sulfurtransferase (3-MST) in the mitochondria (Kimura, 2011). It has been demonstrated that H_2S promotes the formation of long-term potentiation (LTP) (Chen et al., 2017), improves synaptic plasticity remodeling (Li Y. L. et al., 2017), and regulates learning and memory (Whiteman et al., 2011; Li M. et al., 2017; Tang et al., 2018; Zhan et al., 2018). Furthermore, our previous work has confirmed that inhibition of H_2S synthesis contributes to formaldehyde- and homocysteine-induced defects in learning and memory of rats (Tang X. Q. et al., 2013; Li M.H. et al., 2014). Therefore, we investigated whether the inhibition of hippocampal endogenous H_2S generation is responsible for SD-impaired learning and memory.

Autophagy is a catabolic process that digests the useless cytosolic components through invagination of its membrane in order to maintain cell homeostasis and integrity (Barthet and Ryan, 2018; Sharma et al., 2018). Studies demonstrated that the level of autophagy is regulated by a variety of factors, such as oxidative stress, energy balance or aging (Zhang et al., 2016; Loos et al., 2017). However, the change of autophagy level under conditions of SD remains unknown. Accumulating evidence suggests that the disorder of hippocampal autophagy plays crucial role in the formation of cognitive dysfunction in neurological diseases (Ghavami et al., 2014), including Parkinson's disease (PD) (Zhang S. et al., 2013) and Alzheimer's disease (AD) (Zheng et al., 2006; Sharma et al., 2018). Therefore, we speculated that excessive hippocampal autophagy is injurious to the cognitive function of SD-exposed rats.

The present work was to clarify the relationship between hippocampal endogenous H_2S generation as well as autophagy and SD-induced cognitive impairment. We demonstrated that exposure of SD impaired the function of cognition, suppressed the generation of hippocampal H_2S, and stimulated the excessiveness of hippocampal autophagy. These results suggest that inhibited endogenous H_2S generation and excessive autophagy in hippocampus play important role in SD-induced cognitive impairment.

MATERIALS AND METHODS

Reagents

Specific monoclonal anti-LC3, anti-Beclin1, and anti-p62 antibodies were obtained from Cell Signaling Technology, Inc. (Danvers, MA, United States). Specific monoclonal anti-CBS and anti-3-MST were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, United States).

Animals and Experiment Schedule

Adult male Wistar rats (weighing 250–280 g) were purchased from the SJA Lab Animal Center of Changsha (Changsha, China), were housed individually in a constant temperature (25 ± 2°C) and humidity controlled room, the rats were illuminated with artificial light for 12-h light/12-h dark cycle and access to food and water *ad libitum*. Experimental protocol of the study was approved by the Animal Use and Protection Committee of the University of South China. Rats were used according to "3Rs" principles (Replacement, Reduction and Refinement) in all experimental procedures. All efforts were made to minimize animal suffering.

All rats were habituated to the experimenter and laboratory for 1 week pre-handled prior to testing. SD group rats were exposed to the modified multiple platform method (MMPM) for 72 h. The Y-maze was performed one day after SD. The NORT was performed 2 days after Y-maze test. The OLT was performed 2 days after NORT. The MWM test was performed 2 days after OLT. One day after behavioral testing, the hippocampal tissues were collected for detecting the generation of H_2S and the level of autophagy.

Induction of Sleep Deprivation

The method of SD was adopted from the modified multiple platform method. Animals were exposed to the modified multiple platform method (MMPM) for 72 h. In brief, rats were placed on platforms (19 platforms; 6.5 cm in diameter, 15 cm apart edge-to-edge) surrounded by water (24 ± 1°C) which were located 1 cm below the water surface in a water tank (170 cm × 70 cm × 50 cm) where water and food were accessible. The method was used to disturb the total sleep (especially REM sleep). During REM sleep, muscle atonia caused animals to fall into or touch the water and waken. Immediately after SD, animals were submitted to behavioral tasks.

Behavioral Testing

Y-Maze

The Y-maze apparatus is composed of 3 arms with identical dimensions (120°; 55 cm long × 16 cm wide × 20 cm high),was placed inside a room with dim illumination. The floor of the maze consists of sawdust to eliminate olfactory stimuli. Testing was always at the same time and performed in the same room to ensure environmental coincide. The rats prefer to explore a new arm of the maze rather than going back to a previous one. Briefly, each rat was inserted to the center of the Y-maze and allowed to explore freely the three arms during an 8-min session. A rat into an arm was considered valid if its body and tail completely entered the arm. The total number of arm entries and sequence were recorded with video and analyzed later on a computer. An alternation was identified as three consecutive entries in three different arms of the maze (i.e., 1, 2, 3, or 3, 2, 1, etc.). The alternation performance was calculated using the following equation: total alternation number/(total number of entries −2). At the same time, the total numbers of entries in the three arms were used to detecting the activity of rats.

Morris Water Maze

The Morris water maze system consists of water maze device, water maze image automatic collection and software analysis system. Pictures of mice swimming (analog signal) were collected via the camera was introduced to the computer for analog-to-digital conversion to get the relevant data through digital image analysis. This system is a classical program for evaluating the spatial learning and memory of rodents. Water maze device mainly consists of a circular pool containing water (diameter of 180 cm, high 60 cm) and a circular acrylic platform (12.5 cm diameter) placed 2 cm below the surface of the water during acquisition trials. The pool was divided into 4 quadrants, and platform was placed in the first quadrant (target quadrant). The video recorder was placed above the center of the pool, recording the experimental process and stored in a computer (Chengdu Technology and Market Corp, Chengdu, China). The rats were allowed four acquisition trials per day for 5 days conducted in a spaced fashion, and each rat was given a 120-s swim to find the platform. The swimming route and escape latencies to platform (s) were determined in each trial. Twenty-four hours after the last acquisition trial the rats were given a probe trail without the platform, and they were allowed a free 120-s swim to search for the pool. The start position for each animal was in contrast to the platform location and the platform quadrant was referred to as the target quadrant. The times of crossing former platform area and the proportionality of swimming time in target quadrant and Mid-ring were determined. In the visible-platform test, the platform was located 2 cm above the water surface. Swim speed was tested with a visible platform in the water maze to rule out the differences in performance could be owing to non-cognitive factors including stress (Lu et al., 2017) and depression (Yang et al., 2016).

Novel Object Recognition

The Novel object recognition test (NORT) surveys the exploration of familiar and novel objects, which is a part of recognition memory. The test consists of three stages: adaptation, training and testing. During the adaptation period of 2 days, animals were habituated to the opaque empty square box (50 cm × 50 cm × 60 cm) for 5 min each day. In the training stage, the rat was placed into the testing box for 5 min to explore two different objects on opposite sides of the arena, the total approach time for exploring each object were recorded by an experienced researcher blind to treatments. The following acts were considered as the exploration of the object: touching to the object with the head of animal, sniffing to the object and keeping the distance from nose of rat to the objects less than 2 cm. The apparatus include testing box and the objects were cleaned with 70% alcohol at the end of each experiment for every rat. During testing sessions, one familiar object and one novel object of similar size were placed into the same places as in the training phase, and the animals were permitted to explore for 5 min. Calculation of the object recognition memory by measuring the interest in the novel object in testing phase which is called recognition index (RI) was expressed as the time exploring on the novel objects divided by the total time spent in exploring both objects.

Object Location

The Object location test (OLT) following with the same rules on NORT and consist of three identical parts (adaptation, training, and testing). The difference between OLT and NORT is that the exploration time in measuring two same objects but one of them was placed in a new position, also the recognition index (RI) is similar to NORT was calculated as the time exploring on the objects placed in novel location divided by the total time spent in exploring both objects. It evaluates especially spatial memory and discrimination.

Transmission Electron Microscopy (TEM)

Transmission electron microscopy was used to assess the ultrastructural change of hippocampus sections. The volume of hippocampus fragments for electron microscopy were obtained not more than 1 mm × 1 mm × 1 mm and then rapid fixed with 2.5% glutaraldehyde in 0.1 M PBS solution at 4°C for 2–4 h. Afterward, they were washed three times for 15 min each with PBS and then fixed with 1% osmic acid for 2 h. After washed with PBS, tissues were dehydrated in a graded ethanol series, embedded in Epo × 812 overnight. Ultra-thin sections were cut at 60–80 nm thickness and double colored with uranyl acetate and lead citrate, which were subsequently observed under a 1230 type transmission electron microscope (Electron Co., Tokyo) and photographed.

Western Blotting Analysis

The hippocampal tissues were homogenized in extraction buffer (50 mM Tris, pH 7.4, 1% Triton X-100, 150 mM NaCl, 1% sodium deoxycholate, 1 mM NaF, 0.1% SDS, 2 mM Na3VO4, 1 mM PMSF) and then centrifuged at 12,000 rounds/min for 30 min at 4 °C. Protein concentrations were measured using a BCA Protein Assay Kit (Beyotime, Shanghai, China). Equivalent amounts of protein were run on SDS–PAGE (12% for LC3 and; 10% for CBS, 3-MST and Beclin1) and then transferred

to a PVDF membrane. The membrane was blocked using TBS-T buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.05% Tween-20) containing 5% non-fat milk at room temperature for 2 hr and then serially incubated with primary antibodies directed against CBS (1:2000), 3-MST (1:1000), LC3 (1:1000), Beclin1 (1:1000) and P62 (1:1000) overnight at 4°C. After washing with TBS-T three times for 10 min, respectively, the membrane was incubated with secondary antibody (1:5000) in blocking solution at room temperature for 2 h. The membrane was washed again for three times with TBS-T, the bound antibody was were visualized by autoradiographic films (Tanon-5600) and then tegrated optical density of the protein band from Western blot analyses was quantified using Image-J software. Each experiment was repeated at least three times.

Assay of H_2S Generation

To detect the generation of H_2S in hippocampal tissue of rat, unisense H_2S microsensor (a miniaturized amperometric sensor with guard electrode) (Model H_2S-MRCh, Unisense, Aarhus, Denmark) coupled to a unisense picoampere amplifier was used. Hippocampus was homogenized in 50 mmol/L ice-cold potassium phosphate buffer (pH 6.8). After BCA quantitative analysis, the reaction mixture was added to the reaction bottle. The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.4), L-cysteine (20 μl, 10 mM), pyridoxal 5′-phosphate (20 μl, 2 mM), 10% (w/v) tissue homogenate. Adding 1 mol/L NaOH to the central hole of the reaction bottle 0.5 mL, the reaction bottle is blown 20 s by N2 before sealing. Reaction was initiated by a thermostatic water bath for 90 min at 37°C. Then 50% (mass fraction) trichloroacetic acid was added into the reaction system. Finally, the reaction system was incubated at 37 C for 60 min to terminate the reaction. The concentration of H_2S in the solution was determined by the sensitive sulfur electrode method in the central hole. The rate of H_2S formation was calculated and expressed as nmol/(min × mg).

Statistical Analysis

Data are expressed as the mean ± SEM. The significance of the difference between two groups was analyzed by the independent samples *t*-test with SPSS 20 software (SPSS, Chicago, IL, United States). Statistical significance was indicated at $p < 0.05$.

RESULTS

SD Induces a Decrease in Alternation Performance in the Y-Maze Tested

Y-maze test was subjected to detect whether the cognitive function of SD-exposed rats is impaired. As shown in **Figure 1A**, SD-exposed rats showed a significant decline in the alternation performance compared to control group. However, the total arm entries did not change between SD-rats and control group (**Figure 1B**). These data indicated that SD could impair learning and memory process of rats.

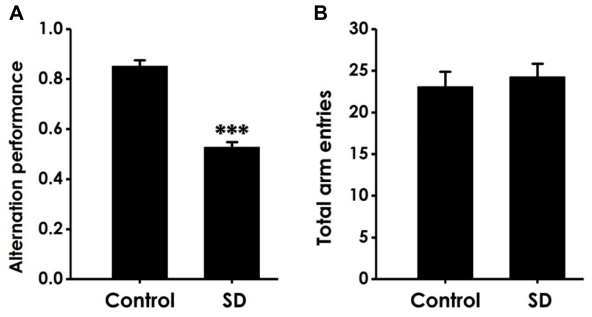

FIGURE 1 | Effect of SD on the alternation performance of rats in Y male test. After exposure of SD for 72 h, rats were tested in the Y male test. The alternation performance **(A)** and the total arm entries **(B)** were recorded. The data are expressed as mean ± SEM (n = 9–11); ***$P < 0.001$, vs. control group.

SD Impairs the Cognitive Function of Rats in Novel Object Recognition (NOR) Test

Next, we used the novel object recognition test to examine the altheration of cognitive function in SD-exposed rats. As shown in **Figure 2A**, the recognition index in SD-exposed rats was markedly decreased compared to control in the test period. However, SD-exposed rats did not change the total object exploration time in the training period (**Figure 2B**) and the test period (**Figure 2C**). These data also indicated that SD impairs the cognitive function of rats.

SD Causes Deficit in Location Memory in Object Location Test

We performed the object location test to extend our observation to a spatial form of cognition. SD did not affect the total object exploration time of rats in the training period (**Figure 3A**) and the test period (**Figure 3B**). However, compared to control groups, the recognition index in test period was significantly decreased in the rats treated with SD (**Figure 3C**), indicating that SD triggered deficit in object recognition memory.

SD Impairs Learning and Memory in the Morris Water Maze Test

To further explore the effect of SD on learning and memory in rats, we investigated the effects of SD on spatial learning and memory using the Morris water maze test. **Figure 4A** shows the representative swimming tracks of rats searching for the underwater platform on the first and fifth training days. On the first training day, there was no difference of the distance in searching for the hidden platform. On the fifth training day, SD-exposed rats exhibited a significant increase in the distance swam compared with the control group. Meanwhile, SD-treated rats exhibited significant higher escape latency on days 5 during training trials compared with control group rats (**Figure 4B**). These data further indicated that SD had an obvious negative effect on spatial learning of rats.

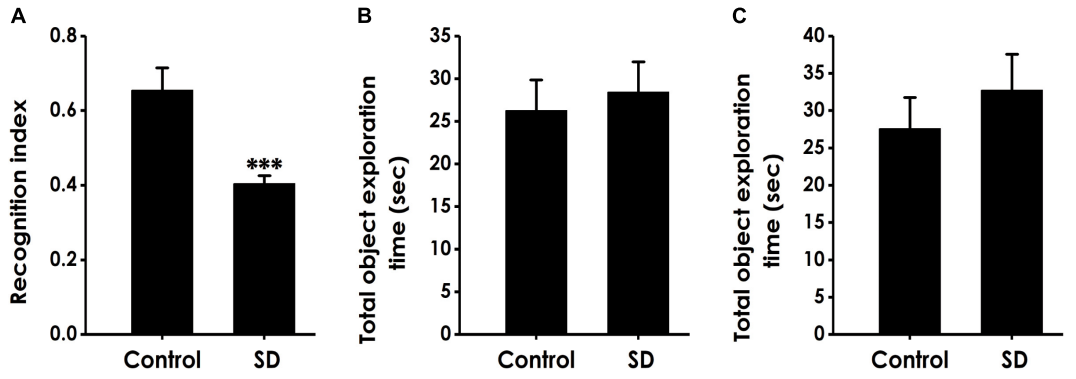

FIGURE 2 | Effect of SD on the object recognition memory of rats. Rats were tested in the novel object recognition test. The recognition index in the test period **(A)** and the total object exploration time in the training period **(B)** or in the test period **(C)** were recorded. Values are the mean ± SEM (n = 9–11); ***P < 0. 001, vs. control group.

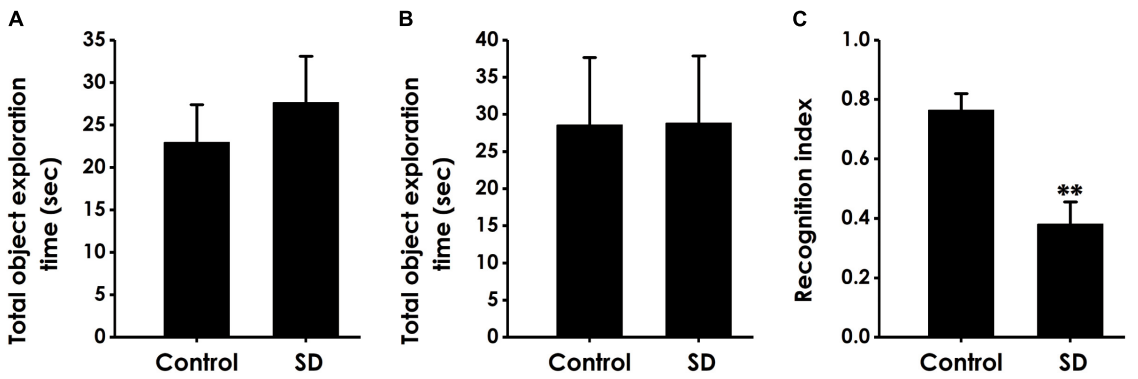

FIGURE 3 | Effect of SD on the spatial recognition memory of rats. Rats were tested in the object location test. The total object exploration in the training period **(A)** and the total object exploration **(B)** as well as the recognition index **(C)** in the test period were recorded. Values are the mean ± SEM (n = 9–11); **P < 0.01, vs. control group.

In the probe trial, the platform was removed and the rats were placed into the quadrant opposite to the target quadrant and allowed to swim freely for 120 s. Rats treated with SD exhibited significantly fewer number of crossing over the platform position (**Figure 5A**) and lower percentage of time in the target quadrant (**Figure 5B**) compared with control group, which indicated that SD impairs the spatial memory of rats.

Ruling Out the Influences of the Changes in Motor Ability and Vision on Learning and Memory in Rats

In order to exclude possible changes in visual acuity and motor ability, we tested the escape latency and the average swimming speed of rats in the visual platform test after we completed the probe test. There was no significant difference in the escape latency (**Figure 6A**) and the average swimming speed (**Figure 6B**) among all rats, which ruled out the possible that the alters in vision and motion contribute to the changes of all parameters in MWM experiment.

SD Causes Excessive Autophagy in Hippocampus of Rats

To explore whether excessive autophagy is involved in SD-induced impairment in cognition, the formation of autophagic vacuoles, as well as the expressions of LC3-II/LC3-I, Beclin-1 and P62 were investigated in the hippocampus of rat (Merenlender-Wagner et al., 2015). As shown in **Figure 7A**, SD-treated rats displayed increase in the formation of autophagic vacuoles. In addition, the ratio of LC3-II/LC3-I (**Figure 7B**) and the expression of Beclin-1 (**Figure 7C**) were significantly increased in the hippocampus of SD-exposed rats. While the expression of P62 was significantly down-regulated in the hippocampus of SD-treated rats (**Figure 7D**). These data indicated that SD exerts excessive autophagy in the hippocampus of rats.

SD Reduces the Expressions of 3-MST, CBS and the Generation of H_2S in the Hippocampus of Rats

To explore whether the inhibition of hippocampal H_2S generation involves in SD-induced impairment in cognition, the

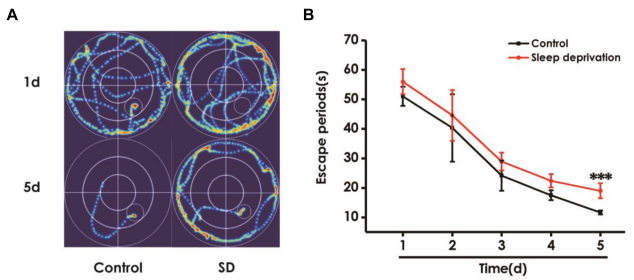

FIGURE 4 | Effect of SD on the spatial learning in the acquisition phase of Morris water maze task. Rats were tested in the Morris water maze task. **(A)** The representative swimming route of rats in 1st day and 5th day in the acquisition phase. **(B)** The escape periods of rats in 1–5 days in the acquisition phase. Values are the mean ± SEM (n = 9–11), $^{***}P < 0.001$, vs. control group.

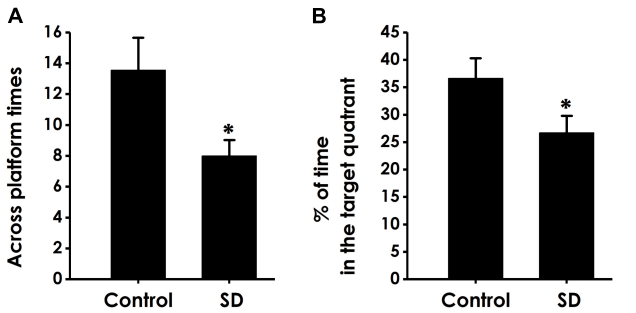

FIGURE 5 | Effects of SD on the spatial memory in the probe phase of Morris water maze task. After finishing the acquisition phase, rats were submitted to the probe trial. The times of crossing platform **(A)** and the proportionality of swimming time in target quadrant **(B)** was analyzed. Values are the mean ± SEM (n = 9–11), $^{*}P < 0.05$, vs. control group.

expressions of CBS and 3-MST as well as the endogenous H_2S generation in the hippocampus of rats were analyzed (Calvert et al., 2010). SD caused decrease in the expressions of CBS (**Figure 8A**) and 3-MST (**Figure 8B**) in the hippocampus of rats. Simultaneously, the endogenous H_2S generation in the hippocampus of rats was significantly inhibited by treatment with SD for 72 h (**Figure 8C**). These data demonstrated that SD reduces the generation of endogenous H_2S in the hippocampus of rats.

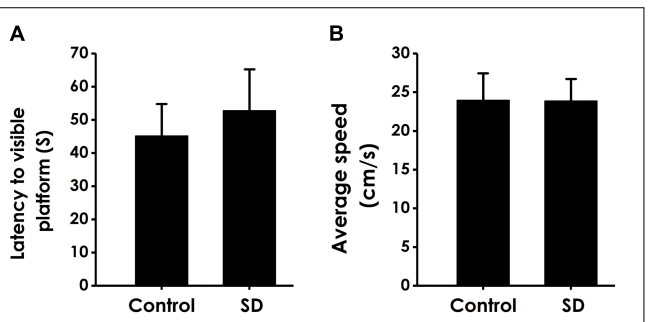

FIGURE 6 | Effects of SD on the vision and motion of rats. After finishing the probe test, rats were submitted to the visible platform test. The latency to reach the platform **(A)** and the average swimming speed **(B)** were recorded. Values are the mean ± SEM (n = 9–11).

DISCUSSION

Sleep deprivation (SD) is considered as a common social phenomenon and is a frequent cause of cognitive impairment (Miller, 2015; Feng et al., 2016; Hao et al., 2018). It is well established that H_2S regulates learning and memory (Nagpure and Bian, 2015; Zhuang et al., 2016; Tang et al., 2018) and that excessive autophagy in hippocampus causes cognitive impairment (Xu et al., 2016). Therefore, we explored the alterations in the hippocampal endogenous H_2S generation and autophagy and the change of cognitive function in SD-exposed rats. Our present work demonstrated that SD impaired the learning and memory of rats and caused the decrease in endogenous H_2S generation and the formation of excessive autophagy in the hippocampus of rats. These novel discoveries provide distinctive insights into understanding the mechanism underlying SD-induced cognitive impairment.

Exposure of SD has certain toxicity in nervous system of humans and animals (Novati et al., 2012; Basner et al., 2013). In agreement with this, sleep plays a key role in the removed of neurotoxic substances that produce in the waking state (Xie et al., 2013). Research has shown that SD impacts the expression and function of glutamate receptor (Ravassard et al., 2009) and neurogenesis (Meerlo et al., 2009) in the hippocampus. In addition, SD interferes with the hippocampal synaptic plasticity and hippocampal long-term potentiation (LTP), contributing to the deficit of cognition in animals and humans (Alkadhi et al., 2013). In our present study, SD-exposed rats showed cognitive impairment in Y maze test, OLT, NORT, and MWM test. Our results are consistent with the finding that exposure of rats with SD leads to the dysfunction of learning and memory (McDermott et al., 2006). Clinical studies show that SD leads to cognitive deficits (Olaithe et al., 2018). However, the molecular mechanisms underlying SD-induced cognitive impairment have not been fully clarified.

The biologic function of H_2S mainly include antioxidation, anti-apoptosis and anti-inflammation for central nervous system (Wei et al., 2014), which imply the protective effect of H_2S on neurodegenerative diseases. It has been reported that physiological concentration of H_2S specifically enhances the activity of N-methyl-D-aspartate receptor and facilitates the hippocampal synaptic plasticity and LTP (Zhang and Bian, 2014; Kamat et al., 2015). Previous work has implicated that exogenous H_2S attenuates diabetes-associated cognitive impairment (Tang et al., 2015; Ma et al., 2017) and has a protective effect from traumatic brain injury-induced cognitive impairment (Karimi et al., 2017; Ma et al., 2017). In addition, we have demonstrated that inhibition of H_2S generation mediates homocysteine-induced cognitive impairment (Li M.H. et al., 2014), which is

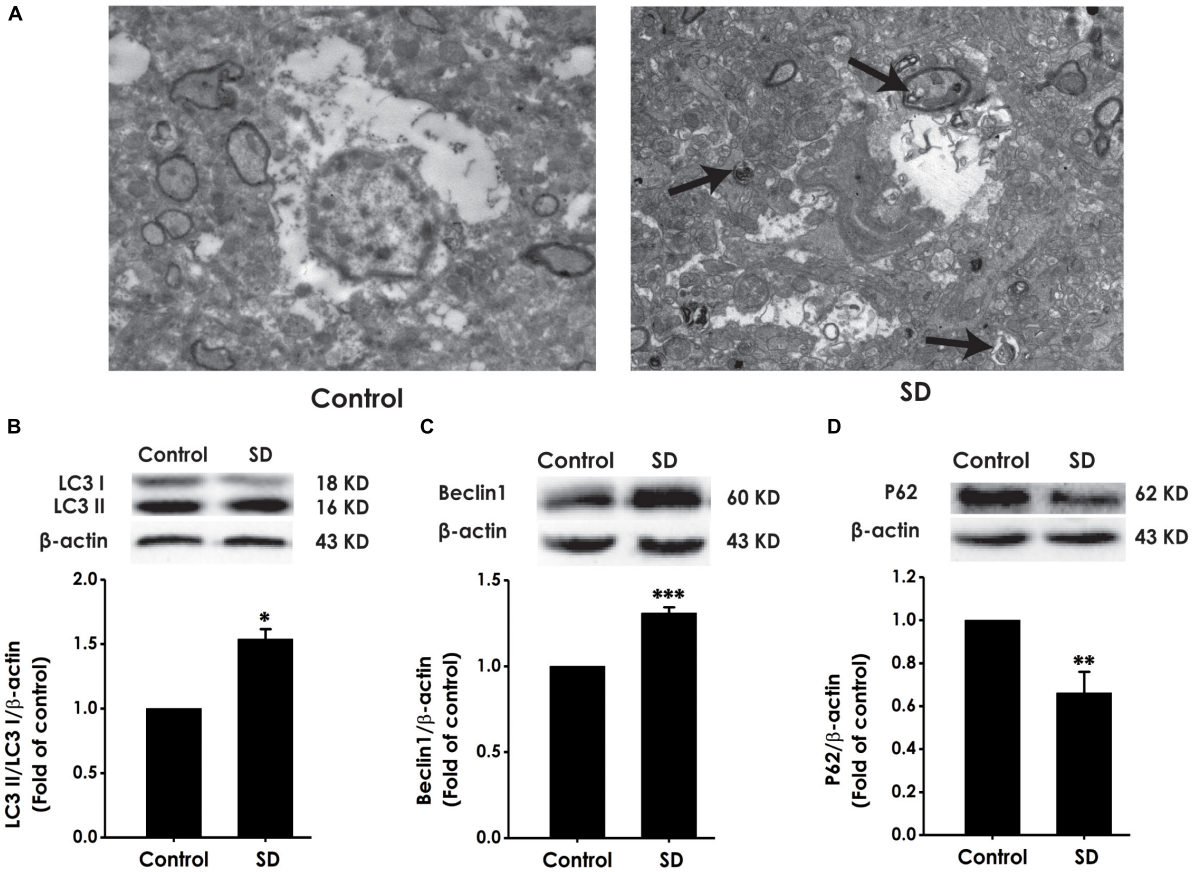

FIGURE 7 | Effects of SD on the autophagy in the hippocampus of rats. After 72 h exposure to SD, the rat's hippocampus was separated. Autophagic vacuoles **(A)** was observed under transmission electron microscope. Arrows indicate autolysosome-like vesicles in the cytoplasm. LC3-II/LC3-I **(B)**, Beclin-1 **(C)**, and P62 **(D)** expressions in the hippocampus of rats were detected by Western blot using anti-LC3, -Beclin-1, and -P62 antibody, respectively. β-actin was used as loading control. Data are reported as the mean ± SEM (n = 3–5); $^{*}P < 0.05$, $^{**}P < 0.0$, $^{***}P < 0.001$, vs. control group.

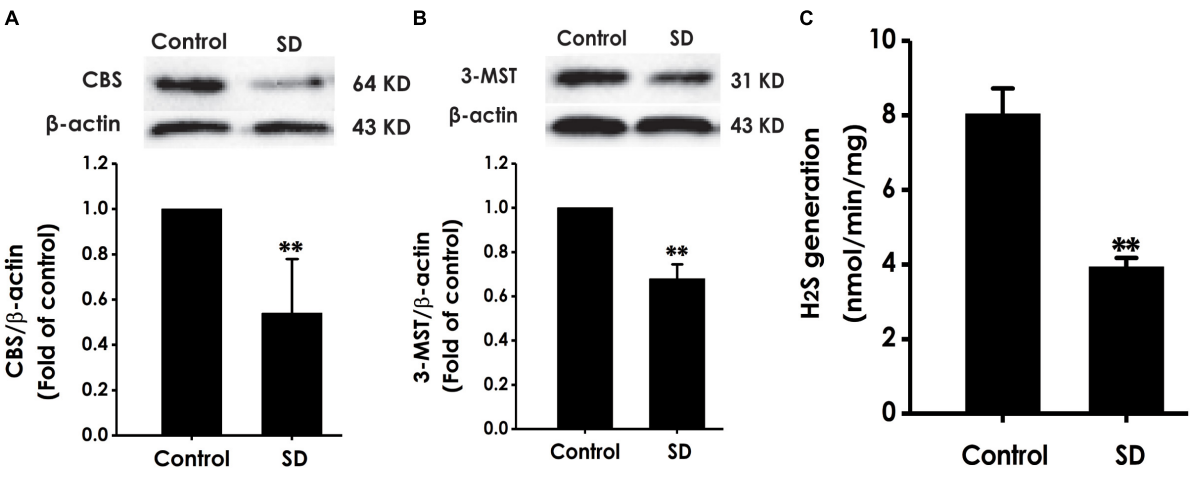

FIGURE 8 | Effects of SD on the expressions of CBS and 3-MST and the generation of H_2S in the hippocampus of rats. After 72 h SD treatment, the hippocampus of rats was homogenized. The expressions of CBS **(A)** and 3-MST **(B)** were measured by western blot analysis using anti-CBS and anti-3-MST antibody, respectively. β-actin was used as loading control. The generation of H_2S **(C)** was analyzed by unisense H_2S microsensor. Values are the mean ± SEM (n = 3–5); $^{**}P < 0.01$ vs. control group.

prevented by exogenous H_2S (Li M. et al., 2017; Tang et al., 2018). Thus, we speculate that inhibited hippocampal H_2S generation may be associated with the pathophysiology of SD-induced cognitive impairment in rats. In our present study, we demonstrated both the decreased expression of H_2S-synthesizing enzyme (CBS and 3-MT) and the inhibition of H_2S generation in the hippocampus of SD-exposed rats. Studies showed that the decreased activity of CBS mediates homocysteine- and formaldehyde-induced cognitive deficits (Tang X. Q. et al., 2013; Kumar et al., 2017). Likewise, the level of endogenous H_2S is decrease in the animal model and the patients of Alzhermer's disease (Liu et al., 2008; Liu H. et al., 2015; Karimi et al., 2017). Thus, it is reasonable to believe that inhibition of hippocampal H_2S generation contributes to SD-induced cognitive impairment.

Autophagy is essential for maintaining metabolic balance by digestion of misfolded proteins and dysfunctional organelles (Mizushima and Komatsu, 2011; Shehata and Inokuchi, 2014). However, it has been reported that excessive activation of autophagy damages synaptic plasticity in hippocampus (Hao et al., 2018) and that excessive activation of autophagy in hippocampus is responsible for cognitive impairment induced by hypoxic-ischemic brain injury (Xu et al., 2016) and sevoflurane (Li X. et al., 2017). Interestingly, inhibition of autophagy has a protective effect on cognitive impairment (Wang et al., 2015, 2017; Kong et al., 2018). So we speculated that excessive autophagy in hippocampus is inseparable from cognitive impairment of SD-exposed rats. Our present study found that the protein expressions of Beclin-1 and LC3-II, which are important for regulation of autophagy, were remarkably upregulated in the hippocampus of SD-exposed rats, while the expression of P62 was significantly downregulated. Meanwhile, the number of autophagosome was also increased in the hippocampus of SD-exposed rats. These data demonstrated that SD induces excessive autophagy in the hippocampus of rats. Interestingly, previous studies showed that the expression of LC3-II was increased in sevoflurane induced cognitive impairment, while p62 was decreased (Li X. et al., 2017), which is consistent with our results. Based on the evidence that excessive autophagy in hippocampus is closely associated with cognitive impairment (Xu et al., 2016; Li X. et al., 2017; Hao et al., 2018), our present results suggested that excessive hippocampal autophagy is an important pathological mechanism involved in the SD-induced cognitive impairment.

CONCLUSION

Taken together, the present study demonstrated that SD causes impairment in cognitive function, inhibition of hippocampal H_2S generation, and excessiveness in hippocampal autophagy. We suggested that SD-caused cognitive impairment may be due to the decreased endogenous H_2S generation and the excessive autophagy in hippocampus. Increasing studies have demonstrated that H_2S inhibits excessive autophagy (Shui et al., 2016; Jiang et al., 2017). Therefore, we suggested that the inhibition of endogenous hippocampal H_2S generation causes the formation of excessive autophagy in the hippocampus of SD-exposed rats. The limitation of this article is that we did not demonstrate whether cognitive impairment induced by SD is improved by the inhibitors of autophagy or exogenous H_2S and that whether other brain regions have the same pathological changes. In the future, we will explore the changes of H_2S generation and autophagy in other brain regions, investigate the protective actions of autophagy inhibitor and exogenous H_2S in SD-induced cognitive impairment, and detect the levels of H_2S in the clinical samples. Based on the neuroprotective role of H_2S, our findings opened a novel avenue that H_2S might be a potential agent for treatment of cognitive impairment induced by SD.

AUTHOR CONTRIBUTIONS

X-QT and WZ were responsible for the experimental design. X-QT supervised this study. S-QY, LJ, and FL analyzed the data. S-QY and LJ wrote the manuscript. S-QY, LJ, FL, H-jW, MX, PZ, C-YW, and Y-RX performed the experiments.

FUNDING

This study was supported by National Natural Science Foundation of China (81671057 and 81771178), the Major Research Topics of the Health and Family Planning Commission of Hunan Province (A2017020), and the Key Projects of the Education Department of Hunan Province (17A187).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

ORIGINAL RESEARCH
published: 27 February 2019
doi: 10.3389/fpsyg.2019.00302

Identification of Key Genes and Pathways in Post-traumatic Stress Disorder Using Microarray Analysis

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OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: 18 October 2018
Accepted: 30 January 2019
Published: 27 February 2019

Citation:
Bian Y, Yang L, Zhao M, Li Z, Xu Y, Zhou G, Li W and Zeng L (2019) Identification of Key Genes and Pathways in Post-traumatic Stress Disorder Using Microarray Analysis. Front. Psychol. 10:302. doi: 10.3389/fpsyg.2019.00302

Introduction: Post-traumatic stress disorder (PTSD) is characterized by impaired fear extinction, excessive anxiety, and depression. However, the potential pathogenesis and cause of PTSD are not fully understood. Hence, the purpose of this study was to identify key genes and pathway involved in PTSD and reveal underlying molecular mechanisms by using bioinformatics analysis.

Methods: The mRNA microarray expression profile dataset was retrieved and downloaded from the Gene Expression Omnibus (GEO) database. The differentially expressed genes (DEGs) were screened using GEO2R. Gene ontology (GO) was used for gene function annotations and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway was performed for enrichment analysis. Subsequently, protein–protein interaction (PPI) network and module analysis by the plugin MCODE were mapped by Cytoscape software. Finally, these key genes were verified in stress-exposed models by Real-Time quantitative (qRT-PCR). In addition, we performed text mining among the key genes and pathway with PTSD by using COREMINE.

Results: A total of 1004 DEGs were identified. Gene functional annotations and enrichment analysis indicated that the most associated pathway was closely related to the Wnt signaling pathway. Using PPI network and module analysis, we identified a group of "seed" genes. These genes were further verified by qRT-PCR. In addition, text mining indicated that the altered CYP1A2, SYT1, and NLGN1 affecting PTSD might work via the Wnt signaling pathway.

Conclusion: By using bioinformatics analysis, we identified a number of genes and relevant pathway which may represent key mechanisms associated with PTSD. However, these findings require verification in future experimental studies.

Keywords: PTSD, bioinformatics analysis, microarray analysis, key genes, key pathways

INTRODUCTION

Post-traumatic stress disorder (PTSD) is defined as affective trauma-related or stressor-related disorder exposed to single/episodic, direct/indirect, or acute/chronic events (Ronzoni et al., 2016; Kim et al., 2018). It manifests with a multitude of clinically significant symptoms including avoidance when re-living the traumatic event, disturbing when reminding the flashbacks and hyper vigilance often last for at least 1 month after the occurrence of the event (Dennis et al., 2016; Eustache et al., 2017).

More and more literatures on the psychopathological consequences of trauma exposure or life-threatening events have focused upon PTSD. The estimated prevalence of PTSD performed in six countries ranged from 3.1 to 61.6% according to the International Consortium to Predict PTSD (ICPP) project (Qi et al., 2018). In a recent meta-analysis of 27 studies including 30,878 ambulance personnel, a 11% prevalence rate of PTSD was found, appearing to be particularity at high risk (Petrie et al., 2018). Similary, in China, the prevalence was reported to range from 1.3 to 62.8% after 2008 *Wenchuan* earthquake (Hong and Efferth, 2016). PTSD can not only cause multisystem disorders with comorbidities both physically and mentally, but also it can lead to a number of negative social consequences such as suicide or violence tendencies. It has brought a significant personal and societal burden.

To date, various researches suggested that pathogenesis of PTSD was associated with autonomic nervous system (ANS), hypothalamic-pituitary-adrenal (HPA) axis, neural circuits and immune system. The underlying pathogenesis of PTSD remains incompletely unknown. Therefore, it is promoting the need to develop a further identifying the etiological factors, molecular mechanisms, and pathways of PTSD to discover novel diagnostic and treatment strategies for PTSD.

Fortunately, with the advances of sequencing and high-throughput DNA microarray analyses, numerous genes and pathways have been demonstrated to be correlated with the genesis and progression of PTSD. For example, Kilaru et al. (2016) found that Neuroligin 1 (NLGN1) might participate in synaptic plasticity, which further suggesting a significant association between Neuroligin 1 (NLGN1) and PTSD. Maheu and Ressler (2017) found that Wnt protein was related to fear- and stress-related disorder. Moreover, various genes, i.e., FK506 Binding Protein 5 (FKBP5) (Young et al., 2015), Dicer 1, Ribonuclease III (DICER1) (Wingo et al., 2015), and Dopamine D2 receptor (DRD2) (Duan et al., 2015) were reported to participate in cellular pathway of PTSD. Also, various gene pathways have been shown to be important, such as mTOR pathway (Oh et al., 2018), ERK pathway (Xiang et al., 2017), and Akt/GSK-3β signaling pathway (Chen et al., 2015), etc. Therefore, identifying differentially expressed genes (DEGs) and pathways, elucidating the interactions network among them, are essential for PTSD.

In this study, we retrieved dataset of mRNA expression microarrays from Gene Expression Omnibus (GEO), and identified a subset of genes as biomarkers in PTSD by using bioinformatics analysis. In addition, several candidate targets for following experimental research were performed. This finding can further help us understand underlying pathogenesis associated with PTSD, and provide initial evidence for future study on potential mechanisms of PTSD.

MATERIALS AND METHODS

Data Acquisition and DEGs Identification

The mRNA microarray expression profile dataset was retrieved and downloaded from the GEO database (available online: http://www.ncbi.nlm.nih.gov/geo). After screening, GSE68077 was obtained for our analysis. The platform for GSE68077 was GPL7202, Agilent-014868 Whole Mouse Genome Microarray 4x44K G4122F (Muhie et al., 2017). This dataset consists of 346 groups including brain transcriptome profiles in mouse model simulating features of PTSD and transcriptome profiling of spleen, blood, and hemi-brain of social stressed C57BL/6 mice exhibiting PTSD like features. The C57BL/6 mice were exposed to SJL aggressor mice for periods of 5 or 10 days (6 h each day) to induce anxiety/stress which parallels to PTSD in human. Organs, blood, and brain regions were collected after 1 day and 1.5 weeks following 5 days trauma-exposed, and 1 day and 6 weeks following 10 days trauma-exposed. In current study, the microarray data of hippocampus 6 weeks after 10 days social stressed was collected for analysis. DEGs were screened using GEO2R, an online analytical tool available in GEO. The $|\log FC| > 1$ and $P < 0.05$ were used as the cutoff values for significantly DEGs. Limma package in the Bioconductor package (available online: http://www.bioconductor.org/) was used for gene differential expression analysis.

Functional and Pathway Enrichment Analysis of DEGs

Gene ontology, a method for annotating genes, was performed to identify potential biological processes, i.e., biological processes (BP), cellular component (CC), and molecular function (MF). The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway was conducted for presenting the annotation and visualization of gene functions. In addition, both GO enrichment and KEGG pathway analysis were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID[1]) (Huang et al., 2007) to understand the biological significance of genes, when P-values <0.05 was considered as cutoff criterion.

Protein–Protein Interaction (PPI) Network Construction and Module Analysis

The Search Tool for the Retrieval of Interacting Genes (STRING[2]) (von et al., 2003), an online tool for annotation of protein cellular

[1]https://david.ncifcrf.gov/

[2]https://string-db.org/

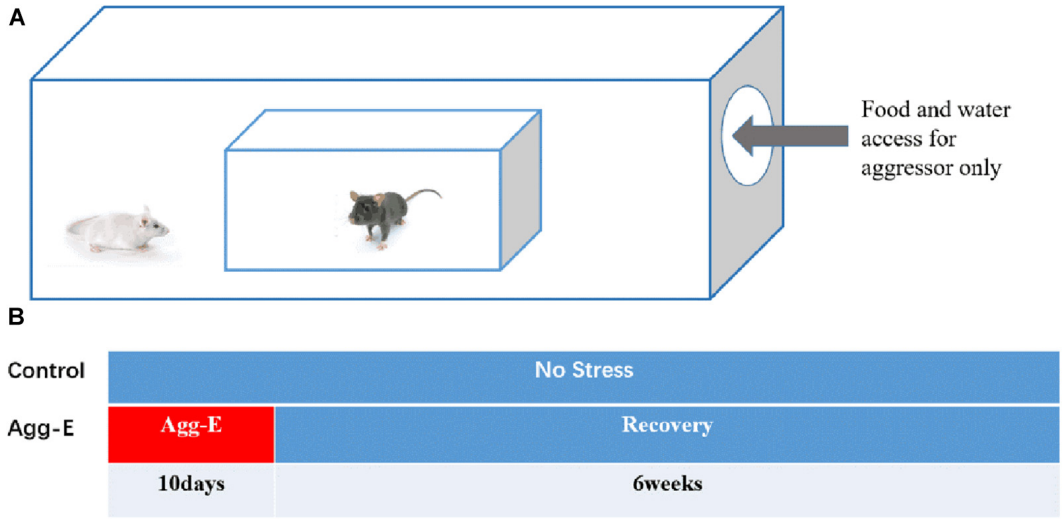

FIGURE 1 | (A) Cage-within-cage configuration for aggressor exposure. **(B)** The protocol timeline of single-housed home cage (10 consecutive days with aggressor exposure and 6 weeks rest).

TABLE 1 | Primers used for qRT-PCR.

Gene	Primer sequence (5′–3′)
GRM2-Forward	CTGTCTCTCTATCTCTCTGC
GRM2-Reverse	TGTGTGTGTGTAACATGATGG
CYP1A2-Forward	AGTACATCTCCTTAGCCCCAG
CYP1A2-Reverse	GGTCCGGGTGGATTCTTCAG
CDH5-Forward	CACTGCTTTGGGAGCCTTC
CDH5-Reverse	GGGGCAGCGATTCATTTTTCT
SF1-Forward	CATGGCAGCAAAGATCCCTC
SF1-Reverse	AAGTCCTCACTCTCATGGCTC
EDN3-Forward	CCCTGGTGAGAGGATTGTGTC
EDN3-Reverse	CCTTGTCCTTGTAAGTGAAGCAC
SYT1-Forward	CTGTCACCACTGTTGCGAC
SYT1-Reverse	GGCAATGGGATTTTATGCAGTTC
CAB39L-Forward	CAAAACGCAGCCTATCGTGGA
CAB39L-Reverse	CTCGTCGTCTGTCCTTTCTTTC
NLGN1-Forward	GGTACTTGGCTTCTTGAGCAC
NLGN1-Reverse	CTTGTTTGGGTATAAAGCCTCCA
GAPDH-Forward	AGGTCGGTGTGAACGGATTTG
GAPDH-Reverse	TGTAGACCATGTAGTTGAGGTCA

localization and biological function, was conducted to predict protein–protein interaction (PPI) information. DEGs were mapped to STRING to evaluate the interaction relationships, with a confidence score >0.9 defined as significant, and PPI integrated networks were visualized by Cytoscape software (Shannon et al., 2003). Then, the plug-in Molecular Complex Detection (MCODE) from Cytoscape was applied to screen the modules of PPI network. Finally, Text mining of gene function prediction was conducted by COREMINE[3] (de Leeuw et al., 2012).

[3]http://www.coremine.com/medical/

Experimental Animals

Aggressor Mice

Six 6-week-old male SJL albino mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The mice were initially weighted 30–35 g. And these mice were individually housed in the plexiglas cages (48 cm × 27 cm, height 20 cm) with free water and food in a 12-h light/dark cycle (lights on 6 AM to 6 PM) for 30 days. The temperature was controlled at 22 ± 2°C and relative humidity was kept at 30–60%. In the meantime, these mice were trained to induce aggressiveness due to isolation (Hammamieh et al., 2012).

Subject Mice

Twelve male C57BL/6N mice (8 weeks old weighing 18 ± 2 g) were provided by the Qinglongshan Experimental Animal Breeding Farm (Nanjing, China). Animal were randomly into two groups: control group and aggressor-exposed (Agg-E) group. Two groups were housed at room temperature 22 ± 2°C with standard condition of 12 h light and 12 h darkness. All mice had plenty food and water freely. The experiments were conducted under the approval of Laboratory Animal Management Committee of Nanjing University of Chinese Medicine (approval ID: 201810A043). All procedures were compliant with the Guidelines of Accommodation and Care for Animals formulated by the Chinese Convention for the protection of vertebrate animals used for experimental and other scientific purposes.

Aggressor Exposure

According to a modified "cage-within-cage resident-intruder" protocol (Porsolt et al., 1977), Agg-E mice were placed in a wire mesh cage that was kept inside the aggressor's large home cage for 6 h. The size of the above cages were 17 cm × 14 cm × 8 cm and 50 cm × 30 cm × 20 cm, respectively, as shown in **Figure 1A**. Similary, the control mice were placed in the same environment

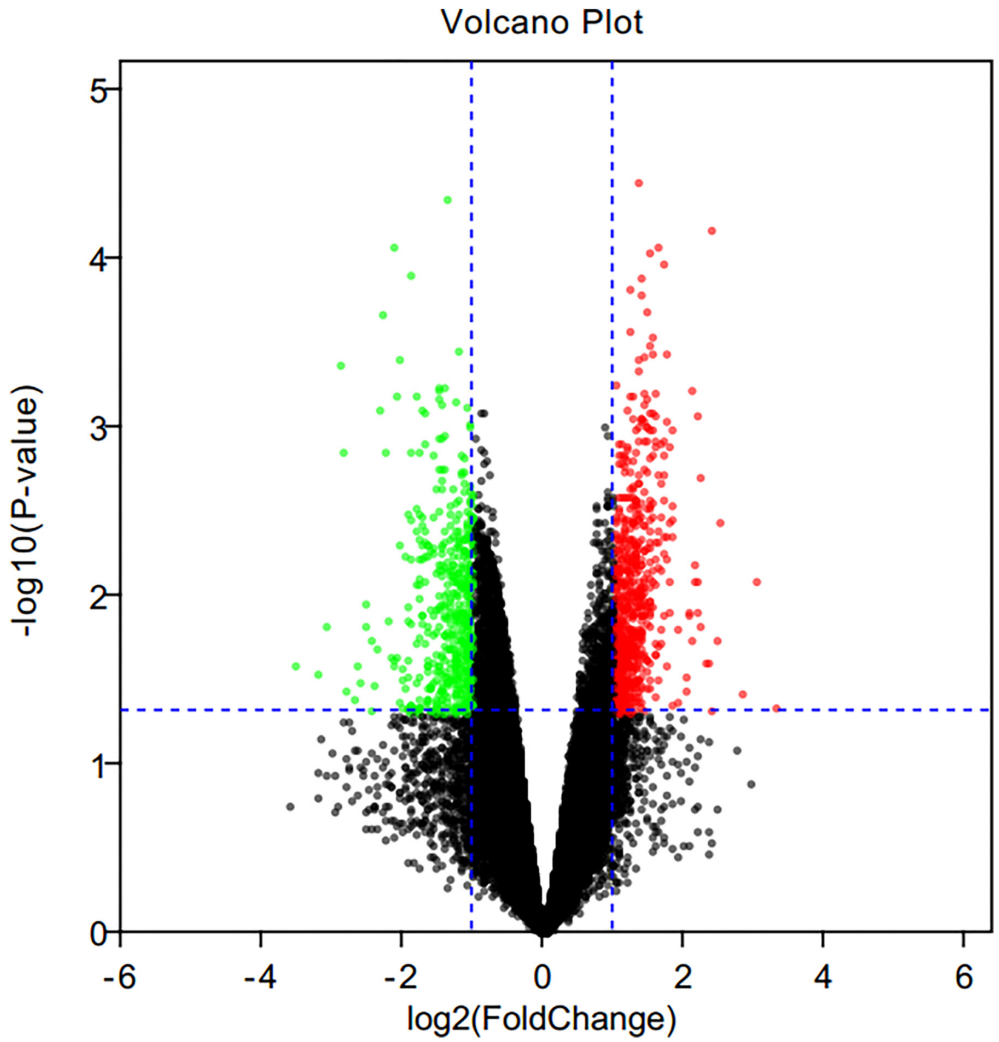

FIGURE 2 | Volcano plot of differential expression genes. Red points as up-regulated genes, green plots as down-regulated genes, and black plots as genes with no significant difference.

TABLE 2 | Gene ontology analysis of differentially expressed genes associated with PTSD.

Term	Function	Gene count	*P*-value
GO:0006874	Cellular calcium ion homeostasis	9	<0.01
GO:0051592	Response to calcium ion	8	<0.01
GO:0006958	Complement activation, classical pathway	7	<0.01
GO:0005886	Plasma membrane	237	<0.01
GO:0050710	Negative regulation of cytokine secretion	4	<0.01
GO:0016055	Wnt signaling pathway	15	0.01
GO:0016757	Transferase activity, transferring glycosyl groups	16	0.01
GO:0007155	Cell adhesion	29	0.03
GO:0030054	Cell junction	40	0.03
GO:0005509	Calcium ion binding	40	0.03

without being exposed by aggressor mice. During each 6 h session, the aggressor mice were given plentiful food and water, while the Agg-E mice and control mice were deprived of food and water. In addition, at one to three random times, Agg-E mice were exposed directly to the aggressor mouse for 1 min or until 10 bites. At the end of each session, the control and Agg-E mice were returned to their home cage where the food and water were plentiful. A total of 10 consecutive days were repeated. After 10 days with aggressor exposure, mice were housed about

TABLE 3 | Kyoto Encyclopedia of Genes and Genomes pathway analysis of differentially expressed genes associated with PTSD.

Term	Definition	Gene count	*P*-value
mmu04610	Complement and coagulation cascades	11	<0.01
mmu04512	ECM-receptor interaction	10	0.01
mmu04723	Retrograde endocannabinoid signaling	9	0.04

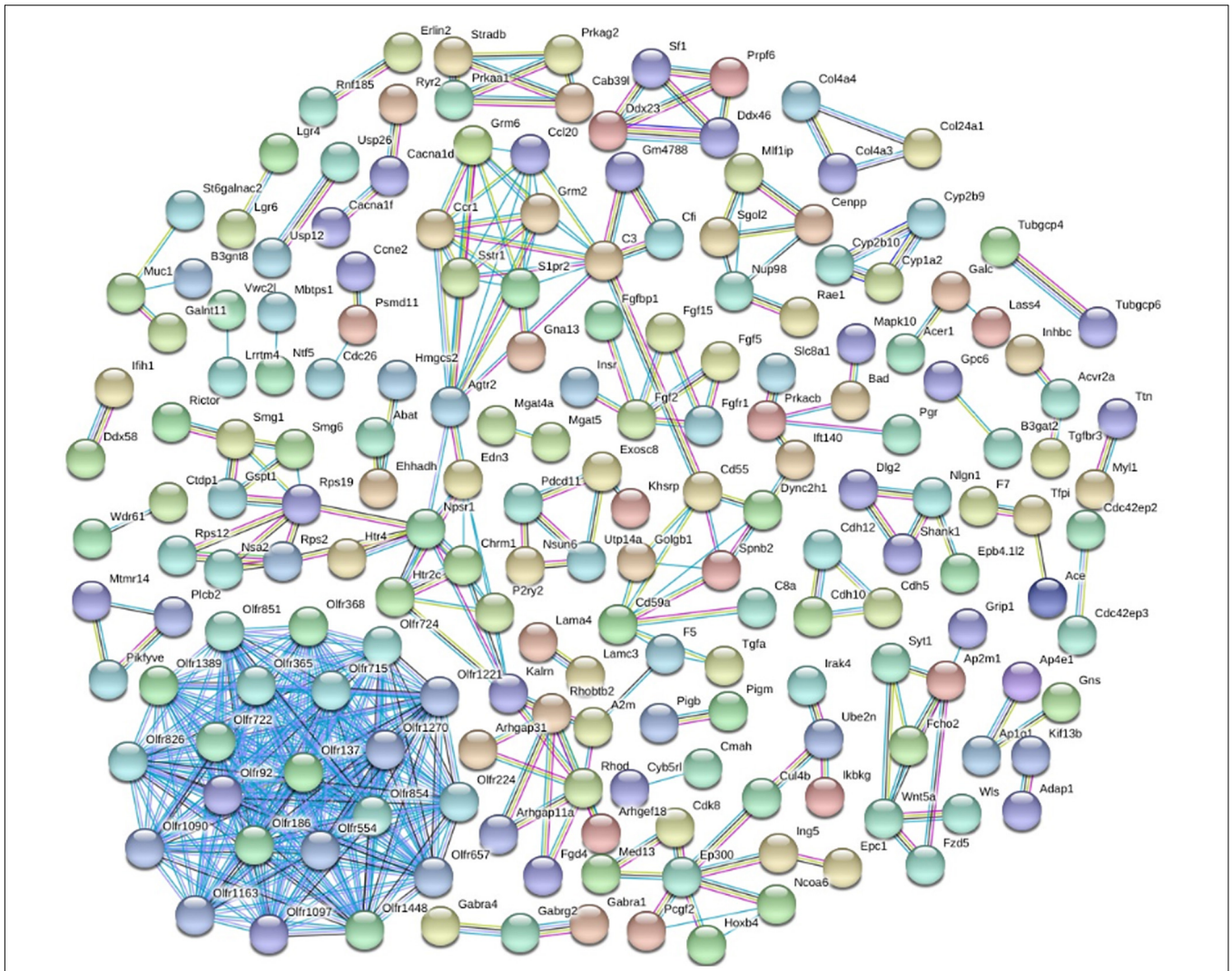

FIGURE 3 | The differential expressed protein–protein interaction network. Proteins were represented with color nodes, and interactions were presented with edges.

6 weeks with food and water *ad libitum*, as shown in the protocol timeline (**Figure 1B**).

Forced Swimming Test (FST), Tail Suspension Test (TST), and Open-Field Test (OFT)

Behavioral tests were performed with Smart3.0 tracking software (Panlab). The subject mice were randomly assigned to two groups (n = 6/group). After 10 days stressed exposure directly or indirectly, the Agg-E group was fed for 6 weeks for recovery. The forced swimming test (FST) and the tail suspension test (TST) were performed to identify the depression-like behavior of the mice in the Agg-E group. Both FST and TST were performed according to previous studies (Porsolt et al., 1977; Steru et al., 1985). Mice were forced to swim in 20 cm water temperature 25 ± 1°C in 2000 ml glass beaker for 5 min. Immobility time in the last 4 min were measured in the FST test. After FST test, mice were allowed to have a rest for 24 h and then hanged for 6 min. Immobility time in the last 4 min were recorded in the TST test. The open-field test (OFT) is used to evaluate the state of autonomic movement, aiming to identify agitation and pathological behavior. The device is a square bucket with a bottom. The bottom surface is divided into 25 small squares. The movement of each mouse was recorded for 5 min by a video camera.

RNA Extraction and qRT-PCR

Hippocampus tissue of both control and Agg-E group were extracted total RNA using TRIzol reagent (Invitrogen), following the manufacturer's instructions. RNA quality and quantity were measured by Nucleic Acid Protein Detector. cDNA was synthesized with total RNA using the First Strand cDNA Synthesis Kit (TaKaRa). The relative expression level of mRNAs was performed by using $2^{-\Delta\Delta CT}$ analysis method. The primers

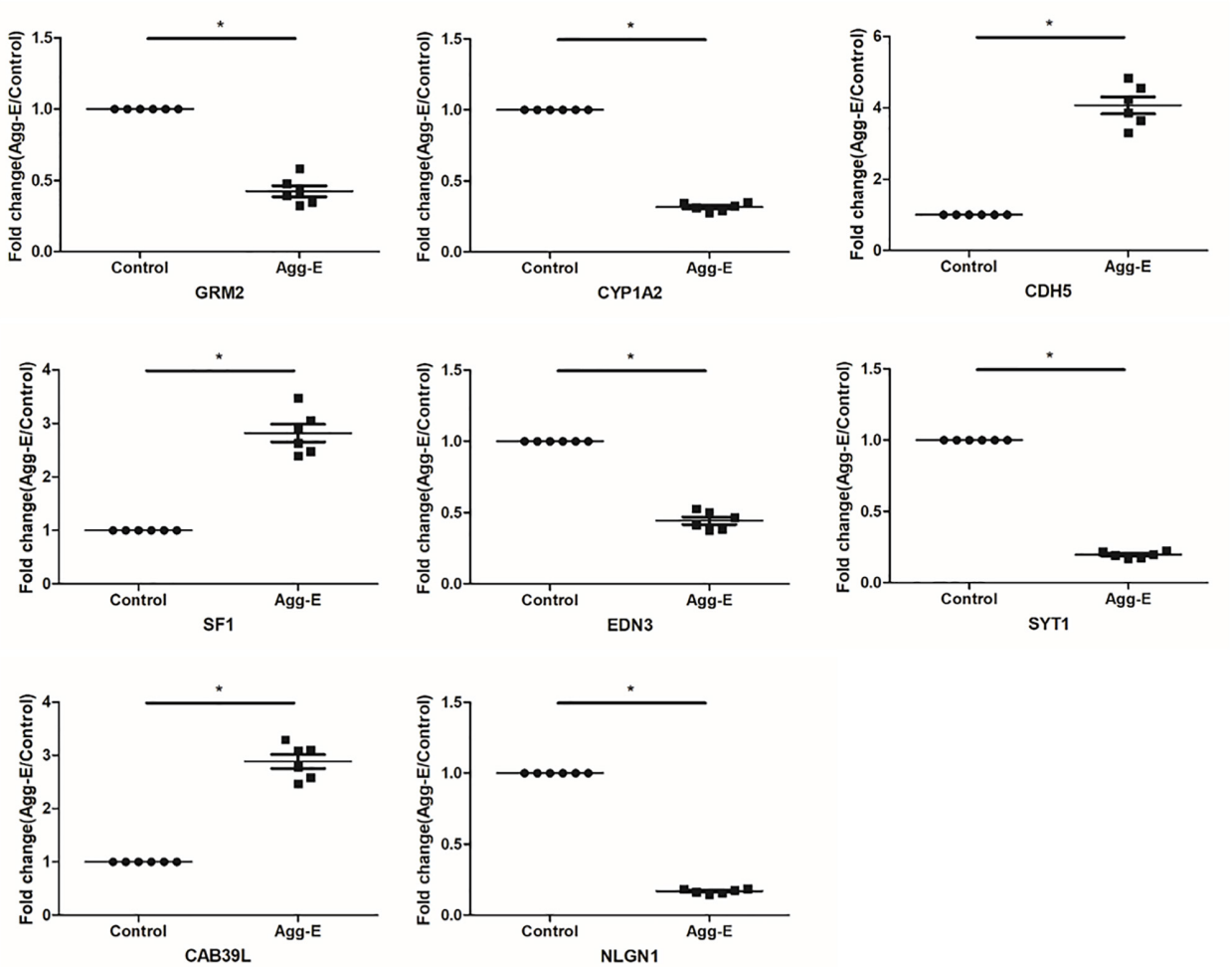

FIGURE 4 | Relative expression level of the "seed" genes in hippocampus region in response to stress exposure. The expression level of mRNAs was conducted using qRT-PCR. Results were shown as mean ± SD, $^*P < 0.05$.

used were as follows (**Table 1**). GAPDH expression served as internal control.

Statistical Analyses

The data of FST, TST and OFT were presented as the mean ± standard deviation (SD) using SPSS19.0 statistical analysis software. The data were analyzed using *t*-test. $^*P < 0.05$ were considered statistically significant.

RESULTS

Identification of DEGs

The mRNA expression profile datasets in hippocampus region consisted of expression data matrix of 41,175 gene probes. Using GEO2R, we identified 1004 DEGs consisting of 583 up-regulated DEGs and 421 down-regulated DEGs. The differential expression genes were shown in **Figure 2**.

Functional Annotations

The 1004 genes were uploaded to DAVID database for biological function assessment, and KEGG pathway enrichment. The results showed that these DEGs were markedly enriched in one of the following 10 biological processes: cellular calcium ion homeostasis, response to calcium ion, complement activation, classical pathway, plasma membrane, negative regulation of cytokine secretion, Wnt signaling pathway, transferase activity, transferring glycosyl groups, cell adhesion, cell junction and calcium ion binding (**Table 2**). We found that the most significantly enriched pathways were retrograde endocannabinoid signaling, ECM-receptor interaction, complement and coagulation cascades (**Table 3**). Gene functional annotations and enrichment analyses indicated that the most associated biological function was the Wnt signaling pathway.

Protein and Protein Network

In order to mine the PTSD-associated genes, we performed PPI network analysis by STRING database, and 415 PPI

pairs were derived (**Figure 3**), which then underwent analysis by Cytoscape to depict the complex relationship (combined score >0.9). Then, 16 clusters were selected from PPI network using the plugin MCODE. MCODE analysis showed that each cluster had one "seed" gene. The "seed" genes were as follows: Olfr1389, Glutamate Metabotropic Receptor 2 (GRM2), Endothelin 3 (EDN3), Golgin B1 (GOLGB1), Synaptotagmin 1 (SYT1), Splicing Factor 1 (SF1), G1 To S Phase Transition 1 (GSPT1), Calcium Binding Protein 39 Like (CAB39L), Exosome Component 8 (EXOSC8), FGF15, Cadherin 5 (CDH5), Collagen Type XXIV Alpha 1 Chain (COL24A1), Cytochrome P450 Family 1 Subfamily A Member 2 (CYP1A2), Myotubularin Related Protein 14 (MTMR14), Neuroligin 1 (NLGN1), and Shugoshin 2 (SGOL2). We found that the main roles of these genes were in cell junction, cell adhesion molecules (CAMs).

Verification of "Seed" Genes and Potential Pathway

To verify the "seed" genes, we used qRT-PCR to identify the expression level of these differentially expressed mRNAs in hippocampal tissue between two groups (**Figure 4**). The results of qRT-PCR showed that all "seed" genes, only GRM2, CYP1A2, CDH5, SF1, EDN3, SYT1, CAB39L, and NLGN1 were differentially expressed ($P < 0.05$, **Table 4**), which were consistent with the microarray datasets. It is suggested that the eight "seed" genes may function as a group, and play a vital role in pathological mechanism to PTSD.

Text Mining of Genes and Pathway With PTSD

To further depict the relationship among the "seed" genes, and the Wnt signaling pathway with PTSD, text mining was conducted using COREMINE. Co-occurrence analysis of the literature was performed using "post-traumatic stress disorder," "Wnt signaling Pathway," and "gene symbols" as search terms. Eight genes (GRM2, CYP1A2, CDH5, SF1, EDN3, SYT1, CAB39L, and NLGN1) were identified in the text-mining searches, as shown in **Figure 5**. We found that seven genes of eight (CYP1A2, CDH5, SF1, EDN3, SYT1, CAB39L, and NLGN1) were correlated with the Wnt signaling pathway. Four genes (GRM2, CYP1A2, SYT1, and NLGN1) were related to PTSD. Moreover, we found that CYP1A2, SYT1, and NLGN1 were associated with the Wnt signaling pathway and PTSD.

TABLE 4 | Relative expression level of eight "seed" genes in hippocampus region.

Gene	Up/down regulation	Fold change (mean ± SD)	*P*-value
GRM2	Down	2.454 ± 0.384	$P < 0.05$
CYP1A2	Down	3.184 ± 0.298	$P < 0.05$
CDH5	Up	4.071 ± 0.579	$P < 0.05$
SF1	Up	2.821 ± 0.407	$P < 0.05$
EDN3	Down	2.288 ± 0.321	$P < 0.05$
SYT1	Down	5.146 ± 0.578	$P < 0.05$
CAB39L	Up	2.887 ± 0.325	$P < 0.05$
NLGN1	Down	5.951 ± 0.596	$P < 0.05$

FST, TST, and OFT

Finally, behavioral evaluations were conducted by FST, TST and OFT. It showed that 10 days aggressor exposure induced a significant increase in immobility time. And there was no significant difference on path length of OFT (**Figure 6**).

DISCUSSION

Post-traumatic stress disorder is a long term, maladaptive stress condition, which is characterized by various symptoms, e.g., fear, depression, avoidance behavior, and impaired hyperarousal (Chitrala et al., 2016). It presents a considerable economic and health burden for families, communities and countries. The occurrence and development of PTSD are complicated. However, the underlying pathogenesis of PTSD is not fully understood. Thus, in this present study, we tried to identify important contributors and elucidate possible molecular mechanism related to PTSD.

Currently, with the development of high-throughput technology, microarrays and next generation sequencing combined with bioinformatics analysis generated numerous datasets including mRNA, miRNA, and lncRNA expression profile. Millions of genes were detected and widely used to predict potential biomarker of PTSD. In this study, we retrieved dataset of mRNA expression microarrays from GEO, and used bioinformatics analysis to identify key biomarkers and associated pathways related to PTSD. A total of 1004 DEGs (583 up-regulated and 421 down-regulated) were found. Using functional annotations and enrichment analysis, we found the function of these DEGs were closely related to the Wnt signaling pathway. By constructing the PPI network and module analysis, a number of "seed" genes were identified. In order to further understand whether these genes were altered in stress-exposed mice exhibiting PTSD-like features, we verified these genes in experimental study by qRT-PCR. The result indicated that these genes obtained from the PPI network involved in PTSD. So we posit that these genes may be served as potential biomarkers of PTSD. And the FST and TST results showed that Agg-E group had a significant increase in immobility time, which suggested that stress exposure can induced depressive-behavior.

In text mining network, three genes (CYP1A2, SYT1, and NLGN1) were found to be associated with the Wnt signaling pathway. We reasoned that the altered genes affecting PTSD might work through the Wnt signaling pathway. It showed that these genes may play key roles in PTSD via the Wnt signaling pathway. This finding was supported by previous study (Vidal et al., 2018), which suggested that the Wnt signaling pathway plays a significant role in neurogenesis and the maturation of hippocampal neurons. Several genetic

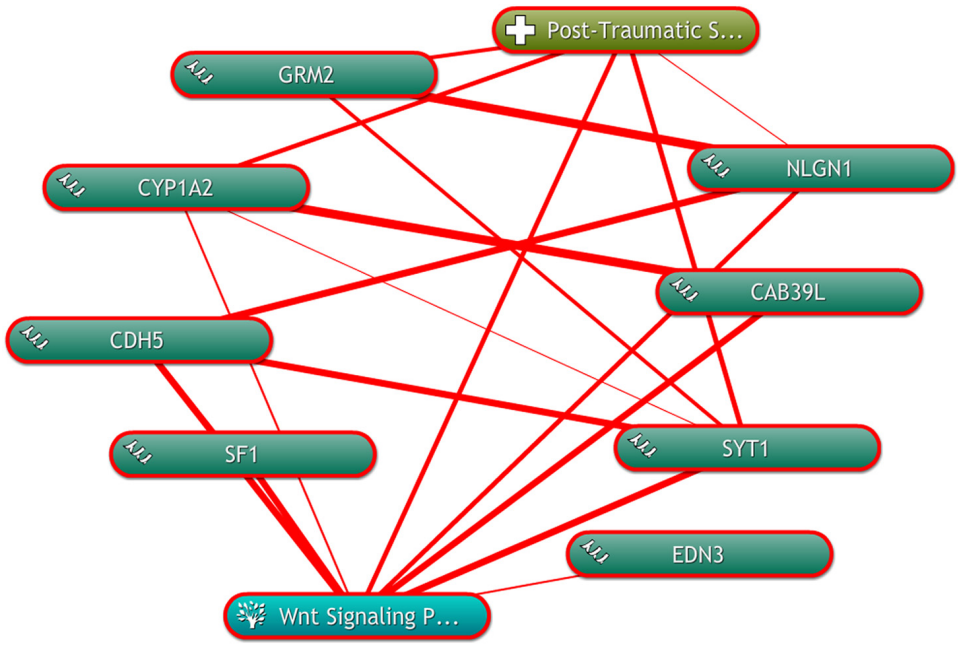

FIGURE 5 | The linear relationship among the "seed" genes, and the Wnt signaling pathway with PTSD by using COREMINE. Three "seed" genes were associated with the Wnt signaling pathway and PTSD. The thicker the line, the closer the connection between the two ends.

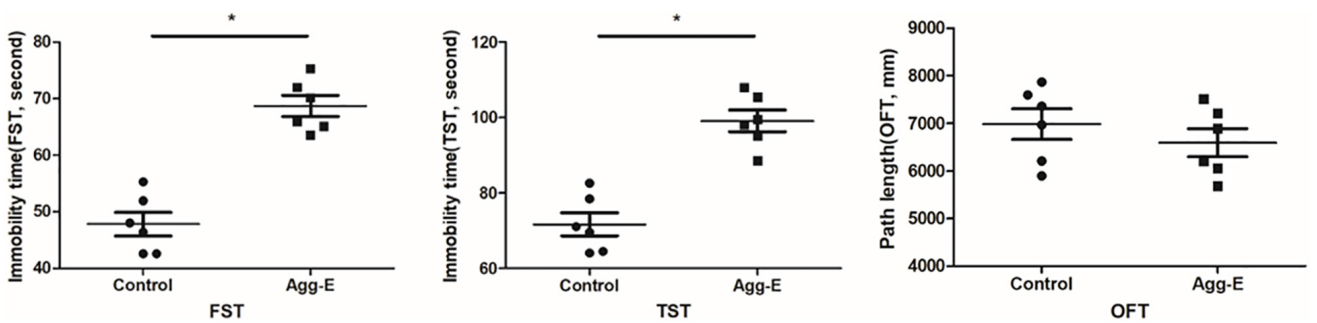

FIGURE 6 | FST and TST immobility time in animal testing and path length of OFT. Results were shown as mean ± SD, $*P < 0.05$.

studies (Inkster et al., 2010; Matrisciano et al., 2011; Wilkinson et al., 2011; Vidal et al., 2018) have reported that the Wnt receptor as well as the signaling pathway downstream were involved in the stress process. Under acute and chronic stress condition, the protein level of secreted glycoprotein Dickkopf-1 (Dkk-1), an inhibitor of the canonical Wnt pathway, showed a significantly higher than that of the controls (Matrisciano et al., 2011). While the expression of disheveled-2 (DVL2), an important protein of the Wnt pathway in nucleus accumbens (NAc) was decreased in chronic social defeat stress models (Wilkinson et al., 2011). Given this evidence, we further argue that the Wnt pathway is critical in pathogenesis of PTSD. The genesis of PTSD is an extremely complex process during which many genetic and epigenetic modifications of driving genes occur. CYP1A2 is concerned with coding a member of the cytochrome P450 enzymes. It is well studied in treating depressive disorder (Hofer et al., 2013). And SYT1 is a membrane protein, a critical mediator for membrane fusion during the neurotransmitter release induced by Ca^{2+}. It can influences synaptic plasticity via the regulation of neurotransmitter release, thus further influencing learning and memory (Zhang et al., 2015). However, no prior reports regarding SYT1 directly associated with PTSD. Further experimental and functional studies are warranted to explore the functional roles of SYT1 related to PTSD. NLGN1, localized at excitatory synapses, plays a critical role in mediating the formation and remodeling of synapses. Previous study (Zhang et al., 2015) found that NLGN1 involved in learning and memory function was closely associated with PTSD. The variation of NLGN1 may lead to higher risk to develop PTSD. In light of our experimental results, we further surmised that these genes may play a positive role on PTSD.

In summary, by using bioinformatics analysis, experimental verification, and text mining, we found that several genes and relevant pathway may represent key mechanisms involved in the development of PTSD. However, these findings require verification in future experimental studies.

AUTHOR CONTRIBUTIONS

YB carried out the design of the study, made the data acquisition, and prepared manuscript with LY. MZ, ZL, and YX conducted the data analysis. MZ, YX, and GZ performed the statistical analysis. WL and LZ provided the several suggestions for manuscript revision.

FUNDING

This work was sponsored by National Natural Science Foundation of China (81603529, 81704084, and 81673982), the Science and Technology Projects of Jiangsu Provincial Bureau of Traditional Chinese Medicine (YB2017002 and YB2015002), the Natural Science Foundation of the Jiangsu Higher Education Institutions (16KJB360002), and supported by Qing Lan Project.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

REVIEW
published: 28 March 2019
doi: 10.3389/fpsyg.2019.00574

An East Meets West Approach to the Understanding of Emotion Dysregulation in Depression: From Perspective to Scientific Evidence

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Depression, an emotion regulation disorder, is a prevalent mental illness in the world. Meanwhile, traditional Chinese medicine (TCM) has been increasingly regarded as a promising and effective alternative therapy approach for patients with depression. Despite many years of research on depression, the current understanding of the pathological mechanism of depression based on TCM theories is still in its infancy. Due to the lack of scientific evidence in the past, TCM is not fully recognized by researchers around the world. This review firstly summarizes the pathogenesis and etiology of depression in terms of both Eastern and Western medical systems. Secondly, it adopts an integrated Eastern and Western approach to propose some plausible neurophysiological pathways linking the liver, spleen, and heart functions explicated in TCM theory. The aim of this theoretical review is to bridge the knowledge gap between Eastern and Western medicine, which may better explain the pathology of depression.

Keywords: traditional Chinese medicine, depression, East meets West, neuroscience, neurophysiological pathway, emotion

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *28 September 2018*
Accepted: *28 February 2019*
Published: *28 March 2019*

Citation:
Ye J, Cai S, Cheung WM and Tsang HWH (2019) An East Meets West Approach to the Understanding of Emotion Dysregulation in Depression: From Perspective to Scientific Evidence. Front. Psychol. 10:574. doi: 10.3389/fpsyg.2019.00574

INTRODUCTION

Depression, an emotion regulation disorder, is one of the most prevalent psychiatric disorders worldwide. This disorder will become the second leading cause of disability by 2020. People with depression will spend approximately 8.2% of their lifespan struggling with the associated disabilities (Ferrari et al., 2013). The prevalence of depression in adolescents is high, accounting for 6% of the population. Recent epidemiological studies indicate that the lifetime rate of depression is 16% (Andrade et al., 2003; Kessler et al., 2003). Depression includes such symptoms as fatigue, depressed mood and anhedonia, irritability, loss of appetite, body weight changes, and sleep disorders. These symptoms may lead to a heavy burden on the patients, their families, their friends, and society (American Psychiatric Association, 2013).

Emotion regulation refers to the interaction between the occurrence, intensity, duration, and expression of emotion (Gratz and Roemer, 2004). It is widely acknowledged that emotion regulation strategies are closely associated with mental health (Aldao et al., 2010). Depression is characterized

by the emotion of sadness and the inability to extract pleasure from positive situations. Previous studies have suggested that patients with depression lack the emotion of anger because of their inability to handle stressful situations (Gu et al., 2016, 2018a). There are many ways for people with depression to regulate their emotions, including coping strategies and motivation (Kring and Werner, 2004; Campbell-Sills et al., 2006). A number of experimental studies on emotion regulation support the view that deficit in emotion regulation can be a crucial clue to understanding the etiology of depression (Soygüt and Savaşir, 2001). Therefore, emotion dysregulation is closely related to depression, and it is vital to understand emotion regulation in order to unravel the pathogenesis of this disorder.

Traditional Chinese medicine (TCM) originated from ancient China and has evolved over thousands of years. Nowadays, a growing number of people around the world are using TCM to prevent or cure diseases. In 2006, there were over 200 million outpatients and 7 million inpatients receiving TCM therapies. Most of the principles of TCM are derived from the philosophical basis of Taoism and Confucianism (General Office of the State Administration of Traditional Chinese Medicine and School of Management of Beijing University of Chinese Medicine, 2006). The main TCM therapies include herbal medicine, acupuncture, acupressure, moxibustion, massage, cupping, and physical exercise such as qigong. TCM theory is based on clinical experience instead of scientific evidence. Western medicine, on the other hand, is based on scientific investigations and tested by animal experiments and clinical trials. The two systems differ in their diagnoses, treatments, and theories (Tian, 2011). Despite a long history of clinical experience, the fundamentals of TCM remain largely unchanged and, similarly and unfortunately, the scientific elements underlying its theories remain largely unknown (Keji and Hao, 2003). Lack of scientific evidence has led to skepticism, criticism, and even rejection of TCM (Ted, 2000).

Given the high prevalence of depression and the increasing attention given to TCM, this theoretical review attempts to explore the etiological mechanism of depression *via* the Eastern and Western or integrative approach. In the long run, this paper will broaden and deepen our understanding of the etiology, signs, and symptoms of depression. Hopefully, this will give us insight into the development of innovative intervention strategies.

TRADITIONAL CHINESE MEDICINE'S VIEWS ABOUT EMOTION AND DEPRESSION

The TCM theory of emotion has a history of more than 2000 years and embraces well-established diagnosis and treatment systems. Many ancient Chinese texts have contents pertaining to the syndromes, etiologies, and treatment of depression caused by extreme emotional changes using the concept of "yu" or "yu-zheng," which literally means "not flowing, entangled, blocked, or clogged" (Ou, 1988). *The Yellow Emperor's Classic of Internal Medicine* is usually considered the earliest Chinese classic medical text in the world (General Office of the State Administration of Traditional Chinese Medicine and School of Management of Beijing University of Chinese Medicine, 2006). It expounds the relationship between emotional changes and the five viscera, namely, the heart, spleen, kidney, liver, and lung. According to TCM theory, emotional change is closely related to the etiology of diseases. The five viscera parallel the five elements (i.e., metal, wood, water, fire, and earth) which are transformed to create joy, anger, sadness, missing, and fear (Veith, 2015). This is derived from the five elements theory which can be used to understand the physiology and pathology of the human body and the etiology and pathogenesis of diseases (Giovanni, 1989). The transformation of emotion is based on the productive cycle of the five elements theory. The interaction of elements and organs is as follows: sadness is related to the lung, and joy can oppose it; fear is related to the kidney, and missing can oppose it; anger is related to the liver, and sadness can oppose it; joy is related to the heart, and fear can oppose it; missing is related to the spleen, and anger can oppose it (Giovanni, 1989; Chen, 1990; Gu et al., 2018a,b). Theoretically, emotional changes have two-way functions. On the one hand, emotional changes may lead to specific diseases. On the other hand, some diseases may result in emotional changes. If emotional changes (i.e., anger, fear, and sadness) can be managed in the short term, this would not bring about negative influences on the human body (Wang et al., 2017). If emotional changes are strong and last for a long time, this will give rise to the dysregulation of the autonomic nervous system (ANS) because it exceeds the adjustable range of physiology and depression will occur.

Many ancient Chinese practitioners proposed definitions of yu. Tao Hongjing (Wu et al., 1963), the author of *Shennong Bencao Jing Jizhu*, a variorum of Shennong's classic materia medica, and a physician of the North and South kingdoms period, reported the treatment of yu using antelope horn. In another text, Chen Wuzhe (Chen and Lu, 1995), who was a famous TCM practitioner in the Song dynasty (960–1,279), proposed the concept of the "seven emotions" which indicated that emotional changes may lead to disharmony of the internal organs and then to yu. Zhang Congzhen (Zhang et al., 2011), who was the most famous TCM practitioner in the Jin dynasty (265–420), put forward the pathogen concept. Mr. Zhang proposed the methods of sweating, emesis, and diarrhea to treat the yu-zheng caused by a pathogen. Although several concepts regarding yu were proposed in ancient times, the most useful concept for understanding the progression of yu is Zhu Danxi's theory of the six depressions, which is regarded as the mainstay of TCM theory for understanding depression. The theory of the six depressions involves the stagnation of either qi, blood, dampness, phlegm, food, or fire, and it is built on earlier Chinese medical texts such as the *Treatise on Cold Damage and Miscellaneous Disorders* and *The Yellow Emperor's Classic of Internal Medicine* (Scheid, 2013; Chen et al., 2015). Zhu Danxi's approach focused on the understanding of disease dynamics. He mentioned that qi was responsible for the movement and transformation of blood, dampness, phlegm, food, and fire. If the qi was stagnant, either blood,

dampness, phlegm, or food would not be able to move or transform properly in the human body. These obstructions of substances might accumulate and eventually turn into fire (Park, 2002) (**Figure 1**).

When Western medicine was introduced into China, its nosology was usually translated into Chinese by referring to the closest TCM concept. In the case of depression, it was translated into Chinese as "yiyu" or "yu" with reference to the yu syndromes in TCM (Ng et al., 2006). Unlike Western medicine, in TCM, diagnosis is based on the syndrome differentiation of diseases or disorders underlying symptom co-occurrence patterns. TCM practitioners discover constraints not only by asking and observing but also by palpating and smelling (Ross, 1985).

The onset of depression is often due to significant emotional changes that are mainly related to the liver (Wang and Lu, 2002; So et al., 2015). In its initial stage, depressive syndrome is mostly classified as "excess type"; in prolonged cases, the classification changes to "deficiency type" or "deficiency-excess type" (Wang and Lu, 2002). Liver qi plays a vital role in the precipitation of depressive episodes, and stagnation of the liver qi is part of the excess-type classification. Conventionally, when there is emotional change, the liver qi is affected first, followed by disharmony of the qi among the five viscera, especially the liver, spleen, and heart, resulting in a loss of regulation of the qi and blood. If the liver fails to control the dispersion, the function of the spleen will be repressed. This will lead to dissipation and harm to the heart qi. If the heart loses its nourishment and the "shen" (spirit) becomes restless, it will lead to an unstable and depressed mood. When stagnation of the qi is prolonged, it will accumulate and transform into fire (Allen, 1990). Previous reviews have supported this theory and suggested that anger leads to deviant dispersion of the liver qi, which then causes depression (Zhao, 1992; Jin and Liang, 1997; Guo and Liu, 2002). A growing body of evidence suggests that anger may lead to liver dysfunction, which means that the liver's function of spreading qi is impaired. Once the liver is unable to maintain its free and unobstructed flow, people may experience depression (Liu, 1991; Zhao and Zhao, 1999; Park, 2002).

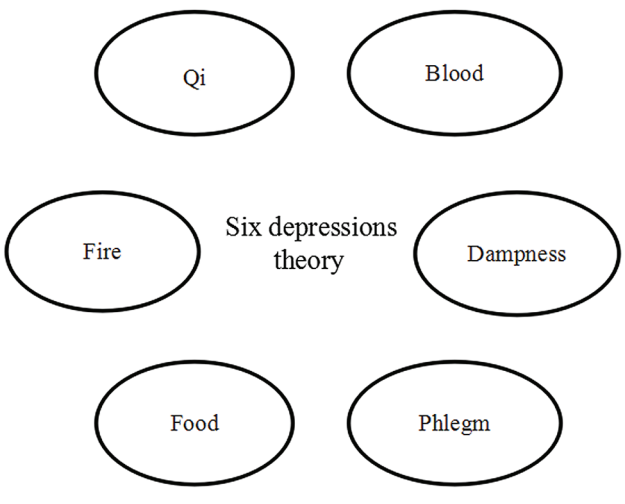

FIGURE 1 | Traditional Chinese medicine theory.

WESTERN MEDICINE'S VIEWS ABOUT EMOTION AND DEPRESSION

More and more studies are investigating the etiology of depression (Krystal et al., 2002; Smith and Vale, 2006; Moret and Briley, 2011; Liu et al., 2015). However, the underlying pathophysiology of depression is still not fully understood. Several possible theories may explain the potential process involved in depression, but neurophysiological factors play a vital causal role in the process (General, 2001).

Regulation of Neurotransmitters

Norepinephrine Theory

Norepinephrine (NE) is responsible for the regulation of cardiovascular activity, pain sensation, and body temperature. Previous studies have shown that there is a close link between NE and anxiety (Schildkraut, 1965; Liu et al., 2015). The possible relationship between depression and disturbance of NE in the brain was first proposed in 1965 (Schildkraut, 1965). In an animal study (Schildkraut, 1965), it was reported that a lower concentration of NE in the brain caused by reserpine might lead to depression. Evidence showed that people with depression had either low or high levels of urinary 3-methoxy-4-hydroxyphenylglycol (MHPG), the metabolite of NE degradation, which indicated there are significant differences in the amount of NE in terms of synthesis and release between people with depression and healthy individuals (Samson et al., 1994). As noradrenergic pathways in the brain arise from the locus coeruleus and project to the frontal cortex, limbic system, and spinal cord, neuroimaging studies suggest that abnormal metabolism in the limbic and paralimbic structures of the prefrontal cortex (PFC) is associated with emotional dysregulation and depression, which might indicate that medicine that increases NE activity in the brain could be one of the most effective therapeutic agents (Drevets et al., 2002).

Serotonin (5-HT) Theory

Serotonin, biochemically derived from tryptophan, is primarily found in the central nervous system (CNS), the gastrointestinal tract, and blood platelets (Young, 2007). There are generally seven serotonin receptor subtypes which exert influences on various biological and neurological processes, such as aggression, anxiety, appetite, sleep, mood, and thermoregulation (Glennon and Dukat 1991; Wesolowska, 2002). Coppen et al. (1965) developed the hypothesis on 5-HT and the treatment of depression in 1965. They proposed that decreased levels of 5-HT in the synaptic cleft might result in depression. A study by Pandey (1997) found that suicidal patients had lower levels of 5-HT compared to normal subjects. A study by Wägner et al. (1990) showed that taking fluoxetine, a selective inhibitor of 5-HT uptake, significantly reduced the content of 5-HT

compared to its original level based on a platelet sample and relieved the syndromes caused by depression. Clinical studies showed that 5-HT_2 receptors were likely to be the candidates involved in the pathophysiology and treatment of depression among various 5-HT receptor subtypes (Hoyer et al., 1986; Nyberg et al., 1993). In addition to 5-HT_2, 5-HT_{1A} receptors have an influence on the regulation of mood. A review suggested that the 5-HT_{1A} receptors were particularly related to antidepressant and anxiolytic responses in human beings (Blier and Ward, 2003). The presynaptic 5-HT_{1A} receptors are located in the raphe nuclei, where they act as cell body auto-receptors to inhibit the firing rate of 5-HT neurons. On the other hand, the postsynaptic 5-HT_{1A} receptors are located in the limbic and cortical regions, where they also attenuate firing activity, which indicates that 5-HT_{1A} receptors bring about a negative feedback influence on firing activity in the brain (Aghajanian and Lakoski, 1984; Blier and De Montigny, 1987; Blier and Ward, 2003).

Dopamine Theory

Dopamine (DA), which participates in emotion regulation, is produced by the dopaminergic neurons in the ventral tegmental area (VTA) of the midbrain, the substantia nigra pars compacta, and the arcuate nucleus of the hypothalamus, and its notable functions are associated with the mediation of mood, behavior, and cognition (Martini, 2015). The relationship between DA and depression was first developed by Molander and Randrup (1976). Willner (1983) found that the concentration of DA was lower in patients with depression compared to healthy subjects. A study with post-mortem human subjects showed that the metabolite rate of DA was critically decreased in suicidal patients with depression, specifically in the regions of caudate, putamen, and nucleus accumbens (Bowden et al., 1997). Evidence from recent studies also supports this finding. An animal study showed that depletion of DA in brain samples was found in animals with behavioral depression after 3 weeks of reserpine injections (Ikram and Haleem, 2017). A clinical study found that the D_2 receptor of DA might be supersensitive in patients with depression compared to controls by means of a novel neuroendocrine challenge test which indicated that dopaminergic mechanisms might be a target of therapeutic interest (Verbeeck et al., 2001).

The Relationship of Possible Factors

Glutamine and λ-Amino Butyric Acid

Glutamine (Glu) and λ-amino butyric acid (GABA) are respectively the main excitatory and inhibitory amino acids in the CNS mediating general mood states (Crabtree et al., 2013). Increasing evidence from clinical studies shows that Glu levels decrease in depressed patients compared with healthy controls (Auer et al., 2000; Liu et al., 2015) and GABA concentrations in the occipital cortex and prefrontal regions of patients with depression also decrease compared with control groups (Sanacora et al., 1999; Hasler et al., 2007). Studies on TCM have mentioned that levels of Glu and GABA might be increased through taking Chinese herbs (Gao et al., 2014; Liu et al., 2015). As the levels of Glu and GABA are vital to maintaining normal brain function, the two neurotransmitter systems may be the possible therapeutic targets in depression (Zorumski et al., 2013).

Gene and Environment Interaction

Previous research on twins has demonstrated that genetic factors play a vital role in the development of depression. Scientific findings show that the heritability of depression accounts for between 31 and 42% of the variance in adolescents' depressive symptoms (Sullivan et al., 2000; Barclay et al., 2015). Scientists have recently raised the possibility that genetic vulnerability factors can interact with environmental factors to make depressive symptoms more severe. An empirical study has suggested that social context will have a function in triggering and compensating for a genetic diathesis (Heath et al., 2002). Also, social context will act as a control to prevent "genetic predisposition behaviors" (Heath et al., 2002). The diathesis-stress process of the gene-environment (GE) interaction might occur when those who have genetic vulnerability are under a stressful environment (Shanahan and Hofer, 2005).

Cognition

Cognition refers to the mental actions or processes of (a) gaining new knowledge and understanding and (b) recalling memories that involve perceiving, recognizing, conceiving, and reasoning. Apart from the factors of neurotransmitters and GE interaction, Beck's cognitive theory of depression also has to be taken into consideration. The cognitive theory of negative automatic thoughts and underlying dysfunctional assumptions schemas were proposed by Beck in the mid-1960s (Beck, 1979). He found that the negative way of thinking came from previously unpleasurable experiences which could guide people's perceptions or interpretations, hence leading to a negative worldview and causing depression (Soygüt and Savaşir, 2001). The cognitive theory of depression indicates that early relevant experiences might result in the formation of dysfunctional beliefs which might lead to negative self-beliefs. When those who have negative self-views about themselves encounter a specific circumstance, they are more likely to feel hopeless and useless and ultimately be depressed (Soygüt and Savaşir, 2001). A study by Allen (1990) based on students showed that negative attitudes toward the future was related to depressive mood, and depression-prone students were found to negatively process personal information, leading to the development of symptoms of depression. Evidence from Abela and D'Alessandro (2002) was in line with previous findings and suggested that dysfunctional attitudes and an increase in depressive mood were significantly associated with students' negative beliefs about the future (**Figure 2**).

AN INTEGRATED EAST MEETS WEST APPROACH TO CLOSING THE GAP

In TCM, "zang fu" can be translated as "internal organs." It may be regarded as a core concept of TCM which views the

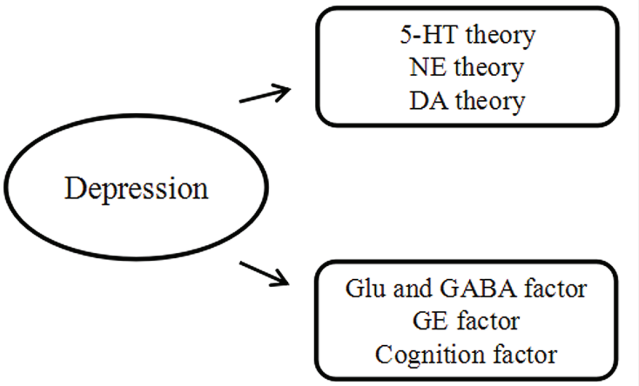

FIGURE 2 | Western medicine theory.

physical body as an integrated whole. It also describes an integrated relationship between mental activities, sense organs, tissues, five solid and six hollow organs, and environment influences (Giovanni, 1989).

The theory of internal organs is entirely different from the anatomical structure originating from Western medicine. However, this does not mean that TCM entirely disregards anatomy. The concept of organs in Western medicine is based on anatomy, whereas the concept of organs in TCM is based on a system concept that embraces anatomy, physiology, and psychology. In TCM, the function of internal organs is basically related to various substances, emotions, tissues, and senses. For example, the basic substances of TCM are qi (energy), xue (blood), jing (essence), shen (spirit), and jin ye (body fluids). Each substance is related to one or more organs (e.g., the spleen governs food qi and influences body fluids, and the heart governs blood).

In Western medicine, the liver, the largest internal organ, has various functions in the body, including the synthesis of proteins, blood clotting factors, triglycerides, cholesterol, glycogen, and the production of bile. However, TCM theorists believe that the liver is responsible for controlling dispersion in all organs and in all directions to ensure the smooth flow of qi throughout the body. This is the most salient of all the liver's functions, especially as far as depression is concerned. With reference to depression, the liver is postulated to be related to the functioning of the neuroendocrine system in Western medicine (Li and Wang, 1985; Yue and Tian, 1995).

To our knowledge, every organ's energy has a normal direction of flow: the qi of some organs flows downward (such as that of the stomach) and the qi of other organs flows upward (such as that of the spleen). The normal direction of the movement of the liver qi is upward and outward in all directions to make sure that the flow of energy is smooth and unimpeded. There are three functional activities of the liver in terms of this movement: regulating emotions, regulating the secretion of bile, and assisting the digestive function of the spleen and stomach (Ross, 1985; Giovanni, 1989).

The emotional state of an individual in fact depends on the smooth flow of energy and blood. When the liver qi flows smoothly, the emotional status of the individual will be calm and peaceful. In contrast, if the liver is not functioning well, the energy of the liver will stagnate, which will then lead to an abnormal increase in liver qi, and give rise to emotional disturbances, such as depression, accompanied by physical symptoms, such as a sensation of oppression in the chest and hypochondriac pain (Giovanni, 1989). Scientific studies of animal and human subjects have provided preliminary support to the postulation that the liver function in TCM is associated with the neuroendocrine system that includes the regulation of the NE system located in the locus coeruleus (LC/NE) and the hypothalamic-pituitary-adrenal axis (HPA) (Yue and Tian, 1995; Wang and Yao 2002; Yan and Xu, 2005; Yue et al., 2005a).

LC/NE System

Studies have explored the symptoms of the abnormal rising of the liver qi that are correlated with a lack of regulation of the ANS (Yue and Tian, 1995), a deficiency of serotonin, and an excessive level of NE (Spiegelhalder et al., 2011; Wei et al., 2012). However, another study claimed that NE level is not related to the severity of depression because of the different stages of depression (Yuan et al., 2004). The LC/NE system may be involved in the regulation of the neuroendocrine system based on the syndrome of liver qi stagnation. The locus coeruleus is the central site of the LC/NE system in the brain, which is the center of the synthesizing adrenergic nerve. The ascending fibers of the adrenergic nerve are mainly projected into the amygdala, hippocampus, and limbic cortex, which are responsible for emotional changes, memory, and behavioral changes. The descending fibers of the adrenergic nerve are mainly projected into the lateral dorsal horn of the spinal cord, which is involved in the regulation of the activity of the sympathetic nerve, and the secretion of catecholamines. It has been suggested that the activated amygdala may stimulate the release of the corticotrophin-releasing hormone (CRH) that increases the activity of the sympathetic nerve *via* the mediating lateral dorsal horn of the spinal cord. Once the sympathetic nerve is activated, adrenaline medulla will release NE and epinephrine (E) due to the activated adrenal gland (Copstead and Banasik, 2010) (**Figure 3**).

A growing number of clinical trials support the association between liver diseases and the lack of ANS regulation (Yue et al., 2005a). A study by Jin (2000) mentioned that there is a positive correlation between increased sympathetic tone activity and the excess type of liver dysfunction, such as loss of appetite and wiry pulse, while there is a negative correlation between increased parasympathetic tone activity and the deficiency type of liver dysfunction, such as weak pulse. A study by Yuan et al. (2004) suggested that NE level is relatively higher in patients with depression compared to people in normal health. As the results on NE level in patients with depression are contradictory, experimental studies to explore this monoamine transmitter concentration in depressed patients would be a promising direction for further research.

The HPA Axis

In addition to the LC/NE system, the regulation of the HPA axis may also be implicated with depression if there is dysfunction

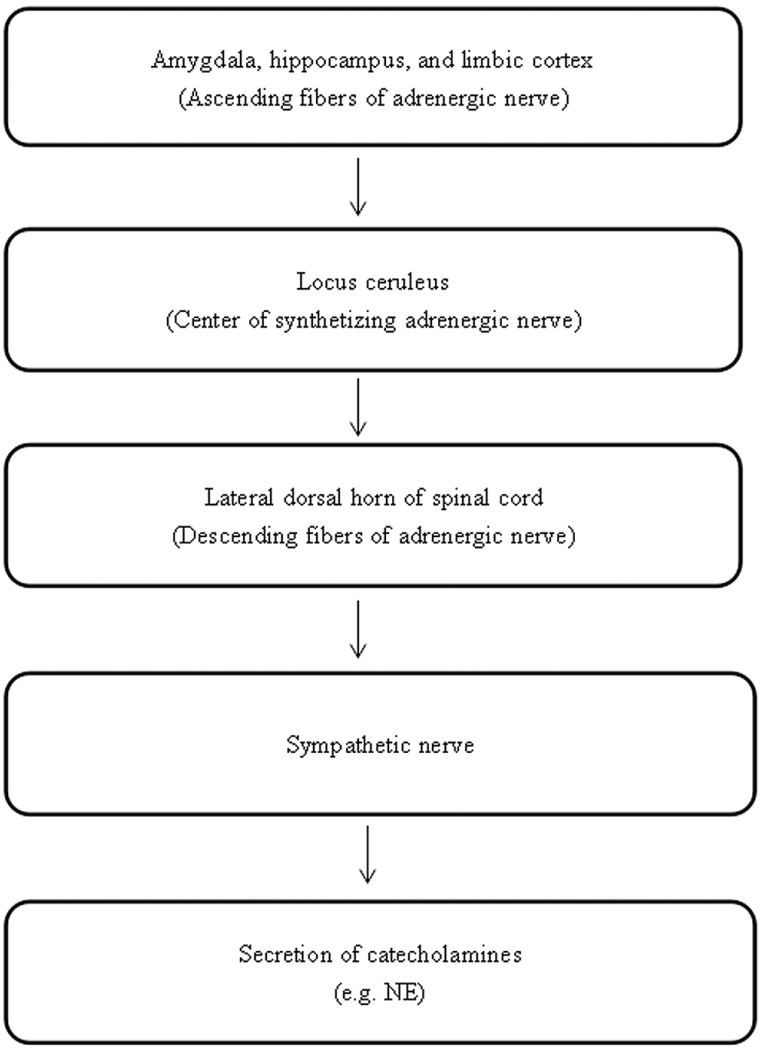

FIGURE 3 | The neuropathways associated with the LC/NE system.

in the liver. The hypothalamus plays a role in the physiology of depression *via* elevation in the activity of the HPA axis (Benca et al., 1992). The significance of the HPA axis in mediating physical manifestations of psychological stress has been well documented (Nestler et al., 2002; Steiger, 2007). The activity of the HPA axis is mainly related to the operation of CRH from the parvocellular neurons of the paraventricular nucleus of the hypothalamus (Steiger, 2007; Gu et al., 2018a). The secretion of CRH will stimulate the release of the adrenocorticotropic hormone (ACTH), secreting cortisol in humans and corticosterone in rats from the anterior pituitary. Most neuroendocrine studies of patients with clinical depression report elevated cortisol secretion and ACTH due to the impairment in the negative feedback system of cortisol to the HPA (Tsang and Fung, 2008) (**Figure 4**).

In addition, the body state named "fight or flight," can be provided by elevated cortisol levels (Wang et al., 2017). Since the negative feedback of the HPA axis and cortisol is impaired, a higher level of HPA axis activity will lead to reduced vagal modulation or excessive activation of sympathetic neurons, resulting in physiological activation, such as increased heart rate, peripheral vasoconstriction, elevated body temperature, and increased body metabolic rate (Kales and Kales, 1984; Kales et al., 1987; Vgontzas et al., 2001; Bonnet and Arand, 2003). The above suggests that depression is closely related to over-secretion of ACTH and cortisol secretion.

ANS Dysregulation

Apart from the function of regulating emotions in the liver, the digestive function of the spleen and stomach also depends on the movement of liver qi in TCM theory. If there is dysfunction in the liver, the digestive activities are impaired. People may exhibit the symptoms of belching, sour regurgitation, and nausea or vomiting. Lastly, the flow of bile is affected by liver function. If dysfunction of the liver occurs, the flow of bile may stagnate, leading to bitter taste in the mouth, belching, or jaundice and, resulting in sleep disturbance. An experimental study found that dysfunction of the ANS could be one of the

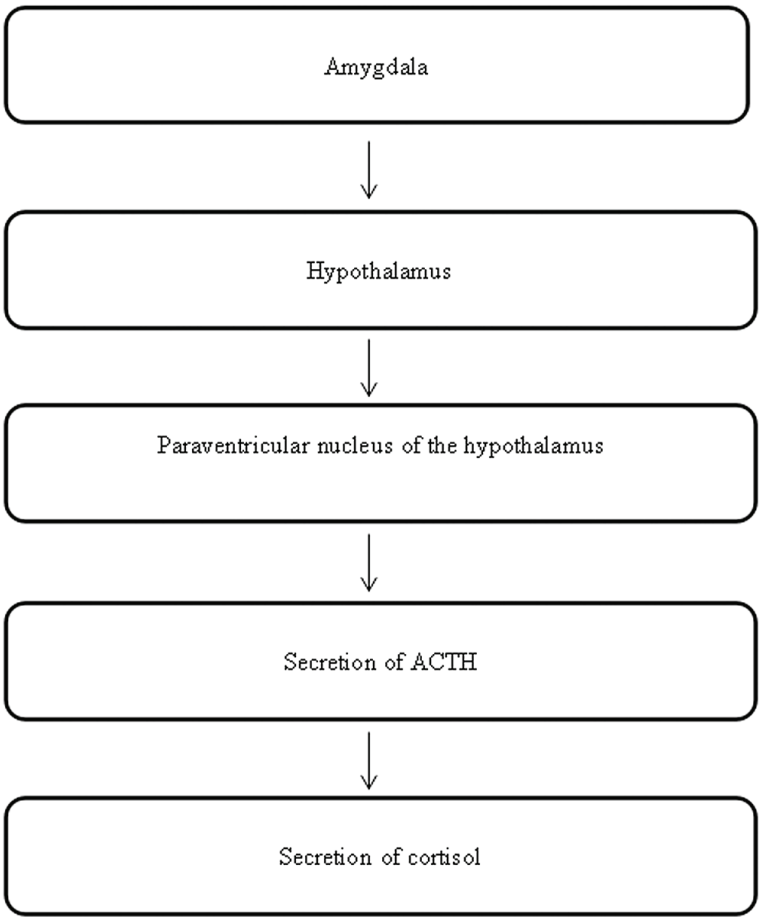

FIGURE 4 | The neuropathways associated with the HPA axis.

reasons for emotional disturbance and functional dyspepsia (Vgontzas et al., 2001). Moreover, studies have shown that there is a correlation between the symptoms of stagnation of liver qi, deficiency of bile secretion, and intestinal malabsorption (Jin et al., 1985; Yue and Tian, 1995) (**Figure 5**).

Tryptophan and Serotonin Deficiencies

The spleen is an abdominal organ which is involved in the production and removal of blood cells, and it is a part of the immune system according to Western medicine. However, the definition of spleen in TCM theory is different, with a broader implication than in Western medicine. It refers not only to the organ itself but also to the functions of digestion (including the pancreas and small intestine) with regard to depression (Yu, 2013).

The primary function of the spleen is to aid the stomach in the digestion of food by transporting and transforming nutrients from food and water, absorbing the nourishment, and separating the usable part of food and water from the unusable part (Giovanni, 1989). Once food and water are ingested, the spleen and stomach work closely together in digesting, extracting, and transporting the essence from food and water to the body. When the spleen is working properly, digestion will be normal and a person will have a good appetite, normal absorption, an adequate energy supply, and regular bowel movements (Giovanni, 1989). As the liver has the function of assisting the digestive functions of the spleen and stomach, if liver does not function properly, it will affect the spleen function, resulting in poor appetite, indigestion, and abdominal distension. In five-element terms, this corresponds to "Wood overacting on Earth."

Previous research aligns with our postulation. It has been reported that patients with dysfunction of the spleen have a low concentration of urine amylase, an insufficient concentration of serum gastrin, and a low frequency of peristalsis of the stomach (Jia et al., 1999; Tao et al., 2005; Zhang, 2006). A review showed that compared to patients suffering from only one gastrointestinal disease, patients with comorbid gastrointestinal diseases are more likely to experience anxiety, depression, and insomnia, with pathogeneses of visceral hypersensitivity, altered gastrointestinal disease motility, infection, and stressful early life events (Yue and Tian, 1995). A study by Lindgren et al. (2012) mentioned that depression was related to the symptoms of poor appetite, heartburn, diarrhea, bloating, constipation, and epigastralgia in pilots. Moreover, tryptophan, which is an indispensable amino acid, and a precursor of

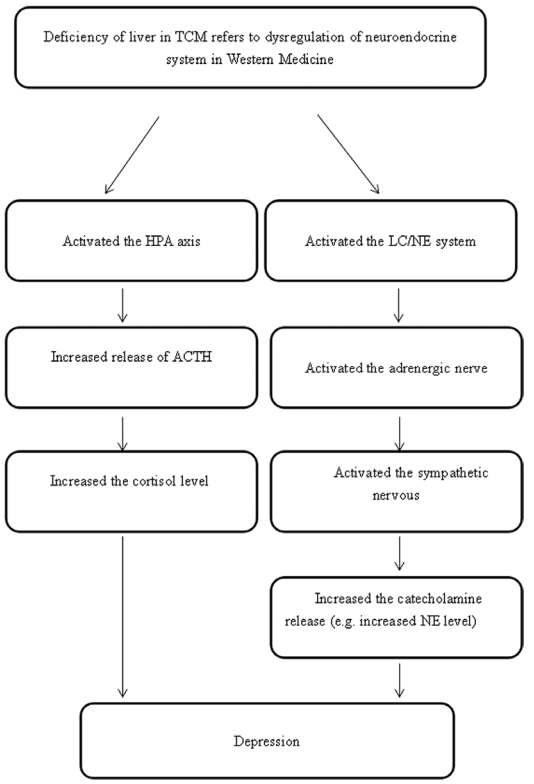

FIGURE 5 | The hypothesized pathological pathways linking liver dysfunction and depression.

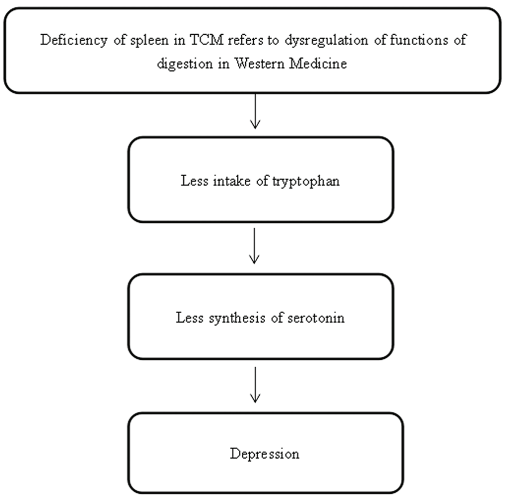

FIGURE 6 | The hypothesized pathological neuropathways linking spleen dysfunction and depression.

serotonin and melatonin, which are thought to regulate mood, is taken from food (Zhang, 2006). Intake of tryptophan has an influence on the regulation of emotional state by influencing serotonin synthesis, and this could be considered as an effective therapy for treating depression (Hartmann, 1982; Shaw et al., 2002; Lieberman, 2003). If there is a lack of food intake that is related to deficiency in tryptophan and eventually serotonin, emotional changes such as depression could happen, which parallels the findings from previous studies (Sainio et al., 1996; Birdsall, 1998; Lieberman, 2003; Le Floc'h et al., 2011; Yao et al., 2011). These studies provided preliminary evidence to support the postulation that stagnation of liver qi and spleen deficiency in depressed people in terms of TCM theory may parallel the abnormal functions of digestion in patients with depression based on the Western medical viewpoint (**Figure 6**).

Hypoactivation in the Frontal Cortex

The heart is a muscular organ which pumps blood throughout the body by a circulatory system that provides oxygen and nutrients, and removes metabolic wastes. However, the function of the heart is more diversified in TCM than in Western medicine. The heart is responsible for the circulation of blood and, at the same time, the regulation of mental activities (Giovanni, 1989; Zhang, 2004; Yue et al., 2005b). According to TCM, the main functions of the heart are to govern the circulation of blood, control the blood vessels, manifest on the complexion, and store the shen, which implies consciousness, mental functions, emotion, and vitality (Ross, 1985; Giovanni, 1989; Zhang, 2004; Yue et al., 2005b).

In TCM theory, a healthy heart is essential for supplying blood to all tissues in the body. When there is a dysfunction in the heart, the circulation of blood is insufficient and the four limbs may be cold. People might exhibit the symptoms of a lower body temperature, and a white or purple complexion (Giovanni, 1989). Also, storing shen, which can be translated as "spirit" or "mind," is a part of the heart's functions. Shen is used to point out the whole field of the emotional, mental, and spiritual aspects of a human being. In this sense, shen not only indicates the heart, but also encompasses the emotional, mental, and spiritual phenomena of all organs. If dysfunction of the heart occurs, there is not sufficient blood to nourish the shen and a person will have difficulty in maintaining a good memory and good mental health; thus, he/she may suffer from depression. Furthermore, the heart is in charge of controlling blood vessels. The function of storing shen depends on adequate nourishment from the heart blood. Therefore, there is a mutual relationship between the function of controlling blood vessels and that of storing mind. As the blood is the root of shen, if the heart blood is sufficient, the mind will be peaceful and happy, and the pulse will become regular and normal. Conversely, if the heart blood is deficient, there is insufficient blood to root the mind, which will result in mental restlessness, depression, palpitations, and a weak or irregular pulse.

A growing number of studies support this ancient theory, showing that, compared with healthy people, patients with depression associated with deficiency of the heart and spleen have lower brain activity in the left frontal cortex region (Feng et al., 2005; Xie, 2007; Wang et al., 2008b). These findings are in line with the findings from Western medicine that major depression is related to decreased activity in the left hemisphere relative to right hemisphere, and to a decline

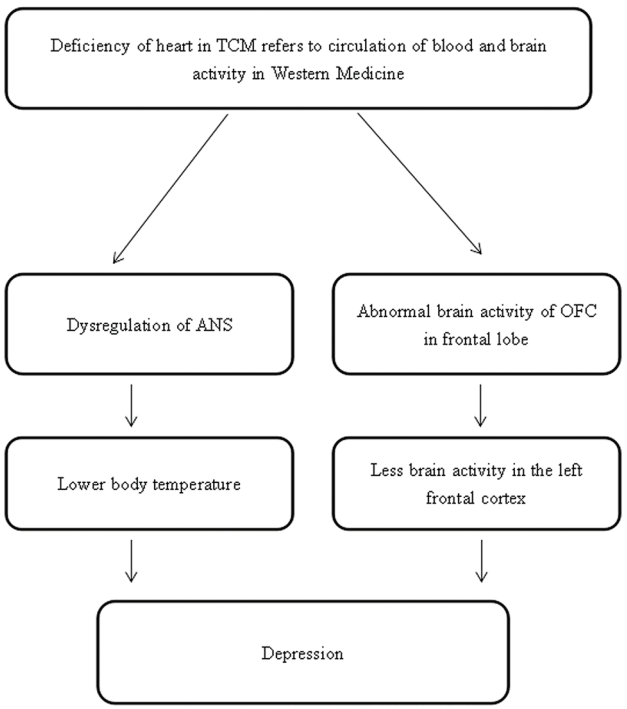

FIGURE 7 | The hypothesized pathological neuropathways linking heart dysfunction and depression.

in the activity of the left frontal cortex in people suffering from depression compared to normal people. Clinical studies reported that depression is related to altered resting-state activity in the PFC, and a growing number of findings from functional and structural imaging studies show that depression is associated with volume reduction in the left subgenual PFC region (Drevets et al., 1997; Öngür et al., 1998; Botteron et al., 2002; Wang et al., 2008a; Ye et al., 2012), because the orbitofrontal cortex (OFC) is involved in cognitive processing and decision-making, and the main function of the PFC is to extract the relevant information about a cognitive experience, so as to modulate the emotion and behavior changes (Feng et al., 2005). Moreover, studies have mentioned that the body temperature in depressed people is lower than in normal healthy people (Zhe, 2004; Lin et al., 2011); this may result from autonomic response dysfunction mediated by central adrenergic activation (Hughes et al., 2006; Hamer et al., 2007; Shinba et al., 2008) (**Figure 7**).

SUMMARY AND THE WAY FORWARD

Two different systems of medicine have been used in parallel to each other for approximately 200 years. TCM is mainly based on observation and experience. In contrast, Western medicine basically relies on scientific investigation. Recent studies in Western medicine suggest that dysregulation of neurotransmitters could be one of the most vital causes of depression; while the classical texts of TCM state that dysregulation of liver qi is the main cause of depression.

Interest in the neuroscientific investigation of TCM for depression has increased dramatically in the past few decades. As the investigation of TCM using neuroscience theories and methodologies is a relatively new field of research, there are limited studies in the available literature. Knowledge of the mechanism that underlies TCM for depression is still in its infancy. However, there is emerging evidence that TCM theory might be illustrated by the changes in neurotransmitters, brain structure and function, and neuroendocrine found in people with depression.

Given the information above, we propose the following postulations linked to the liver, spleen, and heart. In terms of TCM theory, (1) liver function may be explained by the HPA axis and LC/NE system; (2) spleen function may correspond to the digestive system; and (3) heart function may refer to the circulation of the blood and the regulation of brain activity.

Further study using longitudinal study designs and larger sample sizes is recommended to advance our understanding of the mechanism of TCM for treating patients with depression. Moreover, studies applying the integrated approach of East meets West and a rigorous research design are also strongly recommended.

AUTHOR CONTRIBUTIONS

JY and SC searched for articles and wrote the draft version of the manuscript. HT validated the manuscript. HT, SC, WC, and JY revised the manuscript. All the authors read and approved the final manuscript.

ACKNOWLEDGMENTS

The authors wish to thank Ms Wendy So for her valuable suggestions and Ms Duo Ye for her modification of figures.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

ORIGINAL RESEARCH
published: 29 March 2019
doi: 10.3389/fpsyg.2019.00691

Cognitive Control as a 5-HT$_{1A}$-Based Domain That Is Disrupted in Major Depressive Disorder

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Heterogeneity within Major Depressive Disorder (MDD) has hampered identification of biological markers (e.g., intermediate phenotypes, IPs) that might increase risk for the disorder or reflect closer links to the genes underlying the disease process. The newer characterizations of dimensions of MDD within Research Domain Criteria (RDoC) domains may align well with the goal of defining IPs. We compare a sample of 25 individuals with MDD compared to 29 age and education matched controls in multimodal assessment. The multimodal RDoC assessment included the primary IP biomarker, positron emission tomography (PET) with a selective radiotracer for 5-HT$_{1A}$ [(11C)WAY-100635], as well as event-related functional MRI with a Go/No-go task targeting the Cognitive Control network, neuropsychological assessment of affective perception, negative memory bias and Cognitive Control domains. There was also an exploratory genetic analysis with the serotonin transporter (5-HTTLPR) and monamine oxidase A (MAO-A) genes. In regression analyses, lower 5-HT$_{1A}$ binding potential (BP) in the MDD group was related to diminished engagement of the Cognitive Control network, slowed resolution of interfering cognitive stimuli, one element of Cognitive Control. In contrast, higher/normative levels of 5-HT$_{1A}$ BP in MDD (only) was related to a substantial memory bias toward negative information, but intact resolution of interfering cognitive stimuli and greater engagement of Cognitive Control circuitry. The serotonin transporter risk allele was associated with lower 1a BP and the corresponding imaging and cognitive IPs in MDD. Lowered 5HT 1a BP was present in half of the MDD group relative to the control group. Lowered 5HT 1a BP may represent a subtype including decreased engagement of Cognitive Control network and impaired resolution of interfering cognitive stimuli. Future investigations might link lowered 1a BP to neurobiological pathways and markers, as well as probing subtype-specific treatment targets.

Keywords: positron emission tomography, intermediate cognitive phenotypes, major depressive disorder, serotonin, executive functioning, interference resolution, processing speed

OPEN ACCESS

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Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *07 December 2018*
Accepted: *12 March 2019*
Published: *29 March 2019*

Citation:
Langenecker SA, Mickey BJ, Eichhammer P, Sen S, Elverman KH, Kennedy SE, Heitzeg MM, Ribeiro SM, Love TM, Hsu DT, Koeppe RA, Watson SJ, Akil H, Goldman D, Burmeister M and Zubieta J-K (2019) Cognitive Control as a 5-HT$_{1A}$-Based Domain That Is Disrupted in Major Depressive Disorder. Front. Psychol. 10:691. doi: 10.3389/fpsyg.2019.00691

INTRODUCTION

An enduring, but incomplete observation in MDD (Major Depressive Disorder) is of serotonin dysfunction. Serotonin dysfunction is a corollary of the monoamine hypothesis, positing that MDD is associated with relative depletion of monoamines including catecholamines (e.g., dopamine and noradrenaline) and tryptamine (e.g., serotonin) (Wijaya et al., 2018). Parallel and sometimes convergent reports spanning neurochemistry, behavioral pharmacology, neuroimaging and gene have implicated serotonergic dysfunction in MDD. However, while such dysfunction can be thought of as a typical, it is very clearly not a universal characteristic of MDD (Albert and Lemonde, 2004; Kalia, 2005; Surtees et al., 2006). Evidence that serotonin function is relevant in a subset of those with MDD includes a number of different avenues of exploration. First, affective experience and emotional regulation are more dramatically altered after acute tryptophan depletion (ATD), more so in persons with personal or family history of MDD (Rogers et al., 2003; Fusar-Poli et al., 2006; Neumeister et al., 2006; Spring et al., 2007; van der Veen et al., 2007). Second, selective serotonergic reuptake inhibitors (SSRIs) are more effective than placebo in a majority of controlled clinical trials (Rush et al., 2006; Trivedi et al., 2006). Third, functional loci at genes that mediate serotonergic function have been implicated in MDD both alone, and via interaction with early life stress (Hariri et al., 2002, 2006; Sen et al., 2004; Neumeister et al., 2006; Surtees et al., 2006; Gotlib et al., 2008; Mak et al., 2013). Fourth, some of these functional variants in genes (modulating serotonin function) alter brain responses to emotion and brain connectivity (Dannlowski et al., 2008; Kalin et al., 2008; Elton et al., 2014; Wessa and Lois, 2015). These alternations align with a model of how inherited variations contribute to risk for MDD. Fifth, manipulations of serotonin function and/or use of agents with serotonergic effects within animal models can simulate depression and anxiety-like behaviors (Albert and Lemonde, 2004; Bert et al., 2008; Borg, 2008). Sixth, depression is associated with negative cognitive changes including memory and executive function impairments (Burt et al., 1995; Snyder, 2013; Yu et al., 2018), negative affect related to control, success, and rejection (Yeo et al., 2017) and increased negative schema (Stange et al., 2017; Lim et al., 2018). As such, there is continuing pursuit of domains affected in MDD that can be linked to serotonergic function and genes. There is also interest in whether these dimensional features may define a more homogeneous subset for further exploration and targeted treatment. A short review of the relevant links of serotonin dysfunction in MDD and of potential multimodal intermediate phenotypes [IPs (Burmeister et al., 2008; Kalin et al., 2008; Tan et al., 2008; Langenecker et al., 2010; Webb et al., 2016)] is conducted to integrate these separate lines of inquiry.

Imaging Studies of 5-HT$_{1A}$ Function

Evidence of abnormal 5-HT (5-hydroxytyptamine refers to G protein coupled receptors and ligand-gated ion channels, also known as serotonin receptors) function in MDD is building, including for 5-HT$_{1A}$ specifically ($_{1A}$ is a subtype of 5-HT receptor which is the most widespread 5-HT receptor, including within cortex and medial temporal structures). Past human imaging studies of 5-HT$_{1A}$ binding potential (BP) have focused on areas of binding where serotonin receptors are more densely populated, including the raphe, as well as frontal, cingulate and medial temporal cortices (Marazziti et al., 1994; Oquendo et al., 2003; Parsey et al., 2006, 2010; Drevets et al., 2007; Selvaraj et al., 2017; Kranz et al., 2018). 5-HT$_{1A}$ receptors regulate the firing of 5-HT neurons presynaptically in the raphe nuclei and are expressed postsynaptically in many different cortical and subcortical brain regions (Schlumpf et al., 1987; Kaufman et al., 2015; Zanderigo et al., 2018). In the cortex, there are inhibitory properties of the postsynaptic 5-HT$_{1A}$ receptors (Gross et al., 2002; Borg, 2008), plus regulation of the release of glutamate in subcortical structures (Czyrak et al., 2003). A recent review of concentrations of transporter (5HTT), 5-HT$_{1A}$, and 5-HT$_{2A}$ receptors reflect the fact that 5-HTTs are densely populated in subcortical, pre-synaptic regions, whereas, 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors are more dense in cortical regions (Kranz et al., 2010; Kautzky et al., 2018). These same cortical areas support a number of cognitive and affective processes and both the regions and the processes they support are heavily implicated in MDD (Teasdale and Dent, 1987; Heller and Nitschke, 1998; Hugdahl et al., 2003; Phillips et al., 2003, 2008; Drevets et al., 2007; Langenecker et al., 2007c, 2010, 2014; Porter et al., 2007; Disner et al., 2011).

Positron emission tomography (PET) studies, typically utilizing the selective radiotracer for 5-HT$_{1A}$ receptors, $^{[11C]}$WAY-100635, have noted lowered levels of 5-HT$_{1A}$ receptor availability (BP$_{ND}$) within these regions in MDD as well as alterations in 5-HT$_{1A}$ availability pre- and post-treatment (Bhagwagar et al., 2004; Drevets et al., 2007; Moses-Kolko et al., 2007; Hirvonen et al., 2008; Kautzky et al., 2017). Lowered 5-HT$_{1A}$ BP in MDD is the general pattern observed; however, utilizing arterial sampling or a cortical reference region can make a significant impact on the direction of effects [higher or lower (Drevets et al., 2007; Parsey et al., 2010)]. As such, careful verification of reference region/marker equivalence between MDD and healthy comparison (HC) groups is important for PET studies with this radiotracer. Lower brainstem SERT BP was reported in an additional study of depressed suicide attempters (Nye et al., 2013). 5-HT$_{1A}$ disruptions have also been reported in high-risk offspring of those with MDD (Milak et al., 2018).

Animal Models of 5-HT$_{1A}$ in MDD

Given the limited number and variability across human *in vivo* studies, we briefly review the role of 5-HT$_{1A}$ in the pathophysiology of MDD as seen in animal models of depression and human postmortem studies. Animal studies have primarily reported increased 5-HT$_{1A}$ function after chronic SSRI administration (Haddjeri et al., 1998) and increases in anxious and depressive behaviors after 5-HT$_{1A}$ blockade, depletion, or knockout (Olivier et al., 2001;Akil, 2005; Zhang et al., 2006; Richardson et al., 2010). Novel antidepressants including agomelatine and vortioxetine induced modulation of brain-derived neurotrophic factor (BDNF) which is a neurotrophin that serves as a survival factor for neurons (Lu et al., 2018a,b). In a related study, BDNF knock out mice showed a significant attenuation of 5-HT$_{1A}$ receptor function (Hensler et al., 2007).

Acute stress results in decreased 5-HT$_{1A}$ mRNA in the hippocampus (Lopez et al., 1999) and those with knockout or blockade demonstrate memory dysfunction (Sarnyai et al., 2000). Animals with 5-HT$_{1A}$ antagonist acute injection into the dorsal raphe show enhanced social defeat behavior (Cooper et al., 2008).

Stress-sensitive cynomolgus monkeys exhibit a reduced number of 5-HT$_{1A}$ receptors in dorsal raphe after stress exposure (Lima et al., 2009). Similarly, exposure to peer-rearing in rhesus monkeys as an early life stressor generally results in lower *in vivo* 5-HT$_{1A}$ receptor concentrations (Spinelli et al., 2009). Likewise, chronic psychosocial stress in tree shrews results in decreased 5-HT$_{1A}$ receptors in prefrontal cortex, hippocampus, and parietal cortex (Flugge, 1995).

Human Postmortem and Anatomical Studies

Furthermore, in human postmortem studies, lower hippocampal 5-HT$_{1A}$ mRNA is demonstrated in MDD subjects, with death by accident, assault, suicide, or cardiac causes (Lopez et al., 1998) and reduced 5-HT$_{1A}$ receptors in amygdala and hippocampus in suicide completers (Cheetham et al., 1990). Finally, 5-HT$_{1A}$ receptor density is related to gray matter volume cortical thickness in many prefrontal and parietal regions in HCs, but not in MDD (Pillai et al., 2018; Zanderigo et al., 2018). Notably, one recent study used PET binding to subdivide clusters in 5-HT function for anatomical parcellation and alignment with resting state networks (Kautzky et al., 2018). Both 5-HT$_{1A}$ and 5-HT$_{2A}$ demonstrate cortical; distribution and alignment with dorsal attention and frontoparietal networks (clusters 2 and 3), suggesting that is alignment between monamine function and cortical networks.

Tryptophan Depletion and Effects on Cognitive Control and Related Functions in MDD

A potentially convergent line of study is a possibility that divergent serotonergic function for some individuals with MDD is related to abnormalities in executive function and affective processing – these are broad domains within Research Domain Criteria [RDoC (Cuthbert, 2005)] that may constitute IPs for MDD. Executive functioning domains include conceptual reasoning, inhibitory control, verbal fluency, interference resolution, working memory – components of the Cognitive Control network. Difficulties in these skills are present in MDD (Austin et al., 1999; Channon and Green, 1999; Rogers et al., 2004; Langenecker et al., 2007b; Snyder, 2013) and lead to work-related disability and productivity loss (Lee et al., 2018). Links between these executive function skills (and cognitive control network function) and serotonergic function are conducted by reducing synthesis of 5-HT centrally via ATD (Lamar et al., 2009; Smith et al., 1999). ATD has also been shown to result in disrupted affective processing and networks (Phillips et al., 2003), increasing negative emotional experience and decreases in positive affective experience [Roiser et al., 2007; Spring et al., 2007; van der Veen et al., 2007]. ATD also disrupts social cooperation (Wood et al., 2006). Some affective domains of interest for MDD are enhanced memory for negative information and disrupted accuracy in processing of facial emotions [Gur et al., 1992; Langenecker et al., 2005, 2007b; Hsu et al., 2010].

In summary, there is a distinct possibility that disrupted 5-HT$_{1A}$ receptor mediated mechanisms might translate to affective and cognitive domains of dysfunction for MDD. Multimodal studies, encouraged by RDoC, can address convergence of multiple different assays. Here, we hypothesized that lower 5-HT$_{1A}$ BP in MDD [Hypothesis (Hyp) 1] maybe related to dysfunction in affective (bottom-up, Hyp 2) and executive (top-down, Hyp 3) domains (Langenecker et al., 2010, 2014; Disner et al., 2011). These affective dysfunction domains included negative memory bias, (Bradley et al., 1996; Hsu et al., 2010) and impaired emotion categorization (Gur et al., 1992; Langenecker et al., 2005, 2007b). We used executive dysfunction domains - previously identified factors in individuals with bipolar disease, similar to domains reported in MDD (Rogers et al., 2004; Snyder, 2013). These factors do not align perfectly with the RDoC domains within Cognitive Systems, although we note that the RDoC domains are suggestive and not prescriptive (Insel et al., 2010; Sanislow et al., 2010). The broader goal is to utilize dimensional, factor-driven analysis in studies where these are experimentally advantageous over DSM categories. Here, Cognitive Control subsumes the elements of (1) speed (Verbal Fluency and Processing Speed), (2) speed in the context of distracting or competing stimuli (Processing Speed with Interference Resolution), (3) stopping a prepotent response (e.g., regulation, here Inhibitory Control), and (4) balance of decision making within multistimulus sets and changing rules (Conceptual Reasoning and Set-Shifting) (Langenecker et al., 2010; Ryan et al., 2013).

We further investigated relationships of fMRI BOLD responses during a Cognitive Control task based upon 5-HT$_{1A}$ BP in the MDD sample, including fMRI BOLD responses based upon degree of 5-HT$_{1A}$ BP$_{ND}$ in the MDD sample [Hyp 4 of lowered 5-HT$_{1A}$ BP$_{ND}$ correlated with lowered activation in Cognitive Control region(s)]. Cross-modality comparisons are relatively rare in MDD (multimodal imaging can be simultaneous or on separate days), but they illustrate the value in integrating localization, function, and neurotransmitter density (Kalin et al., 2008; Selvaraj et al., 2017; Hamilton et al., 2018; Kranz et al., 2018; Piel et al., 2018). Analyses were also conducted with the HC group to verify the general or MDD specific nature of these relationships (Hyp 5). Exploratory analyses with genetic variants related to serotonergic function were also conducted (Hyp 6) (Wojnar et al., 2009; Villafuerte et al., 2009; Mak et al., 2013; Kautzky et al., 2017; Norgaard et al., 2017; Piel et al., 2018; Zanderigo et al., 2018).

MATERIALS AND METHODS

Participants

Twenty-nine HC and 25 patients with MDD were recruited via newspaper advertisements, campus fliers, and word of mouth with Institutional Review Board (IRB)–approved written informed consent consistent with the Declaration of Helsinki

TABLE 1 | Demographic and clinical information for participants with major depressive disorder and matching healthy control adults.

	MDD (*N* = 25)	HC (*N* = 29)
Age	39.7 (11.0)	37.8 (11.8)
Education	15.0 (2.9)	16.1 (2.3)
Shipley estimated IQ	101.8 (13.1)	105.6 (12.1)
Sex	14F, 11 M	18 F, 11M
HDRS-17*	19.0 (2.6)	0.9 (1.4)
Suicide item (0–4)	0.5 (0.9)	–
Neuroticism*	60.3 (11.2)	40.2 (9.6)
Comorbid anxiety Dx	11 (44%)	–

**Groups differ at p < 0.05. Social anxiety (n = 7), PTSD (n = 2), panic (n = 2), generalized anxiety (n = 1). HDRS, hamilton depression rating scale. T-tests were used to compare groups for age, education, estimated IQ, HDRS, neuroticism. Chi-square was used to compare groups by sex distribution.*

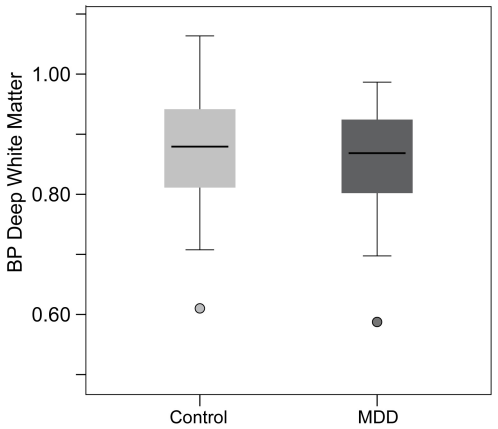

FIGURE 1 | Figure illustrating the 5-HT$_{1A}$ BP values in the deep white matter (DWM) control region. Note that the two individuals (one MDD and one HC) with abnormally high binding potential (BP) in the cerebellar reference region resulted in low values in the DWM region. These two individuals were excluded from all analyses and the MDD and HC did not differ in DWM BP, indicating equivalence in the reference region. Further, no results were changed by including DWM BP as a covariate in analyses.

at the University of Michigan. Diagnosis was confirmed with the Structured Clinical Interview for Diagnostic and Statistical Manual [DSM-IV (American Psychiatric Association, 1994)]. HC subjects were required to be below 5 and MDD subjects above 15 on the Hamilton Rating Scale for Depression for study entry [HRSD, 17 item scale (Hamilton, 1960)], using conservative thresholds for sensitivity and specificity (Naarding et al., 2002; Romera et al., 2011; Sawamura et al., 2018). The groups did not differ in age, sex, years of formal education, or intellectual ability [(Shipley, 1946), all *p*'s > 0.15, **Table 1**]. Other evidence of neurological or psychiatric disorders, other than generalized anxiety and/or social/specific phobia, panic disorder in the MDD sample was exclusionary. Cigarette smokers and those with alcohol abuse or who had used illegal drugs in the past 2 years were excluded. Patients with MDD were unmedicated and had been medication-free for a minimum of 6 months for all potentially psychoactive medications (mean 25.7 months, 14 medication naïve).

Overall Procedure

Neuropsychological measures were typically captured within several days after the intake and diagnosis. fMRI and PET were collected on average 9.5 days (*SD* = 36.8) apart for participants. The SD is large because 2 MDD and 2 HC participants discontinued and then restarted the study about 3–4 months apart. 85% of participants completed all evaluations within 1 month.

PET Scanning and Processing Procedures

Positron emission tomography scanning was conducted using [^{11}C]Way100635. PET procedures were similar to those described previously (Mickey et al., 2008). PET images were acquired with a Siemens/CTI HR^{+} scanner in three-dimensional mode with septa retracted. [carbonyl- 11C]WAY-100635, a specific 5-HT$_{1A}$ receptor antagonist, was synthesized at high specific activity (Hwang et al., 1999). The tracer was administered as a bolus followed by continuous infusion to more rapidly achieve steady-state conditions. Eighteen scans of increasing duration (0.5–10 min) were acquired over a period of 90 min. Raw PET images were co-registered and smoothed with a Gaussian filter (4 mm FWHM). Smoothed images were transformed voxel-by-voxel into parametric maps of tracer transport (K_1 ratio) and specific binding [distribution volume ratio (DVR)] using a modified Logan graphical analysis, with bilateral cerebellar white matter (excluding the vermis) as the reference region (Logan et al., 1996). Non-displaceable binding potential (BP_{ND}) was defined as BP_{ND} = DVR-1 = k_2B_{max}/K_D, where B_{max} is the total receptor concentration, K_D is the dissociation constant, and k_2 is the extracellular concentration of tracer (assumed to be a small and constant value) (Stange et al., 2017). Two individuals (one control and one MDD) showed visible binding in the cerebellum and (as a result) anomalously low global BP_{ND} (2.4–3.0 SDs below the mean, illustrated in **Figure 1**), and were excluded.

Positron emission tomography images were coregistered with MRI images to allow anatomical localization of PET data. Coregistration was accomplished for each subject by alignment of K_1 images with MRI SPGR images using co-registration within SPM2. MRI data were subsequently transformed into standardized coordinates (International Consortium for Brain Mapping; Montreal Neurological Institute) by linear and non-linear warping, and the resulting transformation matrix was applied to parametric PET images.

Although not central to the current study or hypotheses, we specifically addressed the concern that lowered 5-HT$_{1A}$ BP_{ND} in MDD is a function of differences between HC and MDD in the cerebellar white matter reference region. Without an arterial reference point, we instead added a deep white matter (DWM) ROI within the centrum semiovale for test comparisons between MDD and HC subjects. This technique capitalizes on modeling DWM as a constant in the equation. Without any receptors, DWM would be a constant including noise – the only variable free to vary in the equation is BP within the cerebellar

white matter reference region (including noise). There were no significant differences between groups in 5-HT$_{1A}$ BP$_{ND}$ in the DWM of the centrum semiovale [$t(41) = 0.55$, $p = 0.59$]. The reference region BP was equivalent between groups, increasing confidence that effects reported herein are contingent upon inherent regional differences in BP between MDD and HC groups in the regions specified (**Figure 1**).

Candidate Affective and Executive Domains Relevant to MDD

The processing speed with interference resolution includes the trail making test, digit symbol substitution test, stroop color-word test, and response time to targets from the parametric go/no-go test. The parametric go/no-go test was programmed in EPrime 2 completed before the scanning session for practice (Langenecker, 2001; Langenecker et al., 2007a,b,c, 2018a; Votruba and Langenecker, 2013). It was also completed during fMRI. There are three levels of difficulty, including a 3 target Go-only condition, and 2 target alternating target Go/No-go condition, and a 2 target alternating target Go/No-go condition. There are 68 "lure" events so that correct and incorrect rejections of lures can be modeled and analyzed separately.

There are also less prominent potential domains/factors, less strongly linked to risk for MDD or BD, comprising Verbal Fluency with Processing Speed, Inhibitory Control, and Conceptual Reasoning and Set Shifting, and tests from these factors have been demonstrated to be dysfunctional in previous studies of MDD (see Langenecker et al., 2009 for a review). Negative Memory Bias (NMB) was calculated as a subtraction of percentage of negative words recognized from the percentage of neutral words recognized from within the Emotion Words task programmed in EPrime 2 (Hsu et al., 2010). In addition to the Negative Memory Bias, we also used performance accuracy in Emotion Classification of faces as potential Affective Processing domains in MDD that would be linked to abnormal 5-HT$_{1A}$ BP$_{ND}$ (Langenecker et al., 2005).

MRI for Co-registration of PET Images and Collection of fMRI BOLD

One hundred twenty-four high-resolution SPGR axial anatomic images [TE = 5 ms; TR (repetition time) = 24 ms, 45 degree flip angle, NEX (number of excitations) = 2, slice thickness = 1.2 or 1.3 mm, FOV = 24 cm, matrix size = 256 × 256] were performed on each subject with a GE 3T Signa scanner for coregistration of PET images.

The Go/No-go task is a cognitive control task that has been used extensively by our group with fMRI, including in healthy aging, MDD, and bipolar disorder (for review, see Votruba and Langenecker, 2013). The fMRI task includes event-related models for correctly responded "go" events or Hits, correctly rejected "no-go" events or Rejections, and incorrectly responded "no-go" events, or Commissions, modeled with the hemodynamic response function. The steps for processing the data and model building include slice timing, physiological correction, coregistration, normalization, smoothing with a 5 mm FWHM Gaussian filter, and building individual models using SPM2 as described previously (Langenecker et al., 2007c). Contrasts were set up to define activation for Hits, Rejections, and Commissions in a fast event-related model. Imaging parameters include a TR of 2000 ms, FOV of 22 cm, with a 3.0 T GE Signa scanner using a standard radio frequency coil and T2*- weighted pulse sequence. The images were collected using a forward-reverse spiral sequence with 29 axial slices of 4 mm.

Defining Regions of Significant Effect in 5-HT$_{1A}$, and Low and Normal MDD Groups

Differences between groups in 5-HT$_{1A}$ BP$_{ND}$ will be extracted from regions of significant effects (RSEs). 1st 5-HT$_{1A}$ BP$_{ND}$ levels will be converted to z scores based upon mean and standard deviation of BP$_{ND}$ levels for the HC group for each RSE. Then the z scores will be averaged across all RSEs to create a mean Z Group RSE variable across all post-synaptic 5-HT$_{1A}$ regions that differ between groups. Mean Z group RSE will be used as predictor variable in subsequent analyses with performance and fMRI IPs. We will use mean 5-HT$_{1A}$ BP$_{ND}$ PET results in RSEs to define low and normal 5-HT$_{1A}$ BP$_{ND}$ MDD groups in relation to HC 5-HT$_{1A}$ BP$_{ND}$ in the RSEs. These two MDD groups will then be compared to identify regions for fMRI analyses in the imaging contrasts (Commissions, Correct Rejections, Hits) for the Parametric Go/No-go test.

Genotyping for 5HTTLPR and MAO-A

In addition, for exploratory purposes, the relative impact for *5-HTTLPR* and *MAO-A* genotype were evaluated, genes with a significant biological relationship with 5-HT$_{1A}$ BP.

5HTTLPR

Genotyping protocols were performed according to Lesch et al. (1996). The *5-HTTLPR* assay discriminates between two functional *5-HTT* promoter alleles, visualized as DNA bands of 528 bp and 484 bp (long and short alleles, l and s, respectively). Genotypes were grouped in accordance with *in vitro* data on a reduced transcriptional activity of the dominating s allele that leads to a decrease in central 5-HT turnover (Bennett et al., 2002). Ten individuals did not have *5-HTTLPR* genotype obtained (6 HC, 4 MDD).

MAO-A

Genomic DNA was purified from blood using standard methods. The *MAOA* promoter region that contains the upstream VNTR polymorphism (Sabol et al., 1998) was amplified from 10 ng genomic DNA using the primer sequences: Forward 5 CCCAGGCTGCTCCAGAAACATG-3 and Reverse 5′-GTTCGGGACCTGGGCAGTTGTG-3′. Because of the high GC content in the VNTR region, amplification was performed using Invitrogen's PlatinumTaq and PCRX. Twenty-three individuals (12 HC, 11 MDD) did not have *MAO-A* genotype obtained.

Statistical Analyses

First, we identified MDD specific regions of low 5-HT$_{1A}$ BP$_{ND}$ as described in the results section. We compared MDD and HC

TABLE 2 | Regions of significantly effect, with lower 5-HT$_{1A}$ BP$_{ND}$ in MDD relative to HC.

Foci	BA	mm^3	x	y	z	*Z*	*p*
Uncus	20	1344	26	−5	−34	4.38	0.000034
Hippocampus		1536	−25	−13	−20	4.24	0.000053
Parahippocampal	35	1216	−32	−21	−19	3.96	0.00013
Superior temporal	38	960	22	10	−37	4.17	0.000068
Fusiform	37	2624	41	−62	−7	3.78	0.00023
Precuneus	7	832	24	−69	33	3.66	0.00033

There were no regions where MDD group had greater BP relative to the HC group.

groups in 5-HT$_{1A}$ BP$_{ND}$ using a combined threshold of $p < 0.001$ and a cluster minimum of 80mm^3 was used between groups *t*-test in SPM2 (**Table 2** and **Figure 5**). Mean 5-HT$_{1A}$ BP$_{ND}$ was extracted for these Regions of Significant Effects (RSEs) and BP$_{ND}$ was used in group specific linear regressions in SPSS 22 with behavioral performance measures of affective processing (Negative Memory Bias, Emotion Categorization) and executive functioning (Processing Speed with Interference Resolution, Verbal Fluency with Processing Speed, Inhibitory Control, and Conceptual Reasoning and Set Shifting) factors/scores were evaluated as converging, multimodal candidate IPs using MANOVA in SPSS 22. fMRI BOLD activation differences were also investigated in SPM5 factorial model comparisons of normal and low 5-HT$_{1A}$ BP$_{ND}$ MDD groups based upon mean 1A BP$_{ND}$ (with DWM BP as a covariate of no interest). Relationships between fMRI BOLD signal differences were evaluated subsequently with correlations with RDoC Domains scores in SPSS 22. These domains were also evaluated in exploratory analyses for serotonin-related genetic effects using *5-HTTLPR* and *MAO-A*.

RESULTS

Defining Regions of Low 5-HT$_{1A}$ Binding Potential in the MDD Group

Regions of significant BP differences between HC and MDD groups (HC > MDD) were used to define low 5-HT$_{1A}$ BP$_{ND}$ MDD regions of significant effect (hereafter RSE). The whole brain analyses in MDD vs HC with 5-HT$_{1A}$ BP$_{ND}$ indicated six RSEs of lower 5-HT$_{1A}$ BP$_{ND}$ in the MDD group relative to the HC group. These regions, predominantly temporal, are reported in **Table 2** and **Figure 5**, defining 10–20% reduction in post-synaptic 5-HT$_{1A}$ BP$_{ND}$ in MDD across these regions. There were no regions where MDD group had greater BP$_{ND}$ relative to the HC group. Half of the MDD group was below the 5th percentile of 5-HT$_{1A}$ BP$_{ND}$ for the Z normed average of the RSEs relative to the HC group.

Impact of Low 5HT1a BP$_{ND}$ on Executive Functioning and Affective Processing IPs in MDD

We investigated the linear relationship of IPs (e.g., Processing Speed with Interference Resolution, Inhibitory Control, and Negative Memory Bias) with mean rank 5-HT$_{1A}$ BP using regression in SPSS 22. Negative Memory Bias accounted for 16.4% (no difference after accounting for age) and Processing Speed with Interference Resolution accounted for 26.3% (35.4% after accounting for age effects) of mean rank BP$_{ND}$ in the MDD group (covarying DWM DVR, *p*'s = 0.074, 0.029, respectively, **Figure 2**). No other IPs were significantly related to mean rank 5-HT$_{1A}$ BP$_{ND}$ in the MDD group. In the control group, <1% variance in processing speed with interference resolution or negative memory bias was accounted for by mean rank BP$_{ND}$. Negative memory bias and processing speed with interference resolution were non-significantly correlated ($r = -0.33$, $p = 0.17$).

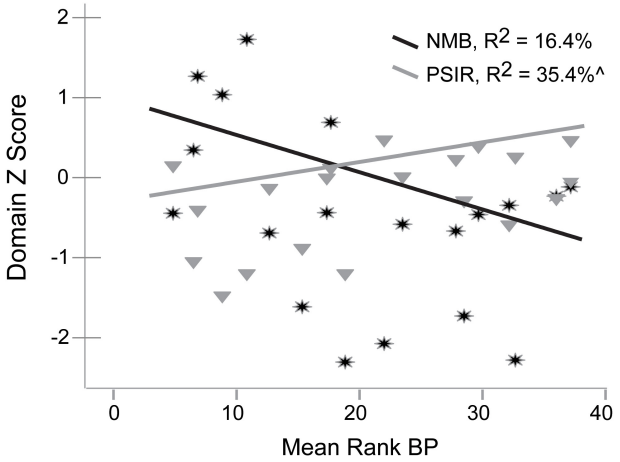

FIGURE 2 | Relationships of Negative Memory Bias (NMB) and Processing Speed with Interference Resolution (PSIR) variables with mean rank 5-HT$_{1A}$ BP in the MDD group.

Lowered Mean 5-HT$_{1A}$ BP in Relation to fMRI BOLD Responses to Hits, Rejections, and Commissions Within the MDD Group

It was expected that abnormalities in executive functioning domains based upon mean 5-HT$_{1A}$ BP$_{ND}$ would also be related to BOLD fMRI differences. As this has not been evaluated in published studies, there was no clear expectation of hyper or hypo (our hypothesis) activation during the Cognitive Control task for low vs normal 5-HT$_{1A}$ BP groups. These contrasts in SPM5 included in separate models for BOLD responses during Hits, Correct Rejections, and Commissions. Rejections and Commissions are used to calculate Inhibitory Control, which would be expected to be related to 5-HT$_{1A}$ BP$_{ND}$ based upon relationships illustrated in **Figure 3**. Processing speed with interference resolution includes response speed to Hits and Conceptual Reasoning with Set Shifting includes Hit accuracy, suggesting that these Hit events should also be related to 5-HT$_{1A}$ BP$_{ND}$. There were some individuals without fMRI scans, some with abnormal DVR in the cerebellum (see section "Materials and Methods"), and some with low IQ, leaving, 17 MDD subjects

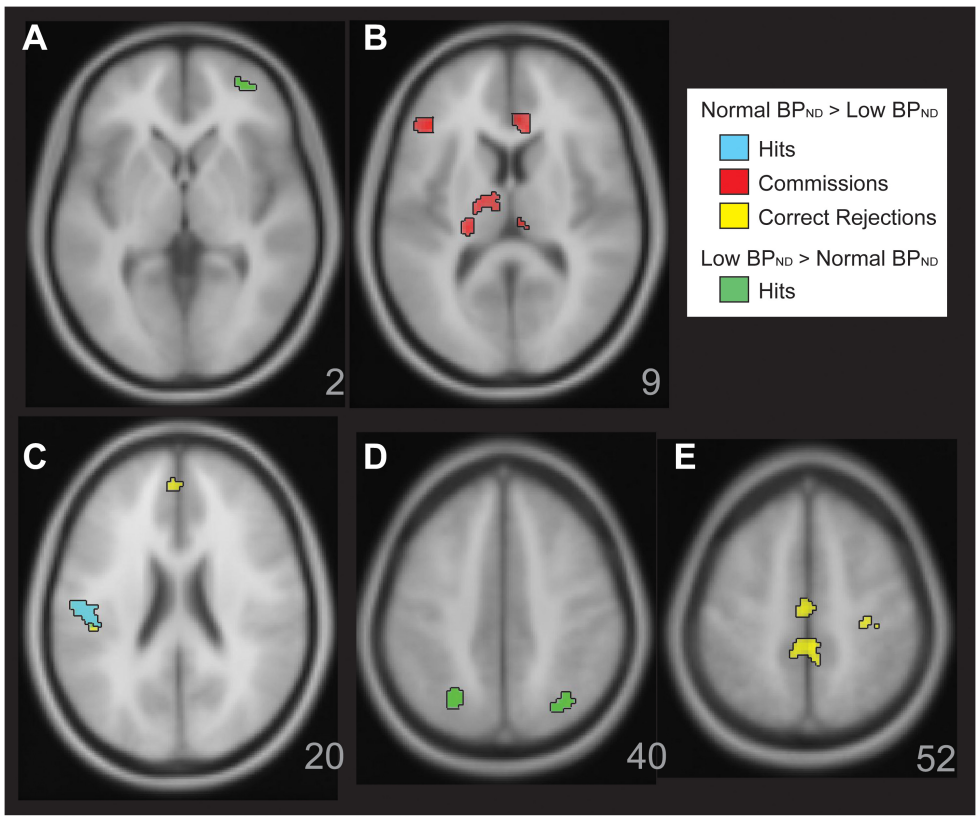

FIGURE 3 | Figure illustrates significant BOLD activation relationships with 5HT-1a BP in the MDD group. There was greater BOLD activation with normal 5-HT$_{1A}$ BP MDD in the Parametric Go/No-go test. This is shown for correct Hits (Panel **C,D**, cyan), for Commissions (Panel **B**, red), Correct Rejections (Panels **C,E**, yellow). There were also a few areas of greater activation for correct Hits with low 5-HT$_{1A}$ BP in the MDD group (Panels **A,D**, green).

available for fMRI analyses (divided into normal and low, in a model with 19 HCs). Whole brain analyses were conducted using combined height and extent thresholds with 3dClustSim ($p < 0.005$, $k > 55$, 1000 Monte Carlo simulations, $p < 0.05$ whole brain adjusted).

Within the MDD sample, there was a general pattern of greater activation with increasing mean 5-HT$_{1A}$ BP$_{ND}$. For Correct Rejections (yellow, **Figure 3**, Panels C, E), this was observed in dorsal anterior cingulate, postcentral gyrus, left posterior insula, and mid cingulate gyrus RSEs. There was increasing activation in rostral anterior cingulate, left inferior frontal gyrus, bilateral dorsal medial thalamus, and pulvinar RSEs with greater mean 5-HT$_{1A}$ BP$_{ND}$ in relation to Commissions. There was greater activation for Hits in a left posterior insula RSE related to mean 5-HT$_{1A}$ BP$_{ND}$ (cyan, **Figure 3**, Panel C). The exception to this general pattern of increased activation with increasing mean 5-HT$_{1A}$ BP in MDD was observed for Hits in bilateral superior parietal lobule and right anterior inferior frontal gyrus, where there was decreasing activation as mean 5-HT$_{1A}$ BP$_{ND}$ increased (green, **Figure 3**, Panels A and D).

We further investigated dimensional, linear links between these multimodal IPs using pairwise correlations between the mean rank 5-HT$_{1A}$ BP$_{ND}$, mean rank for the combined fMRI BOLD RSEs (by condition), and behavioral performance parameters. These fMRI BOLD RSE clusters were combined by condition and group difference for purposes of data reduction, with the resulting mean Z BOLD RSE scores highly correlated with all individual clusters (r's > 0.59 for Commission clusters, r's > 0.68 for Correct Rejection clusters, r's > 0.78 for Hits clusters, p's < 0.001). As illustrated in **Figure 4**, the mean Z fMRI BOLD RSEs were significantly correlated with mean rank 5-HT$_{1A}$ BP for Hit BOLD RSE Normal > Low ($r = 0.73$, $p < 0.001$, **Figure 4**, Panel A), Hits mean Z BOLD RSEs Low > Normal ($r = -0.80$, $p = 0.0001$, Panel B), Commissions mean Z BOLD RSEs Normal > Low ($r = 0.87$, $p = 0.0001$, Panel E), and Rejections mean Z BOLD RSEs Normal > Low ($r = 0.77$, $p = 0.0001$, Panel C). PSIR Z score was positively correlated with fMRI BOLD Hit RSE (Normal > Low, $r = 0.54$, $p = 0.02$) and fMRI BOLD Commission mean Z RSEs (Normal > Low, $r = 0.53$, $p = 0.03$, Panel F). Negative Memory Bias Z was significantly positively correlated with fMRI BOLD Hit mean Z RSEs (Low > Normal, $r = 0.50$, $p = 0.04$) and negatively with fMRI BOLD Rejections mean Z RSEs (Normal > Low, $r = -0.59$, $p = 0.01$, Panel D). Information from 19 HCs with all modalities of measurement included – are added to these scatterplots (**Figure 4**) for comparison. The scatterplots indicate individual level differences in fMRI for Cognitive Control that relate to 5-HT$_{1A}$ BP$_{ND}$ and PSIR.

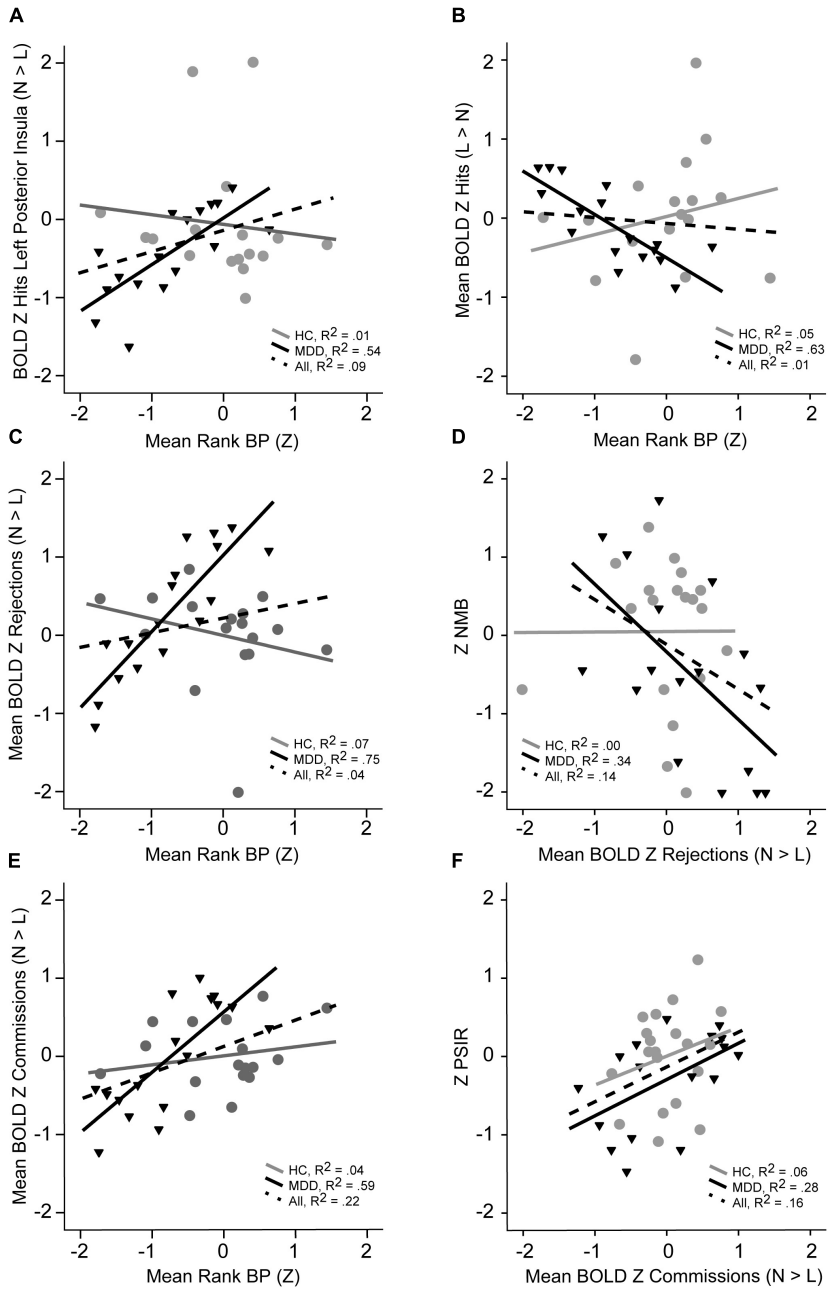

FIGURE 4 | Illustration of linear relationships by group for 5-HT$_{1A}$ BP, fMRI BOLD signal, and neuropsychological performance measures (MDD in black, HC in gray, dashed line for both). Panel **A** shows the relationship between BOLD activation or Hits in left posterior insula with mean 5-HT$_{1A}$ BP rank. Panel **B** illustrates the relationship between mean BOLD signal RSEs for correct Hits that are greater in normal relative to lower 5-HT$_{1A}$ BP in MDD with mean 5-HT$_{1A}$ BP rank. Panel **C** depicts the relationship between mean BOLD for correct rejections and mean 5-HT$_{1A}$ BP rank. The relationship between mean BOLD for correct rejections and NMB in shown in Panel **D**. Panel **E** illustrates the mean BOLD for commission errors with mean 5-HT$_{1A}$ BP rank. The relationship between mean BOLD for commission errors and processing speed with interference resolution is shown in Panel **F**.

Exploratory Analyses of *5-HTTLPR* and *MAO-A* Effects in Executive Functioning and Affective Processing IPs

Next, we expected that the low functioning forms of either the *MAO-A* and *5HTTLPR* genotypes would be associated with poorer performance irrespective of group for candidate genes in exploratory analyses. Given the small sample size, and the relatively weak link between Cognitive/Affective IPs and functional polymorphisms that might impact 5-HT$_{1A}$, the probability of type II error is high. The MANCOVA for *5-HTTLPR* (diagnosis as covariate) was significant for Conceptual Reasoning and Set Shifting [$F(1,29) = 4.29$, $p = 0.047$,

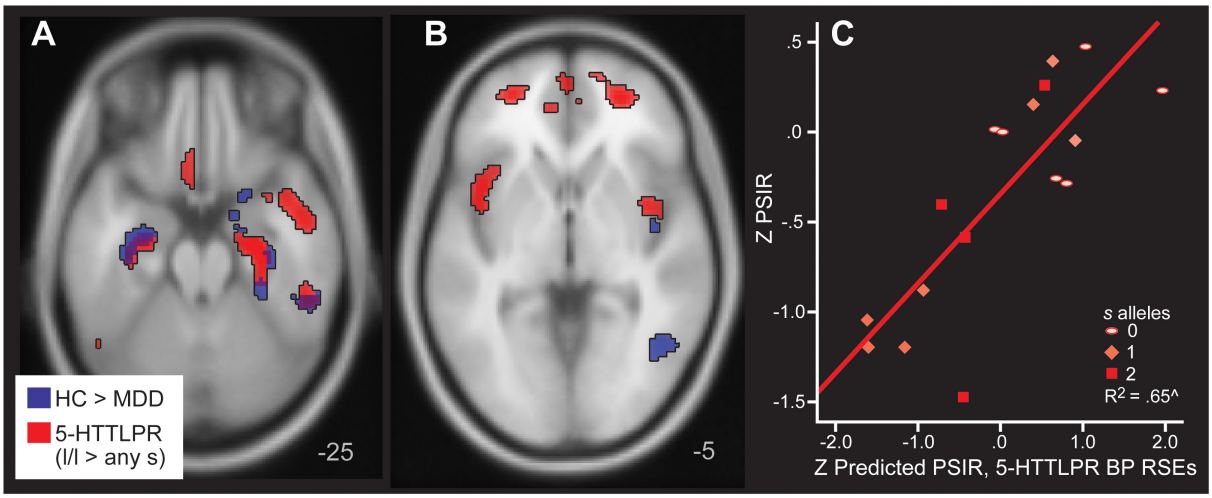

FIGURE 5 | Areas of greater 5-HT$_{1A}$ BP in HC relative to MDD (blue) and in *l* homozygotes for *5-HTTLPR* relative to *s/s or l/s* (red) in Panels **A,B**. Note that the blue clusters are the same as those listed in **Table 2**, which were used to define the regions of significant effect that defined mean rank BP regressors. They are included for comparison with the *5-HTTLPR* analysis here to show the similarities in location and direction. Panel **C** depicts the actual and predicted PSIR values. The predictions are based upon mean 5-HT$_{1A}$ BP values from the regions of significant effect (RSEs) in the *5-HTTLPR* analysis.

E^2 = 0.13] and Processing Speed with Interference Resolution [$F(1,29)$ = 5.93, p = 0.02, E^2 = 0.17], with poorer performance in *s* allele carriers irrespective of group status. For definition of high and low functioning *MAO-A* genes, the intermediate genotype group of women was placed into the low functioning allele group based upon prior results (Austin et al., 1999) There was a significant effect for genotype on Conceptual Reasoning and Set Shifting [$F(1,26)$ = 5.73, p = 0.02, E^2 =0 .18] and a trend for Emotion Categorization [$F(1,26)$ = 3.35, p = 0.08, E^2 = 0.11]. Those with low function alleles for *MAO-A* performed better on Conceptual Reasoning and Set Shifting and marginally worse on Emotion Categorization.

5-HT$_{1A}$ BP$_{ND}$ Links to *5-HTTLPR* Genotype and Relationship With PSIR

Next, we evaluated specifically the effect of *5-HTLPPR* genotype on 5-HT$_{1A}$ BP$_{ND}$ reductions, covarying for disease group. Group results based upon disease (from **Table 2**) and genotype are displayed in **Figure 5** (Panels A and B). Those with the *5-HTTLPR s* allele, irrespective of disease status, exhibited lower 5-HT$_{1A}$ BP$_{ND}$ in fronto-temporal regions, overlapping with temporal regions that were lower in those with MDD. There were additional frontal regions of lower 5-HT$_{1A}$ BP$_{ND}$ in *s* allele carriers irrespective of diagnosis.

Mean rank order BP based upon *5-HTTLPR* was averaged across all 16 RSEs of greater BP in *l/l* homozygotes relative to the *s* allele carriers. Regression was used to predict Processing Speed with Interference Resolution based upon *5-HTTLPR* and also using mean rank BP from the *5-HTTLPR* RSEs. Fifty-two percent of Processing Speed with Interference Resolution was explained by 5-HT$_{1A}$ BP$_{ND}$ regions with significantly low BP extracted in those with an *s* allele (covarying age and DWM DVR, B = 0.79, p = 0.001, **Figure 5**, Panel C). 5-HT$_{1A}$ RSEs defined by MDD vs control and by *5-HTTLPR* were highly correlated (r = 0.88, p < 0.001) in 5-HT$_{1A}$ BP$_{ND}$. MDD and *5-HTTLPR s* allele were retained as independent variables in this analysis. No other regression models reached significance when using mean rank BP from the *5-HTTLPR* RSEs to predict Negative Memory Bias, Emotion Categorization, Inhibitory Control, or Conceptual Reasoning with Set Shifting.

DISCUSSION

The present study is the first to link abnormal 5-HT$_{1A}$ BP$_{ND}$ measures in unmedicated, symptomatic patients with MDD to objective performance and imaging markers of illness, in this case interference resolution, a component of Cognitive Control. The separation of interference resolution performance by 5-HT$_{1A}$ levels is marked, with a medium-large effect size. The abnormal 5-HT$_{1A}$ BP$_{ND}$ is also related to fMRI BOLD hypoactivation changes in a Cognitive Control domain during Inhibitory Control, the Parametric Go/No-Go Test. The impact of *5-HTTLPR* genotype upon interference resolution and 5-HT$_{1A}$ is modest and significant. The results follow previous studies showing links between 5-HT$_{1A}$ BP$_{ND}$ values with clinical factors such as anxiety symptoms, treatment outcome, genetics, and sex (Bhagwagar et al., 2004; Drevets et al., 2007; Hirvonen et al., 2008; Miller et al., 2008; Parsey et al., 2010). There is also evidence that lower 5-HT$_{1A}$ levels are present when there are increased depression symptoms in the context of epilepsy, Parkinson disease and in chronic stress without depression (Jovanovic et al., 2008). Using objective, but simpler, performance measures to identify subjects with a higher probability of abnormal 5-HT$_{1A}$ BP$_{ND}$ could have substantial benefits for clinical, genetic and research studies. The executive functioning measures used to derive the processing speed with interference resolution

variables are inexpensive to administer and are easily employed in subject recruitment (even clinical) settings (Langenecker et al., 2007b; Dawson et al., 2017). These measures could be used to select individuals for treatments or research protocols that specifically target 5-HT$_{1A}$ receptor functioning and for subtype-specific pharmacotherapy treatment trials. Analogs of these performance measures are already present in animal models to further aid in strategies for better understanding the neurobiology and genetics of depression and for new treatment development.

In the data presented it was striking that Cognitive Control, and not Negative Memory Bias (inverted effect) or Emotion Categorization, was related to lower 5-HT$_{1A}$ BP$_{ND}$ in MDD. Indeed, recent studies have demonstrated that executive functioning measures, an umbrella domain term that includes Cognitive Control, are perhaps most critical in understanding increased risk for MDD, and are observed in the remitted state, and in family relatives of those with mood disorders (Clark et al., 2005; Bora et al., 2009; Peters et al., 2017). A recent review illustrated how executive dysfunction for those with MDD is substantial and fairly consistent across well-powered studies (Rogers et al., 2004). Some existing literature, although mainly with small N studies, suggest that executive functioning is also a good predictor of treatment response and functioning in MDD (Kampf-Sherf et al., 2004; Taylor et al., 2006; Jaeger et al., 2007; Dawson et al., 2017). Executive functioning also can be used to predict recurrence (Langenecker et al., 2018a) and workplace disability (Lee et al., 2018).

A recent review of 5-HT$_{1A}$ receptor studies suggested that there is a weak relationship between 5-HT$_{1A}$ and cognitive function (Borg, 2008). One of four studies in healthy controls have illustrated a relationship between 5-HT$_{1A}$ BP$_{ND}$ and cognitive performance (Yasuno, 2004). A pilot study in those with Alzheimer's disease, mild cognitive impairment (MCI), or neither suggested a relationship of decreased 5-HT$_{1A}$ BP$_{ND}$ with poorer MMSE in the entire sample, and with learning and memory in the healthy control and MCI participants (Kepe et al., 2006). Another study suggests that gray matter thickness in key limbic regions is positively associated with 5-HT$_{1A}$ (Kraus et al., 2012). A similar study shows these associations in fronto-limbic regions (Zanderigo et al., 2018). In our control sample we replicate the pattern of non-significant relationships of 5-HT$_{1A}$ BP$_{ND}$ to cognitive and affective measures. However, in MDD, we demonstrate a significant positive relationship of 5-HT$_{1A}$ with Negative Memory Bias and Inhibitory Control, and a significant negative relationship of 5-HT$_{1A}$ with Processing Speed with Interference Resolution.

fMRI BOLD signal changes for correct rejections and errors of commission resulted in hypoactivation in critical regulatory and inhibitory regions for those with lower 5-HT$_{1A}$ BP$_{ND}$. These low BOLD signals were related to Negative Memory Bias and Processing Speed with Interference Resolution. Notably, those MDD with lower 5-HT$_{1A}$ BP$_{ND}$ levels show increasing difficulties with poorer set-shifting and processing speed. In contrast and with an intriguing result, MDD subjects with higher/normative 5-HT$_{1A}$ BP$_{ND}$ levels exhibited significant Negative Memory Bias and increased activation in regulatory regions during successful rejection. As a result, increased need for recruitment in the mid-dorsal and rostral anterior cingulate for successful rejection may reflect poorer Inhibitory Control in those without lower 5-HT$_{1A}$ BP$_{ND}$ levels. This observation is confirmed by performance above normal levels in Inhibitory Control as 5-HT$_{1A}$ BP$_{ND}$ levels decreased. Those with higher/normative5-HT$_{1A}$ BP$_{ND}$ 5-HT$_{1A}$ BP$_{ND}$ levels tended to have worse Negative Memory Bias, Inhibitory Control, and increased BOLD recruitment for successful rejection of prepotent stimuli. Although the sample is quite small, these results reaffirm with other work that there likely many circuits, neurotransmitters, and behaviors associated with subtypes of MDD that are heretofore unclear (Webb et al., 2016; Kling et al., 2018).

Further, *5-HTTLPR* appears to be related to both 5-HT$_{1A}$ BP$_{ND}$ levels, and executive functioning performance, irrespective of illness. Notably, samples of this small size often suffer from difficulty with replication and should be interpreted very cautiously. There are some clues, however, that interference resolution might explain the inconsistent findings in emotion processing studies of *5-HTTLPR* from other studies as well. For example, affective processing and executive regulation can be in dynamic opposition to one another in some contexts, or in the case of psychiatric illness, there may be excessive responses in the former *and* weaker control in the latter (Phillips et al., 2003; Kampf-Sherf et al., 2004; Langenecker et al., 2007c, 2014). Presence of the low functioning alleles of *5-HTTLPR* may reflect a relatively weaker executive functioning system, resulting in a stronger environmental dependence in the development of and execution of emotion regulation (Jacobs et al., 2006; Dannlowski et al., 2008; Lohoff et al., 2014; Piel et al., 2018). In non-stressful environments, this weakness is less likely to result in problematic outcomes, but could still result in excessive responses to negative emotional stimuli (Sen et al., 2004; Surguladze et al., 2008). This pattern of diminished regulation skill in those carrying the short allele may be exaggerated in MDD (Dannlowski et al., 2008). In high demand, high stress, negative environments, there may be greater difficulty in regulating negative emotional responses, and greater difficulty in shifting from one emotional state to another for those with low functioning *5-HTTLPR* alleles (Jacobs et al., 2006; Neumeister et al., 2006; Piel et al., 2018). The lack of regulation or emotional flexibility to environmental demands could then perpetuate depressive symptoms.

Use of screening tools like PSIR measures, with knowledge of convergent results with the 5-HT$_{1A}$ BP$_{ND}$ levels may be one dimensional way of increasing the homogeneity in MDD samples. Such increased homogeneity could lead to more targeted, precision medicine trials. For example, preselecting individuals based upon poor Cognitive Control could lead to a larger percentage with low 5-HT$_{1A}$ BP$_{ND}$, facilitating identification of related biomarkers for treatment. As we already know that weaker CC is a predictor of poor treatment response, greater likelihood of recurrence, these individuals might benefit from different treatment algorithms [e.g., TMS trials (Kampf-Sherf et al., 2004; Januel et al., 2006; Siegle et al., 2006; Langenecker et al., 2007c,d, 2018a,b; Levkovitz et al., 2009; Drysdale et al., 2016; Crane et al., 2017; Dawson et al., 2017; Natania et al., 2018)].

It is also notable that Emotion Categorization accuracy, although related at a trend level to MAO-A genotype, was not related to 5-HT_{1A} BP_{ND} levels or *5-HTTLPR*. More surprisingly, higher mean rank BP was associated with *greater* Negative Memory Bias in MDD. Those with lower 5-HT_{1A} BP_{ND} exhibited no evidence of a Negative Memory Bias, suggesting that using mean rank BP to illustrate dimensional functioning in MDD could be defined in part by the segregation of Affective and Executive Domains. In reality, though, risk for MDD is likely defined by affective dysfunction, executive dysfunction, *or* dysfunction in both systems, and it is unlikely to be so clearly illustrated based upon results from one ligand. Several recent studies reported mixed results in links between serotonin genes and emotion reactivity (Kranz et al., 2018; Piel et al., 2018).

The main limitation of the study is the sample size. We were able to obtain a relatively large sample for a multimodal study, and we were also able to find strong biological links between different measurement modalities. Samples of this size only reliably find large and very large effect sizes. We did obtain robust results consistent with our hypotheses. Another potential limitation of the present study relates to the broader difficulty within the field to agree upon the best reference strategy for calculating 5-HT_{1A} BP_{ND}. Although we have shown equivalence in our reference region between groups. There are challenges to the reference region approach that appear to be surmountable by excluding gray matter and vermis, such as in this work (Parsey et al., 2010), and verifying equivalence of the reference region, as we have done. Animal models, especially those of 5-HT_{1A} knockout mice, suggest that there may be lower 5-HT_{1A} availability in MDD and similar states, resulting in decreased serotonergic regulation. Likewise, postmortem data also suggests decreased 5-HT_{1A} mRNA in MDD. These findings are contrasted with others suggesting that higher 5-HT_{1A} in mice can mimic aspects of autism and not anxiety/depression. We contend that the present results strengthen evidence that lower 5-HT_{1A} BP_{ND} is a viable IP in MDD. This is in part upon the clear lack of BP difference in a deep white matter region, the presence of a behavioral performance correlate, links to fMRI BOLD, and link to a genetic marker *5-HTTLPR*. At the very least, the fact that 5-HT_{1A} BP_{ND} is altered in some individuals with MDD is clear. Until the discrepant reference region/correction methods can be resolved, directionality is still contested. Individual studies will have to demonstrate equivalence of reference ranges/structures. Furthermore, newer radioligands such as (Elton et al., 2014) MPFF might have more sensitive and stable properties for investigation of 5-HT_{1A} BP_{ND} with MDD subjects absent these reference region concerns (Lothe et al., 2012).

In addition, fMRI is expensive and requires extensive equipment for set-up and analysis. A lower cost alternative to measure hemoglobin changes during task may be functional near-infrared spectroscopy (fNIRS) (Ho et al., 2016). As a number of the imaging regions identified here were cortical, it is possible that fNIRS could be used less expensively and with a broader range of patients to understand the relationship of hemodynamic changes to lowered 5-HT_{1A} function and cognitive control (McKendrick et al., 2015). Finally, neuropsychological testing, fMRI and PET were typically collected on separate days and locations. The anatomical cross-localization could be off in such instances, and the measurements could be weakened by day-session specific parameters (Selvaraj et al., 2017; Hamilton et al., 2018). As the relationships were quite robust, there may have been additional links that were missed.

5-HT_{1A} function has a number of associations with other chronic diseases that are often comorbid with MDD, including coronary artery disease and obesity (Vickers and Dourish, 2004; Ramage and Villalon, 2008; Quek et al., 2017; Ho et al., 2018), and changes in 5-HT_{1A} function are coassociated with changes in pro-inflammatory cytokines including interleukin-1 beta (IL-1β), IL-17, and tumor necrosis factor – alpha (TNF-α) (Aune et al., 1993; Lu et al., 2017; Ng et al., 2018). These studies suggest that 5-HT_{1A} shares functions that are related to, but extend beyond the phenotype of MDD, which in light of the present study, further confirms a need for homogeneous subsets that can be used to explore specific biological pathways for illness and recovery.

CONCLUSION

In conclusion, the present study offers promising new evidence that biomarkers for MDD can be found and objectively measured. The heterogeneity of MDD has been problematic in pursuing these biomarkers, and identification of subtypes of MDD may, in the end, prove to be the most fruitful in linking biomarkers, to phenotypes, to genetic risks, and ultimately to personalized medicine. The phenotypic heterogeneity of MDD, combined with prior attempts at a "one size fits all" biomarker approach to MDD has been one limiting factor in this complex illness. Future work can capitalize upon the relationship between 5-HT_{1A} BP_{ND} abnormalities and executive functioning in MDD. These links could also be pursued more broadly in other psychiatric conditions within the RDoC initiative, as executive functioning disruption is not specific to MDD.

ETHICS STATEMENT

The work was approved by the University of Michigan IRB. Written informed consent was obtained from all participants.

AUTHOR CONTRIBUTIONS

SL designed the study, analyzed the data, and wrote and edited the manuscript. BM performed the PET and cognitive analyses, and wrote and edited the manuscript. PE, SK, and TL performed the PET analysis, and wrote and edited the manuscript. SS performed the genetics analysis, and wrote and edited the manuscript. KE performed the analysis, and wrote and edited the manuscript. MH and SR wrote and edited the manuscript. DH performed the fMRI and PET analyses, and wrote and edited the manuscript. RK designed PET, and edited the manuscript. SW and HA designed the study and edited the manuscript. DG performed the genetics, analysis and edited the manuscript. MB designed the study, performed the genetics analysis, and edited the manuscript. J-KZ designed the study, and wrote and edited the manuscript.

FUNDING

NIMH grants P01 MH42251 (J-KZ, Elizabeth A. Young, HA), K23 MH074459 (SL), and the National Alliance for Research on Schizophrenia and Depression (NARSAD, SL, J-KZ). SL was supported by RDoC grant MH101487. Also supported by the General Clinical Research Center at the University of Michigan (NIH grant RR00042), now MICH-R CTSA (UL1RR024986).

ACKNOWLEDGMENTS

We thank Virginia Murphy-Weinberg, RN, MS, Emily M. Briceño, Leslie M. Guidotti-Breting, Allison M. Kade and the technologists of the PET Center and fMRI Laboratory at the University of Michigan for their assistance in the performance of the studies. We thank Colin Hodgkinson, NIAAA, NIH for assistance in genetic analyses.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

REVIEW
published: 24 April 2019
doi: 10.3389/fpsyg.2019.00781

A Model for Basic Emotions Using Observations of Behavior in *Drosophila*

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Emotion plays a crucial role, both in general human experience and in psychiatric illnesses. Despite the importance of emotion, the relative lack of objective methodologies to scientifically studying emotional phenomena limits our current understanding and thereby calls for the development of novel methodologies, such us the study of illustrative animal models. Analysis of *Drosophila* and other insects has unlocked new opportunities to elucidate the behavioral phenotypes of fundamentally emotional phenomena. Here we propose an integrative model of basic emotions based on observations of this animal model. The basic emotions are internal states that are modulated by neuromodulators, and these internal states are externally expressed as certain stereotypical behaviors, such as instinct, which is proposed as ancient mechanisms of survival. There are four kinds of basic emotions: happiness, sadness, fear, and anger, which are differentially associated with three core affects: reward (happiness), punishment (sadness), and stress (fear and anger). These core affects are analogous to the three primary colors (red, yellow, and blue) in that they are combined in various proportions to result in more complex "higher order" emotions, such as love and aesthetic emotion. We refer to our proposed model of emotions as called the "*Three Primary Color Model of Basic Emotions*."

Keywords: basic emotions, core affection, monoamine, evolution, instinct, emotion flow, *Drosophila*

OPEN ACCESS

Edited by:
Wenfeng Chen,
Renmin University of China, China

Reviewed by:
Weiwen Wang,
Chinese Academy of Sciences, China
Leonid Perlovsky,
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Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *25 December 2018*
Accepted: *21 March 2019*
Published: *24 April 2019*

Citation:
Gu S, Wang F, Patel NP, Bourgeois JA and Huang JH (2019) A Model for Basic Emotions Using Observations of Behavior in Drosophila. Front. Psychol. 10:781. doi: 10.3389/fpsyg.2019.00781

INTRODUCTION

Emotions are fundamental to human life (Kvajo, 2016); when expressed pathologically, psychiatric disorders of emotional regulation, such as depressive and bipolar disorders, are leading causes of medical disability. Despite the importance of emotion in human health and illness, scientists struggle to reach consensus on the constructs underlying emotional phenomena and experiences (LeDoux, 1995; LeDoux J., 2012). In addition, controversy abounds over the definitions of emotion, the number of discrete, fundamental emotional states that exist, and the degree to which different emotions have distinct neurophysiological signatures (LeDoux, 1995; Damasio, 1997). Insects, such as *Drosophila*, offer animal models for studying the mechanisms of fundamental emotional processes (Anderson and Adolphs, 2014; Hoopfer, 2016; Shpigler et al., 2017). It is important to use the powerful analytical tools available in invertebrate model organisms to understand

the evolutionary origins and neurobiological underpinnings of emotions. Darwin (1876) found that insects use certain behaviors to express feelings homologous to human emotions, such as stridulation, which is the act of creating sounds by rubbing certain body parts together and can represent a range of various emotions in some insects. Darwin (1876) assumed that emotion-associated behavioral phenotypes are easily recognizable in many species, including insects (Anderson and Adolphs, 2014).

We have developed a theory of primary emotions using behavioral observations of *Drosophila*. Basic emotions are internal states induced by basical bodily changes, and can in turn induce genetically "hardwired" instinctual behaviors. They are highly conserved throughout evolution, and exhibit certain functional and adaptive properties that are shared across a wide phylogenetic range. For example, emotions such as fear and anger are thought to have evolved in response to fundamental life challenges and threats. Anderson and Adolphs (2014) suggested that these primary emotions (when combined) provide a framework for creating various types of secondary emotions, such that elements of primary emotions can be combined with the experience of other, higher order emotions that are more affected by specific learning and experience. Using this approach, primary emotions are observable in evolutionarily diverse organisms, allowing us to functionally "dissect" the mechanisms of the presumed associated internal emotional states and their externally manifest behaviors. There are many reports associating presumed fear and anger emotions with "fight or flight" behaviors in *Drosophila* (Kravitz and Fernandez, 2015). From analysis of these basic emotions and their associated behavioral phenotypes in animal models, we elucidate mechanisms of basic emotions in humans and propose to utilize this insight to define the mechanisms of disorders of emotional regulation.

EMOTION THEORIES

During the last century, the two most widely accepted theories in affect studies are basic emotion theory and dimensional theory. However, these two theories have been contradictory to each other, and have been described as being in a "100 years war" against each other (Lindquist et al., 2013; Barrett and Russell, 2015). The difference lies in whether emotions are characterized as discrete entities or an independent dimension (Bestelmeyer et al., 2017). Here we give an integrative theory in which we propose that these two theories not necessarily contradictory.

Basic Emotion Theory

Basic emotion theory has been very influential for more than half a century, providing inspiration for interventions in psychopathology (Saarimaki et al., 2016; Celeghin et al., 2017; Williams, 2017; Hutto et al., 2018; Song and Hakoda, 2018; Vetter et al., 2018; Wang et al., 2018). Theories about basic emotions originated from ancient Greece and China (Russell, 2003). Current basic emotion theory started with Darwin (1872) and Ekman (2003), and later (Tomkins, 1962), subsequently followed by Ekman (1984), and Izard (1977), then by many current psychologists (Ortony and Turner, 1990; Panksepp, 2007; Scarantino and Griffiths, 2011; Gu et al., 2016, 2018; Saarimaki et al., 2016; Hutto et al., 2018). Basic emotion theory proposes that human beings have a limited number of emotions (e.g., fear, anger, joy, sadness) that are biologically and psychologically "basic" (Wilson-Mendenhall et al., 2013), each manifested in an organized recurring pattern of associated behavioral components (Ekman, 1992a; Russell, 2006). Izard (1977) argued that the basic emotions are preserved because their biological and social functions are essential in evolution and adaption; he further suggested that basic emotions have innate neural substrates and universal behavioral phenotypes (Shpigler et al., 2017). In a special issue of *Emotion Review*, several research psychologists outlined the latest thinking about each theoretical model of basic emotions (Plutchik, 1962; Ekman and Friesen, 1969; Ekman, 2003; Izard, 2010, 2011; Ekman and Cordaro, 2011; Levenson, 2011; Panksepp and Watt, 2011; Tracy and Randles, 2011).

Basic emotions evolved to handle fundamental life tasks, e.g., fear and anger can aid survival by influencing an organism to either flee for safety or fight to defend itself. The elements of basic emotions can be combined to form complex or compound emotions (Ekman, 1992b). Even though many psychologists have accepted the theory of basic emotions, there is no consensus about the precise number of basic emotions. Robert Plutchik proposed eight primary emotions: anger, fear, sadness, disgust, surprise, anticipation, trust and joy, and arranged them in a color wheel. Ekman proposed seven basic emotions: fear, anger, joy, sad, contempt, disgust, and surprise; but he changed to six basic emotions: fear, anger, joy, sadness, disgust, and surprise. However, a recent study found that disgust and anger shared similar wrinkled nose, and fear and surprise shared raised eyebrows (Jack et al., 2014). The differences between anger and disgust and the differences between fear and surprise, are thought to have developed later for social functions and not for survival *per se* (Mansourian et al., 2016). As such, Jack et al. (2014) proposed that we humans have four basic emotions: fear, anger, joy, and sad. Notably, other authors have also proposed fear, anger, joy, and sadness as four basic emotions (Gu et al., 2015, 2016; Wang and Pereira, 2016; Zheng et al., 2016). As Izard said: people need the category label of *fear* to explain flight to one another for safety, *anger* to explain the frustration of blocked goal responses, *joy* (or its equivalent) to explain the pride of achievement, and *sadness* to explain the experience of a life-changing loss (Izard, 2007).

Dimensional Theory of Emotion

Dimensional studies of emotions originated from Wundt (1897), later followed by Scholsberg (1954), who proposed that emotions can be defined by three-independent dimensions: pleasant-unpleasant, tension-relaxation, and excitation-calm. Later, many others found that the last two dimensions are actually overlapping. Ekman (1957) also proposed a pleasant-unpleasant and active-passive scale as sufficient to capture the difference among emotions. Then Russell's (1980) invented the circumplex, and proposed that all emotions can be arranged in a circle controlled by two independent dimensions: hedonic (pleasure-displeasure) and arousal (rest-activated) (**Figure 1**, left) (Russell, 1980; Russell and Barrett, 1999; Posner et al., 2005;

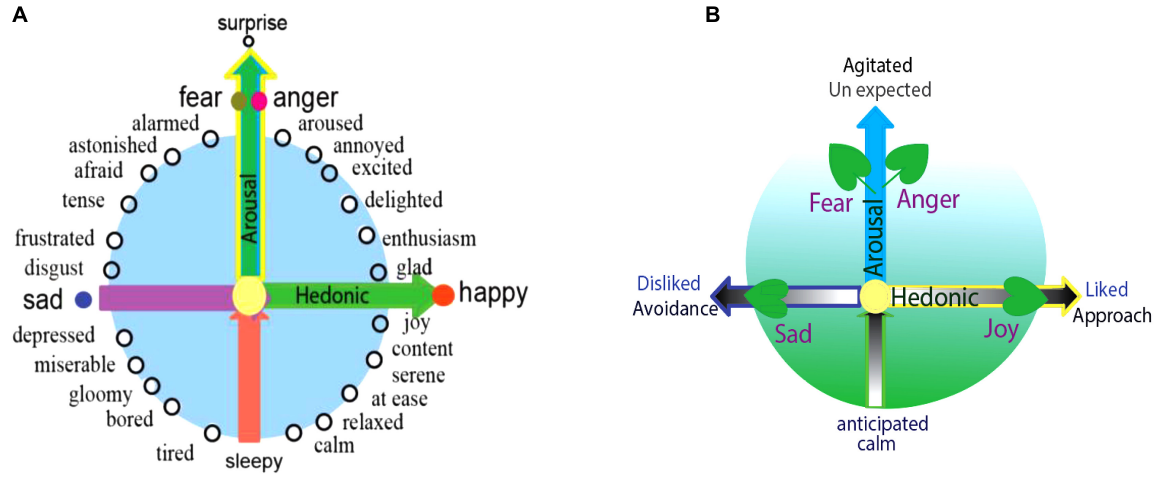

FIGURE 1 | Core affects and basic emotions. **(A)** All emotions, including the basic emotions, can find their locations in the circle of the Circumplex, which means that different emotions have different *arousal or hedonic parameters*. The reason for basic emotions being "basic" is that they can also be arranged on the special location in a two-dimension coordinate plane. And the typical locations of the four basic emotions suggest that they have different parameters (core affects): happiness and sadness are due to the hedonic value of the stimulus (physiological needs), while the fear and anger depend on the way the stimulus occurs (safety needs). **(B)** The locations of basic emotions in the dimensions are also decided by the stimulus that induce them, and also in the behavioral responses. The stimulus has two features: whether it fits into our needs (hedonic value) or whether it happens expected (arousal). The behavioral responses the emotion induced has two features: approach (hedonic value) and agitation (arousal value).

Barrett and Russell, 2015). The horizontal axis of the circumplex is hedonic and the vertical axis is arousal; accordingly, the different location of each emotion on the quadrant reflects varying amounts of hedonic and arousal properties (**Figure 1**; Posner et al., 2005).

Integration of Basic Emotion Theory and Dimensional Theory

Basic emotion approach differs from the dimensional approach in that the latter suggests that emotions are fundamentally the same, only differing in intensity or pleasantness (Ekman, 2003), while the former proposes that emotions are composed of limited number of basic emotions. Here we propose an integrative approach wherein basic emotions also differs in the intensity or pleasantness, like all other emotion. Therefore, basic emotion theory is not contradictory to dimensional theory. The dimensional approach proposes that every emotion has different amounts of hedonic and arousal value. The hedonic (pleasure-displeasure) and arousal (rest-activated) value, which can be called core affects (Russell, 2003; Barrett et al., 2007), are essential features of all emotions, including basic emotions (Gu et al., 2016). Therefore, basic emotions, like all other emotions can find their location in the circumplex.

The specificity of basic emotions on the circumplex is that they located on the axis of the dimensions, which might be the reason that they are "basic." Happiness and sadness are on the opposite sides of the horizontal dimension, implying that they are a reflection of the hedonic value of the stimulus and unrelated to the safety value (**Figure 1**, left). Fear and anger are on the vertical axis, implying they are based on the safety value of the stimulus and independent upon the hedonic value of the stimulus. Because of their special location, the basic emotions (fear or anger) have "0" amounts of hedonic value, and the basic emotion of joy or sadness has "0" amounts of safety value. Therefore, the reason for basic emotions being "special" lies in that they only represent only one core affect, because of their specific location. Thus, we introduce a prerequisite conditions for basic emotions: *Basic emotions should locate on the axis of the two emotional dimensions*. Emotions located on the axis of the dimensions are basic emotions, which might suggest that we have four basic emotions: fear-anger, joy, and sadness. Complex emotions can also find their locations on the quadrant, such as love or aesthetic emotions.

Factors Affecting the Locations of Emotion in the Dimensional Plane

Ekman (1992b) said all emotions differ in the stimulation events, appraisals, behavioral response, and physiological responses. The locations of all emotions on the dimensions can be defined by these factors. According to the appraisal theory, emotions are internal states which are activated by stimulation events. Every stimulation event has two features: whether it is fit for our needs (hedonic value), and whether it happens as expected (arousal) (**Figure 1**, right). These two features correspond to the two core affects: the hedonic value that represents a physiological need, and the safety value represents the way the stimulus happens (Wang and Pereira, 2016; Zheng et al., 2016). The two-dimensional coordinate plane or the core affects also represents these values of the stimulus: whether it happens as expected, and whether it is fit for our needs (Gu et al., 2016). Lazarus distinguished two kinds of appraisals at a stressful stimulation (**Figure 1**, right) (Izard, 1977). The first is automatic,

unconscious, fast-activating, is related to harm and threat, and induces fearful emotion to motivate avoidance and withdrawal, whereas the second appraisal (he named reappraisal) is conscious and associated with coping (Lazarus, 1999; Zheng et al., 2016). Fear and anger both result from unexpected stressful events, and while fear is associated with feelings of uncertainty, anger is associated with planning to cope with the stressful situation (**Figure 1**, right) (Moons et al., 2010). Ultimately, fear and anger depend on the manner the stimulation event occurs (Gu et al., 2016), or fear and anger are "twin" emotions, they are two sides of one coin (Gu et al., 2015), and they locate on the top of the vertical dimension.

The locations of emotions on the dimensional plane are also predicated by the behaviors they might induce. Emotion is an internal state, not a behavior (Baker, 2004). Emotion is a tendency of behavior (Roseman, 1984), because the emotion can be separated from the behavioral actions it induces. For example, we can block the actions associated with angry emotion. However, the emotion induced behaviors have two features: the direction and the tension of the behavior (LeDoux, 1998; LeDoux and Brown, 2017). These features can be reflected on the dimensions: hedonic value decides the approach/avoidance, the vertical dimensions decides the tension of the behavior (Wang and Pereira, 2016; Zheng et al., 2016). Thus, the locations of the emotions on the dimension can also be determined by the behaviors they might induce. Fear and anger can induce "flight or fight," which have opposite directions: fear is in the negative direction (Certel et al., 2010), while fight is in the positive direction. Analogously, joy and sadness can induce approaching or avoidance, respectively (Arnott and Elwood, 2009).

Therefore, like what Lazard suggested, in face of a stressful situation, the individual will collect energy to cope with the situation with "fear or anger" emotions, and induce "fight or flight" actions. After coping with the situation, the individual will have an opportunity for reappraisal, which Lazarus named as emotion-focused coping (Izard, 1977). If the individual have successfully coped with the stressful situation, he/she will feel happy; otherwise, he will feel sad (Aldwin, 1994; Lazarus, 1999). Therefore, Frijida said, sadness is acceptance of the failure, without more efforts to fight (Frijda, 1986). In all, the two dimensions of emotions not only represent the two different feature of a stimulus (hedonic value and safety value), but also represent two features of the behaviors they induced: direction (approach or avoidance) and activation.

EVOLUTIONARY ASPECTS OF BASIC EMOTION

Human emotions are poorly understood even though there is a long venerable tradition of research directly at understanding them. One problem is that physiologists and psychologists who study emotions do not necessarily look at emotion enough from an evolutionary standpoint (Ramachandran and Jalal, 2017). This is unfortunate because as Dobzhansky famously said, "Nothing in biology makes any sense except in the light of evolution" (Ramachandran and Jalal, 2017). However, comparative psychologists have also struggled with the problem of emotion because emotions cannot be directly observed or measured, and we have no access to the animals' subjective experiences (Ramachandran and Jalal, 2017). Human being and some other animals (such as primates) can consciously know the subjective feelings (Kamitani and Tong, 2005; Kuppens et al., 2013); but other animals, who have no power of self-observation and cannot consciously know their internal states; they still have emotions (Perlovsky, 2016a). For the invertebrate animals, an emotion is just an internal state, which includes the need, drive, or tendency to a kind of behavior (Mesquita and Frijda, 2011).

We can only study emotions in the invertebrate animals depending on the animal's behavior and physiology-emotional expression (Cosmides and John, 1995). Darwin (1872) was first to study the animal behaviors for emotions, and he suggested that even insects use certain behaviors to express feelings homologous to human emotions. In order to survive, animals need to sense their environment, evaluate the surrounding stimuli with their internal needs, and initiate appropriate behavioral responses (Kaun and Rothenfluh, 2017). Evolutionarily, emotional behaviors should first have direct survival value that was honed by natural selection, and these behaviors are adaptive responses to the environment that increase the chances of survival (John and Leda, 1990; Cosmides and John, 1995). The basic emotions were selected through evolution in order to promote the survivability of the species in their specific primitive environment (LeDoux J.E., 2012). Therefore, the basic emotion related behaviors are the naturally born instinct behaviors, which are evolutionarily adaptive (Ramachandran and Jalal, 2017). In addition, these basic emotion related behaviors are manifested as stereotypical behavioral phenotypes.

Basic Emotions Are Related to Instincts

Basic emotions are thought to be universal as they are related to the most basic needs of the body, or the bodily instinctual needs (Schoeller et al., 2018). These few emotions constitute the most ancient and noticeable emotions, while other emotions (complex emotions), such as aesthetic emotions, are related to higher needs of our human experiences (Perlovsky, 2012). Grossberg and Levine, 1987 proposed a famous theory about instinct, which considers that the instinct resembles "neural sensors that measure vital parameters important for survival" (Grossberg et al., 1987; Perlovsky, 2016c), "for example, a low blood glucose level specifies an instinctual need for food" (Schoeller et al., 2018). Therefore measurement of glucose level is a kind of neural sensor. Grossberg and Levine theory proposed that emotions are the neural signals that connect instinctual sensor with the conscious brain, to make the instinctual needs to be consciously known to the brain, to make the instinct conceptually recognized-understood (Fontanari et al., 2012). William James also proposed that feelings are derived from sensing the body states (Damasio and Carvalho, 2013). Therefore, emotion is a kind of internal neural activity, whose major function is to sense the bodily needs, and then motivates behaviors depending on the external stimulus (Schoeller et al., 2018). Therefore basic emotions indicate satisfaction of instincts (Fontanari et al., 2012).

Remarkably, studies in *Drosophila* confirmed these hypothesis. Recent studies reveal that a small number of specialized central brain neurons in *Drosophila* brain directly sense specific circulating macronutrients (Pool et al., 2014), monitor systemic energy balance and alter feeding probability based on internal nutritional state (Pool and Scott, 2014). In addition to internal nutrient sensors, the flies can distinguish sugars based solely on caloric content in the absence of sweet taste detection (Dus et al., 2011). Furthermore, there is accumulating evidence that all animals have the central sensors to directly detect the levels of circulating carbohydrates and amino acids (Hao et al., 2005; Domingos et al., 2013). These studies thus identify central brain mechanisms that sense availability of specific nutrients, convert it to a change in neuromodulator output, and promote or inhibit feeding (Pool and Scott, 2014).

The basic emotions are evoked by sensing the basic bodily instinctual needs, while the "complex" emotions, including love, aesthetic emotions are evoked by higher cognitive needs of human being. In Maslow's Hierarchy of Needs, physiological needs and safety needs might be directly related to basic emotions, while the other needs, such as the need for love, esteem and self-actualization, are related to feelings such as aesthetic emotions (Zheng et al., 2016). Kant (1951) said aesthetic emotions are related to need of knowledge in human being. Perlovsky also proposed that human beings have the special need for knowledge, which was named "knowledge instinct." Aesthetic emotions are related to the knowledge needs, are related to learning, or understanding, and they are shown in many fields, such as mathematics, music, or even language (Perlovsky, 2015; Schoeller and Perlovsky, 2016). Aesthetic emotions can also be seen in creativity, and they are also related the NE and DA neuromodulator (Gu et al., 2018). *In all, the emotions are evoked by these sensing for bodily needs, and underlined by the neuromodulator release, and will promote some behaviors*.

Basic Emotions Are Primitive

Basic emotions are basic due to the fact that they fit for primary life needs, and are developed early phylogenetically and ontogenetically (Ekman, 2003). The idea that invertebrates exhibit basic forms of emotions is increasingly accepted (Arnott and Elwood, 2009). Animals spend a significant amount of time seeking and selecting food for eat, while striving to avoid being eaten (John and Leda, 1990; Cosmides and John, 1995). These two forms of needs, which can be called hedonic and safety values, are two equally important, independent needs (Zheng et al., 2016). In Maslow's Hierarchy of Needs, physiological needs were proposed to be superior to safety needs, with the idea that safety needs emerge only once physiological needs are satisfied (Maslow, 1948). However, an animal will not eat in a dangerous situation since the primary objective in such situations is assurance of safety (Zheng et al., 2016). Wild animals navigating the rich environments will face complicated situations with many uncertainties; accordingly, the evolution of an adaptive mechanism to first perform a safety check of the surroundings is critically important for its survival (Gu et al., 2016; Zheng et al., 2016). Maslow (1948) also said that "practically everything looks less important than safety, even sometimes the physiological needs which being satisfied, are now underestimated. A man may be characterized as living almost for safety alone" (Maslow, 1948). Therefore, in our previous paper, we proposed that safety needs might be more basic (Zheng et al., 2016). Anyway, *physiological needs and safety needs represent two equally important dimensions of human needs, which are independent from each other*. Darwin said "the fear emotion does not depend on experiences; instead it depends absolutely on heritage." Exposure of laboratory rodents to a predator, such as a cat, elicits defensive behaviors even if they have never been exposed to cats before; therefore, such behaviors appear to be innate and not experiential, as Darwin posited. The *fear emotion might be the most crucial emotion* for survival via eliciting defensive responses, such as fighting, and thus has been preserved throughout evolution (Olsson and Phelps, 2007).

Basic Emotions Manifest as Stereotypical Behavioral Phenotypes

Emotion is a kind of internal drive, which can exert a powerful effect on behavioral choice (Pereira and Murthy, 2017). Basic emotion related internal drive can induce behaviors that are supported by genetically hardwired neural circuits and are critical for animals' survival. Several major categories of innate behavior, such as feeding, reproduction, aggression, and sleep, are observed across animal species. As hierarchical systems comprised of behavioral subprograms, innate behaviors are not only robust but can also fluctuate in intensity and adapt to an organism's internal and external contexts (Kim et al., 2017). Fear is an important hereditary gift that aids survival by protecting against dangerous situations (Becker, 1997). All animals have specific behaviors to defend against predators, and Darwin found that "even insects express fear, anger, jealousy and love, by their stridulation" (Darwin, 1998). At stressful situation, emotion anger develops after fear emotion disappear, and manifests as fighting to cope with stressful situations, which can be easily observed in invertebrates. For example, the stings of bees, spiders, flies, ants, and scorpions are powerful tools to protect the organisms from predators. In addition, fighting also serves in the acquisition and defense of vital resources, such as food, shelter, or access to mates, such as what Sturtevant reported the fighting behavior in *Drosophila* (Chen et al., 2002). In stressful situations in which two males are courting the same female, he wrote "in such cases they (males) may be seen to spread their wings, run at each other, and apparently butt heads. One of them soon gives up and runs away. If the other then runs at him again within the next few minutes he usually makes off without showing fight" (Chen et al., 2002; Kim et al., 2018). Although it is difficult to determine whether fighting behaviors in insects are manifestations of an internal emotional state of anger like in humans, the function of fighting behaviors of invertebrates is very similar to the defensive behaviors of mammals; e.g., biting, clawing, hissing, and arching the back are fighting behaviors by which mammals threaten dangers away. Furthermore, like mammals, emotions in invertebrates are transient internal states, such that anger in insects manifests after fear to cope with the stressful situation (Zheng et al., 2016). It is necessary for survival even in *Drosophila*, and fight-or-flight behaviors can be easily seen in *Drosophila*,

the flies are ready to fight with wings up for threat at a stressful situation, and after a series of stereotypical fighting behaviors one fly admits failure and fly away (Darwin, 1872).

Basic Emotions Gain New Meanings Throughout Evolution

Despite the universal nature of basic emotions, new behaviors of emotions have emerged via evolution (Ekman, 1992b). Some emotion related behaviors might be learned, e.g., the stress-related emotions of fear and anger have also got new meanings over time. While the original meaning of fear entails life-threatening situations with fight or flight behaviors in response to predators, new meanings of fear are generalized to any situations that occur unexpectedly. In addition the original meaning of happiness is associated with pleasurable feelings and generates approach and consummator behaviors, eventually leading to behavioral reinforcement (Schultz et al., 1997), the new meaning of happiness also gained new meanings through evolution: whether the organism is able to cope successfully (Yurkovic et al., 2006).

Consistently, the neuromodulator dopamine (DA), is known to mediate unconditioned pleasure and reward from food, sex, and drugs, but recent findings suggest that DA is also involved in the anticipatory, preparatory, approach, and coping phases of reward behavior (Schultz et al., 1997; Sandoval and Seeley, 2017). This is consistent with Lazarus's reappraisal theory about happiness and sadness, which posits that happy and sad emotions are related to the success or failure of coping with stressful situations, respectively (Lazarus, 1999). The happiness in insects parallels that of mammals in several aspects. In *Drosophila*, happy emotion is expressed as locomotive activity (Mansourian et al., 2016; Wang and Sokolowski, 2017), which also happens after successful coping. Depending on studies in *Drosophila*, Kim et al. (2018) proposed that winning is perceived as rewarding, while losing is aversive. Presentation of food promotes a state of elevated locomotive activity, which can be controlled by DA. Furthermore, during courtship, males extend their wings horizontally and vibrate them to generate a "song" that attracts females, and the optogenetic activation of specific brain interneurons that control the courtship song can lead to persistent singing for several minutes (Dickson, 2008; von Philipsborn et al., 2011; Inagaki et al., 2014). In contrast, *Drosophila* raise their wings vertically into the "wing-threat" position during antagonistic interactions with conspecific males, and after a series of stereotypical defensive behaviors, one fly lowers its wings to admit failure and retreats (Chen et al., 2002).

BIOLOGICAL ASPECTS OF EMOTIONS

Given that basic emotions evolved to handle fundamental life tasks, it would follow that there must be biological patterns that drive each emotion. Ekman (1992b) proposed that basic emotions have many characteristics that distinguish one emotion from another, such as universal signals, distinctive physiology, and automatic appraisal influenced by both ontogenetic and phylogenetic past. Therefore, basic emotions not only provide information through behavioral expression to conspecifics, but specific biological changes prepare the organism to respond differently to various states of emotion. However, many studies using fMRI failed to get consistent results about specific neural basis for specific basic emotions (Lindquist et al., 2012), which lead to many psychologists try to give up the basic emotion theory (Lindquist et al., 2013; Barrett and Russell, 2015). Here we review basic emotions in *Drosophila*, whose brain structure are totally different from that of humans, but the neuromodulator are similar among all these animals. Numerous studies have pointed to an important role for neuromodulators (e.g., DA, 5-HT, and NE) in emotional process (Pereira and Murthy, 2017). Neuromodulators are believed to control the internal states related to emotions, mood, and affects, and exert critical influences on emotion related behaviors (Watanabe et al., 2017). Therefore, we propose that *emotion is an internal state, whose neural substrate is the neuromodulator* (Kim et al., 2018). For example, norepinephrine is a potent modulator of brain-wide states such as arousal (Suver et al., 2012; Pereira and Murthy, 2017).

Neural Basis for Basic Emotions

Insects lack homologs of vertebrate forebrain structures involved in emotional processing, such as the nucleus accumbens (NAc), prefrontal cortex (PFC), amygdala, and hippocampus; however, insects have evolved structures such as the mushroom body and central complex that show many functional and anatomical similarities to the mammalian structures that mediate basic emotions (Lin A.C. et al., 2014; Lin S. et al., 2014). The monoamine system in invertebrates (Klemm et al., 1985) and non-mammalian vertebrates (Perry and Capaldo, 2011) has been implicated in stress by numerous studies. Comparative anatomical studies of the neurons releasing OA, DA, and 5-HT have shown striking similarities among different insect species (Konings et al., 1988; Dacks et al., 2005) and point to a stereotypic pattern of neurons that are widely distributed in the central nervous system (CNS) of flies. In *Drosophila* brain, there are about 100 octopaminergic neurons (Sinakevitch et al., 2005), which can be divided into two kinds of neurons: interneurons and efferent neurons. The efferent neurons project diffusely to almost all the neuropil structure in the brain (Sinakevitch et al., 2005). Like the NE in vertebrates, the counterpart of OA, some octopaminergic neurons have been prove to be implicated in aggression (Ramirez and Pearson, 1991; Schroter et al., 2007; Andrews et al., 2014). Similarly, there are approximately 40 5-HT neurons in the *Drosophila* brain, whose innervations are spread around the feeding apparatus and also the ring gland (Sitaraman et al., 2008). On the contrary, the dopaminergic neurons project to the mushroom bodies to control behaviors (**Figure 2**; Zhang et al., 2007). Similar to 5-HT, DA neurons are interneurons, which connect different regions of neuropil. DA neurons show hydroxylase-immunoreactivity, and locate in the central body, anterior protocerebrum and other scattered region (Han et al., 1996). A cluster of approximately 130 dopaminergic neurons that innervate the horizontal lobes of the mushroom body was implicated sugar reward (Burke et al., 2012; Liu et al., 2012; **Figure 2**).

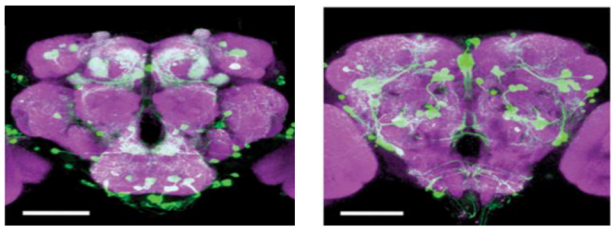

FIGURE 2 | DA cells in the *Drosophila* brain. An anterior view of DA neurons in the *Drosophila* brain. Labeling of DA cells and processes was achieved by a tyrosine-hydroxylase enhancer trap driving expression of green fluorescent protein (White et al., 2010), Scale bar, 100 μm (courtesy of Dr. Frank Hirth, King's College London).

Norepinephrine – Stress

The norepinephrine system of mammals influences various aspects of the animal's life, including *Drosophila*, and they can modulate a variety of physiological processes and behaviors in response to stress. Stressful stimuli induce a metabolic and behavioral adaptation, leading to enhanced energy supply, increased muscle performance, increased sensory perception and a matched behavior. This so-called "fight or flight" response can be seen in both vertebrates and invertebrates. Cannon said: "The highest level of modulatory monoamine input occurs during "fight or flight" behavioral situations."

Fighting is a primitive behavior and is regarded as one of the fundamental instincts behind innate animal behaviors by Konrad Lorenz and Nikolaas Tinbergen, the founders of modern neuroethology (Asahina, 2017). Fighting in *Drosophila* offers a unique opportunity for studying basic emotions because of its stereotypical actions and its easily identified genetic resources. The fighting behavior in *Drosophila* is activated by pheromones and internal states, such as hunger. *Drosophila* recognizes the sex, species and even the food quality by chemosensation (Wang et al., 2011; Clowney et al., 2015). For example, 11-cis-vaccenyl acetate is a specific male odor chemical, and has been shown to be involved in male-male fighting (Wang and Anderson, 2010; Liu et al., 2011). The yeast smell which might signify food quality can induce female aggression (Ueda et al., 2002; Nelson and Trainor, 2007; Asahina et al., 2014). Even though these cues can induce fighting, the internal status of the animals can affect the fighting too, for example, prior defeat can reduce fighting behavior in many animal species (Penn et al., 2010; Hammels et al., 2015). In addition to past fighting experience, fighting behavior can also be influenced by the outcome of other behaviors, including recent mating success, hunger levels, and sleepiness. For example, recent mating experience can increase fighting tendency (Yuan et al., 2014), whereas sleep deprivation can decrease the frequency of fighting (Kayser et al., 2015). Lorenz already found the importance of internal states in fighting and suggested that fighting threshold can be lowered by an aggressive drive (anger) (Asahina, 2017).

It might be due the fact that increase octopamine can modulate the flight-or-fight response by affecting chemosensation responses (Stevenson et al., 2005; Ramdya et al., 2015). Amines like dopamine, tyramine, octopamine, and serotonin, were among the first molecules to be implicated in *Drosophila* aggression, in large part because previous studies in lobsters and crickets suggested strongly that monoamine neuromodulators affect aggression (Asahina, 2017). It has been well-known that the function of NE system is to induce fight-or-flight behaviors and serve to help the organism cope with dangerous environment (Zheng et al., 2016). Following exposure to a threat, NE is released from sympathetic nervous system to the blood, and directly affects the heart rate, and triggers the glucose release. NE is also released from locus coeruleus in the central nervous system. LC neurons project very profusely to most regions of the brain, and can influence the whole brain (Yurkovic et al., 2006). Octopamine (OA) in insects is a neuroactive substance that has a chemical structure that closely resembles NE and can function as a neurohormone for basic emotion (Arnott and Elwood, 2009; Lovheim, 2012; Asahina, 2017). The activity of octopaminergic neurons was initially discovered in octopus's salivary glands, and has since been shown to mediate stress-response in many invertebrate (Sinakevitch et al., 2005; Stevenson et al., 2005; Burke et al., 2012). In addition, OA has been shown to be involved in fighting and also in elevated flight in crickets (Gammie et al., 2005; Stevenson et al., 2005; Asahina, 2017). In male *Drosophila*, octopamine is necessary to maintain levels of aggression (Zhou et al., 2008). A null mutation in the gene that encodes tyramine-β-hydroxylase (TβH), which catalyzes the OA synthesis, can significantly reduce fighting and aggression (Andrews et al., 2014; Asahina, 2017), which suggest its role in maintenance of fighting (Andrews et al., 2014). Even though activation of octopaminergic neurons or exogenous administration of octopamine, the invertebrate counterpart of noradrenaline can activate aggression (Schretter et al., 2018), whether an octopaminergic signal is sufficient to elevate levels of aggression remains unclear. For example, even though OA plays a role in fighting or aggression, artificially administering OA or an OA-agonist results in mixed effects on aggression (Hoyer et al., 2008; Kayser et al., 2015). In addition, overexpression of TβH does not increase fighting (Hoyer et al., 2008), instead it induced conflicting outcomes (Certel et al., 2010; Kayser et al., 2015). This might be due the fact that OA and NE systems are the substrates for both fear and anger, or fight and flight behaviors. OA-dependent modulation of organs and tissues is mainly elicited through muscle action, especially in terms of its impact on the "fight or flight" response. Flight is also an important and critical behavior in flying insects. Here the "flight" means flying away, instead of the normal "fly." Neuromodulation of insect "flight" has thus far been attributed primarily to biogenic amines (Brembs et al., 2007). However, there are few studies in *Drosophila* about the "flight" behavior (Shen, 2012). However, studies about the innervation pattern in the periphery also support the idea that the OA/TA system is crucial for insects to switch from a dormant to an excited state, by a positive modulation of muscle activity, heart rate, and energy supply, and a simultaneous negative modulation of physiological processes like sleep (Agrawal and Hasan, 2015; Pathak et al., 2105; Pauls et al., 2018).

Dopamine – Reward

Octopamine was historically considered to be the signal for reward in insects, only recently has dopamine been linked to motivated behavior and rewarding reinforcement in fruit flies (Burke et al., 2012). The hedonic hypothesis of DA was first proposed by Wise and posits that DA in the brain plays a critical role in the subjective pleasure associated with positive reward and that a reduction in DA results in a loss of pleasure (Bozarth and Wise, 1980; Gu et al., 2016). Afterward, many studies have proved the role of DA in reward signaling (Dougherty et al., 1999; Matthews et al., 2016), notably many pharmacological and behavioral studies have confirmed the important role of medial prefrontal DA system in reward behaviors (Keleman et al., 2012). In addition, studies of the mechanisms of some drugs of abuse also support the role of DA in reward system by increasing presynaptic release of DA and inhibiting DA reuptake, such as cocaine and amphetamine (Narita et al., 2008; Del Campo et al., 2011). In contrast, decreased striatum DA responses were reported in detoxified cocaine abusers. Together, these studies suggest an involvement of DA neurotransmission in the reward process (Matsumoto et al., 2016), and this hypothesis has significantly impacted theories of drug addiction, and motivation since it was first introduced.

However, recent DA studies have opened this theory to reexamination (Berridge and Kringelbach, 2015). The incentive salience hypothesis has recently been accepted, which suggests that the major function of DA is anticipatory, preparatory, approach, instead of unconditioned pleasures from food, sex, or drugs (Schultz et al., 1997; Sandoval and Seeley, 2017). Therefore, Hailan Hu proposed that happiness = reward minus predicted reward (Dickson, 2008; Hu, 2016; Matsumoto et al., 2016), which means surprise can enhance happiness (Lazarus, 1999). Similar in *Drosophila*, OA (surprise) was thought to be involved in reward in insects (Burke et al., 2012), including *Drosophila* (Zhang et al., 2007; Liu et al., 2017). It is found that OA can trigger activation of dopaminergic neurons (Burke et al., 2012; Kahnt et al., 2012). Analysis of the β-adrenergic-like OCTβ_2R receptor suggest that this OA-dependent reinforcement requires an interaction with dopaminergic neurons that control appetitive motivation (Burke et al., 2012; Kahnt et al., 2012). These evidence suggests clear roles for DA in reward-related processes in invertebrates (Ma et al., 2016), including motivation behavior and nutritional valuation of reward (Arnott and Elwood, 2009).

Historically, DA has been suggested to be most prominently associated with reward and punishment (Liu et al., 2012), recent findings from *Drosophila* confirmed all these functions, as well as additional roles in the interplay between external sensation and internal states (Burke et al., 2012; Kaun and Rothenfluh, 2017). It is believed that drug addiction is due to the mechanism of drugs of abuse "hijacking" the dopaminergic "reward" circuit and thus these artificial rewards reinforce associated behaviors. Recent complicating dopaminergic involvement in addiction has been proved to modulate internal state of the animals (Kim et al., 2017). Many drugs of abuse, such as alcohol stimulate locomotion, can induce *Drosophila* hyper-locomotion, and PPM3 DA neurons in mushroom body has been suggested to be involved in changing the activity and arousal states of the flies (Kim et al., 2017). Different kinds of drugs with different stimulation salience can induce different dopaminergic activity in distinct DA neurons in the mushroom body.

Serotonin – Punishment

Although DA release mediates reward, inhibition of DA should induce punishment. However, evolution created a separate process for punishment, and while it may seem redundant, 5-HT has been known to play a critical role in punishment. It is found that approximately 90% of 5-HT is secreted by gut chromaffin cells in response to noxious food, thus inducing vomiting or diarrhea (Beyeler, 2016; Zheng et al., 2016). Some plants exploit this function of 5-HT by expediting passage of seed through the digestive system. In addition, some animals such as scorpion and wasp stings also use 5-HT to induce pain (Gu et al., 2016). Aversion is separated from reward in the evolutionarily lower animals (Beyeler, 2016). The nematode, *Caenorhabditis elegans*, can also be infected by pathogenic bacteria, even though it feeds on bacteria. Indeed, exposure to pathogenic bacteria can enhance the 5-HT release, which can induce negative reinforcing reflex in *C. elegans*. However, the function of 5-HT gets more complicated during evolution, for example, it is found that some 5-HT neurons are involved in the positive rewarding process (Liu et al., 2012; Ries et al., 2017). It is found that some 5-HT neurons in dorsal raphe nucleus fire consistently during acquisition of a variety of rewards, including sex (Isosaka et al., 2015). Some studies reported a correlation between 5-HT level and positive emotions in rodents (Kvajo, 2016). Therefore, it might be too complicated to differentiate the functions of DA and 5-HT in such highly evolutionary creatures such as mammals and human beings. As such, *Drosophila* offered a very good model to test the functions of DA or 5-HT in positive or aversive reinforcement (Zhang et al., 2016).

The emotions are internal states evoked by sensing for bodily instinctual needs, and underlined by neuromodulator release, and will promote some behaviors. In mammals, these neuromodulators are primarily monoamines, which are highly interconnected with a network of modulators and transmitters important for complex behaviors. Neuropeptides and monoamines are very often expressed in combination with each other and with neurotransmitters. Possible interactions between neuromodulators and neurotransmitters are interesting and important issues. A dozen neuromodulator systems in *Drosophila* have been implicated to date in basic emotion, including serotonin, octopamine, acetylcholine, glutamate, and GABA, and many neuropeptides, and many of these neuromodulator systems appear to be functionally conserved throughout evolution, including orthodox for mammalian peptidergic signals tachykinin, cholecystokinin, neuropeptide Y, Neuromedin U and insulin (Pool and Scott, 2014). However, work in *Drosophila* suggests that the rewarding drives are majorly gated through DA neurons, or DA plays a central role in creating the motivational drive underlying many behaviors (Kim et al., 2017). In male flies, dopaminergic neurons of the ventral nerve cord promote persistent copulation, and a subset of dopaminergic neurons innervating the mushroom

FIGURE 3 | Three-primary-color model of core affects. The three monoamine neuromodulators are the substrates for three core affects (norepinephrine-stress, dopamine-reward, 5-HT-punishment). And the three core affects constitute the basic emotions: stress-fear and anger, reward-happiness or joy, punishment-sadness or disgust.

body is required for persistent courtship. Similarly, even neuropeptide are shown to affect *Drosophila* aggression, such as neuropeptide F, a functional homolog of vertebrate neuropeptide Y. However, NPF-expressing neurons seem to play a more general role in modulating male behavioral patterns when potential competitors are present (Asahina, 2017). Unlike octopamine, which is clearly an important neuromodulator for aggression, NPF has also been regarded as neuromodulator of feeding behavior across animal species, and activation of NPF signaling mimics the hunger states. In addition, there is no specific population of NPF expression neurons, unlike the neurons for three monoamines (Asahina, 2017; Ryglewski et al., 2017).

Therefore, we propose that the monoamine neuromodulators underlie the three core affects of basic emotions; specifically, NE is related to the fight-or-flight responses at stressful events, DA is involved in reward, and 5-HT is related to punishment (**Figure 3**). Consistently, a paper in PNAS proposed that the use of *Drosophila* as a model for circuit dissection of internal states can promote behavioral changes associated with winning or losing after coping: Winning is perceived as rewarding, while losing is aversive (Kim et al., 2018).

CONCLUSION

In this review, we propose a framework for the evolutionary study of emotions based on behavioral observations of *Drosophila*. From analysis of molecules and neural systems to observational study of behaviors and social functions, the *Drosophila* model is a powerful tool to understand the evolutionary origin and neurobiological underpinnings of emotions (Anderson, 2016; Kim et al., 2018). The brain structure of *Drosophila* are totally different from that of humans, but they have similar neuromodulators and innate states (Kim et al., 2018). Numerous studies have pointed to an important role for neuromodulators (e.g., DA, 5-HT, and NE) in the emotional process (Pereira and Murthy, 2017). Neuromodulators are believed to control the internal states related to emotions, mood, and affects, and exert critical influences on emotion related behaviors (Watanabe et al., 2017).

Emotion Is an Innate State, Whose Neural Substrate Is the Neuromodulator Release

We demonstrated that basic emotions are primitive, internal states that have gained new meanings and new external behavioral expression via evolution in order to meet organisms' biological, social, and functional needs (Ekman, 1992b; Anderson and Adolphs, 2014). Reward, punishment, and stress are the three most primitive features of the four basic emotions (happiness, sadness, fear, anger) and are driven by the three monoamine neuromodulators (DA-reward, 5-HT-punishment, NE-stress). These three monoamines are not only the substrates for the four basic emotions, but we posit that these monoamines combine in varying degrees to ultimately create various higher order emotions, much like the way different colors can be created from the three primary colors; we call this the "Three Primary Color Model of Basic Emotions."

This paper establishes a new theory of emotion. A scientific theory in psychology, similar to those in physics, is its elegance and beauty in describing a vast area of knowledge from few basic principles, or use few fundamental principles to describe a vast area of knowledge (Perlovsky, 2016b). Traditional psychology is a "soft" science that does not develop models of the mind based on few principles, describing vast areas of knowledge, and making experimentally verifiable predictions (Perlovsky, 2016b). Here we introduced the very simple model for basic emotions, a very simple theory about emotions. It might be an oversimplification to categorize monoamine simply as an aggression-promoting neuromodulator, but we hope our hypothesis can help understand the basic emotion theory. For validation, detailed studies of the specific behavioral expressions of states of relative excess or deficit of the neurotransmitters 5-HT, NE, and DA may offer confirmatory observations supporting this model of emotions.

AUTHOR CONTRIBUTIONS

FW and SG designed the manuscript. FW, JB, and JH wrote the manuscript. NP revised the manuscript.

FUNDING

This work was supported by a grant from Foundation of Humanities and Social Science in Ministry of Education in China (FW, 19YJAZH083).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychology

ORIGINAL RESEARCH
published: 26 April 2019
doi: 10.3389/fpsyg.2019.00845

The Childhood Maltreatment Modulates the Impact of Negative Emotional Stimuli on Conflict Resolution

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It has been reported that negative emotional stimuli could facilitate conflict resolution. However, it remains unclear about whether and how the impact of negative emotional stimuli on conflict resolution varies depending on childhood maltreatment. To clarify this issue, seventy-nine subjects were required to perform an arrow Eriksen Flanker Task which was presented in the center of emotional pictures. The present study found a significant interaction effect of childhood maltreatment and emotion on executive attention scores in reaction times (RTs) that reflect conflict resolution speed. For subjects in high childhood maltreatment, negative pictures elicited smaller executive attention scores in RTs than neutral and positive pictures, while neutral and positive pictures elicited similar executive attention scores in RTs. By contrast, for subjects in low childhood maltreatment, executive attention scores in RTs were similar across three conditions. These results suggest that the speed of conflict resolution is enhanced in high, instead of low, childhood maltreatment in situations of negative stimuli. This finding extends our understanding of the interaction among emotion, childhood maltreatment and conflict resolution.

Keywords: executive attention, conflict resolution, childhood maltreatment, arrow Eriksen Flanker Task, emotion

OPEN ACCESS

Edited by:
Fushun Wang,
Nanjing University of Chinese Medicine, China

Reviewed by:
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Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *29 May 2018*
Accepted: *29 March 2019*
Published: *26 April 2019*

Citation:
Meng X, Gao S, Liu W, Zhang L, Suo T and Li H (2019) The Childhood Maltreatment Modulates the Impact of Negative Emotional Stimuli on Conflict Resolution. Front. Psychol. 10:845. doi: 10.3389/fpsyg.2019.00845

INTRODUCTION

Child maltreatment is a global phenomenon affecting the lives of millions of children all over the world (Stoltenborgh et al., 2015; Viola et al., 2016). A recent research reported that across the globe the overall estimated prevalence rates assessing maltreatment ever during childhood were 12.7% for sexual abuse, 22.6% for physical abuse, 36.3% for emotional abuse, 16.3% for physical neglect, and 18.4% for emotional neglect (Stoltenborgh et al., 2015). Existing research has amply demonstrated that exposure to childhood maltreatment is associated with a significantly increased likelihood of multiple forms of psychopathology, including depressive disorder (Nanni et al., 2012; Hovens et al., 2016), anxiety disorder (Bruce et al., 2012; Choi and Sikkema, 2015), bipolar disorder (Pavlova et al., 2016), attention-deficit/hyperactivity disorder (Briscoe-Smith and Hinshaw, 2006) and common psychiatric disorders (Kim et al., 2009; Keyes et al., 2012). It is thus imperative to

understand the mechanisms underlying the association between childhood maltreatment and later psychopathology to break the continuity between the two.

It has been proposed that one possible mechanism for the association between childhood maltreatment and later psychopathology is that childhood maltreatment increases sensitization to negative emotional stimuli (Pollak et al., 2001; Sandre et al., 2018). Existing research has demonstrated that childhood maltreatment influences attention bias to negative signals (Pine et al., 2005; Shackman et al., 2007). Using event-related potentials (ERPs) technique, a study conducted by Shackman et al. (2007) found that relative to controls, abused children overattended to task-relevant visual and auditory anger cues, and they also attended more to task-irrelevant auditory anger cues (Pine et al., 2005; Shackman et al., 2007). Furthermore, individuals with childhood maltreatment tend to have difficulties in disengagement of attention from threatening events (Pollak and Tolley-Schell, 2003). Specifically, in a selective attention paradigm using emotional faces as cues, Pollak and Tolley-Schell (2003) found that physically abused children demonstrated delayed disengagement when angry faces served as invalid cues, suggesting the influence of childhood maltreatment on individual's selective attention to threat-related signals. And the enhanced attention to threat further facilitates both the development and maintenance of emotional disorders (Li et al., 2008; Yuan et al., 2009, 2014, 2015; Meng et al., 2015, 2016).

For the influence of emotion on executive attention control, Easterbrook (1959) influential hypothesis argued that increased emotional arousal of negative stimuli may result in narrowing attention breadth and reducing interference of distracting or irrelevant information (Easterbrook, 1959). In consistence with this view, recent findings observed the facilitation effects of negative stimuli on the processing of conflict resolution (Dennis et al., 2008; Finucane and Power, 2010; Kanske and Kotz, 2010, 2011). For example, when subjects were engaged in identifying the print color of a central target word and ignoring the flanker words above and below the target word, Kanske and Kotz (2011) observed that reaction times (RTs) to incongruent stimuli, in which the target and flanker colors are different, are faster when these stimuli are emotional negative compared to neutral. In addition, when subjects were required to complete a modified version of the Attention Network Test after the presentation of emotional pictures, Finucane and Power (2010) observed that in comparison with neutral stimuli, fear stimuli reduced RTs to a target. Taken together, since individuals with childhood maltreatment experiences tend to develop sensitization to negative stimuli, and negative stimuli could facilitate conflict resolution, it is likely that childhood maltreatment could modulate the impact of negative stimuli on conflict resolution. Specifically, negative stimuli would elicit increased emotion arousal for subjects in high childhood maltreatment, which would narrow their attention and facilitate their conflict resolution. Nevertheless, to date, this hypothesis has not been examined.

To clarify whether and how the impact of negative emotional stimuli on conflict resolution depends on childhood maltreatment, we asked subjects to perform an arrow Flanker Task which was presented in the center of emotional pictures. The arrow Flanker Task (Eriksen and Eriksen, 1974) is a frequently used interference paradigm to investigate conflict resolution (Posner et al., 2007; Finucane and Power, 2010). In the arrow Flanker Task, participants respond to a target arrow presented among strings of flanker arrows, which are either identical with the target (congruent conditions) or different from the target arrows (incongruent conditions). Typically, incongruent conditions elicit slower RTs and more error rates than congruent conditions. Conflict resolution efficiency usually was evaluated by executive attention scores that were calculated by subtracting their responses during congruent conditions from their responses in incongruent conditions (RTs incongruent conditions – RTs congruent conditions; ERs incongruent conditions – ERs congruent conditions) (O'Toole et al., 2011). Specifically, higher scores in ERs indicate reduced conflict resolution accuracy, and higher scores in RTs reflect reduced conflict resolution speed. Given that childhood maltreatment is associated with diminished executive functioning in children, adolescents, and adults (Perez and Widom, 1994; Porter et al., 2005; Minzenberg et al., 2008; Spann et al., 2012). We hypothesize that compared to subjects in low childhood maltreatment, subjects in high childhood maltreatment would show rapid slow conflict resolution in the measurement of executive attention scores in RTs and in ERs. Furthermore, based on previous studies showing individuals with childhood maltreatment experiences tend to develop sensitization to negative stimuli, and negative stimuli could facilitate conflict resolution (Easterbrook, 1959; Dennis et al., 2008; Finucane and Power, 2010; Kanske and Kotz, 2010, 2011), we hypothesize that compared to subjects in low childhood maltreatment, subjects in high childhood maltreatment would show rapid conflict resolution in the measurement of executive attention scores in RTs during the presentation of negative emotional stimuli. As there is no conclusive finding in previous research on the effect of negative emotional stimuli on conflict resolution in the measurement of executive attention scores in ERs, we would not make specific hypothesis for the modulating effect of childhood maltreatment on the impact of emotion on conflict resolution in the measurement of executive attention scores in ERs during the presentation of negative emotional stimuli. This study facilitates the understanding of the mechanisms underlying the association between childhood maltreatment and later psychopathology.

MATERIALS AND METHODS

Subjects

Seventy-nine (43 males, 36 females, mean age: 20.5 years; SD: 2.31) students from Nanyang Normal University were recruited for the experiment. All the subjects were right-handed, and had self-reported normal or corrected-to-normal vision. In addition, subjects reported that they were healthy and free of any reported affective disorders. Each subject provided informed

consent prior to the experiment. The experimental procedure was conducted in accordance with guidelines of the 1964 Declaration of Helsinki (World Medical Organization, 1996) and approved by the ethics committee of the School of Psychology in Southwest University.

After consent procedures and before experiment, subjects were required to complete the Childhood Trauma Questionnaire–Short Form (CTQ-SF) scale. The CTQ-SF was used for assessing childhood maltreatment (Bernstein et al., 2003). It is composed of five subscales, including sexual abuse, physical neglect, emotional abuse, physical abuse, and emotional neglect. There are 28 items in total in CTQ-SF and responses are rated on a 5-point scale (ranging from "never true" to "very often true"). According to the childhood maltreatment median score (childhood maltreatment median = 35), we grouped subjects into either a low childhood maltreatment group (n = 41, the average score of children maltreatment is 30.27) or a high childhood maltreatment group (n = 38, the average score of children maltreatment is 41.87). The low childhood maltreatment group has lower scores in CTQ-SF than the high childhood maltreatment group [$F(1,78) = 122.92$, $p < 0.001$].

Emotion Induction Stimuli

To avoid the cultural bias in Chinese subjects when the International Affective Picture System was adopted to elicit emotion (Huang and Luo, 2004), the current study selected the emotional pictures from the native Chinese Affective Picture System (CAPS; Bai et al., 2005). In the present study, 112 positive pictures, 112 neutral pictures, and 112 negative pictures were used. The present study included six blocks and each block consisted of 168 trials (grouped into three conditions: positive, neutral, and negative). The size and resolution of all these emotional pictures used in the present study were identical. Additionally, the contrast of the monitor was set to a constant value across subjects.

Valence and Arousal Assessment

In order to examine the validity of the three pictures sets (negative, neutral, positive), another sample of subjects (n = 41; 18 males, 23 females; age ranged from 18 to 25 years; mean age: 21.27) was recruited to rate the valence (negative – neutral – positive) and arousal (low arousal level- high arousal level) of the 336 pictures on a self-reported 9-point rating scale (SAM; Lang et al., 1997). The valence significantly differed amongst negative, neutral, and positive pictures [$F(2,80) = 265.902$, $p < 0.001$]. Positive pictures (6.728) were rated more positive than were neutral pictures (5.369) [$F(1,40) = 113.701$, $p < 0.001$] which, in turn, were rated positive compared with the negative pictures (2.943) [$F(1,40) = 233.754$, $p < 0.001$]. Also, the arousal level significantly differed amongst negative, neutral and positive pictures [$F(2,80) = 49.069$, $p < 0.001$]. Negative pictures (6.44) were rated more arousing relative to positive pictures (5.342) [$F(1,40) = 32.683$, $p < 0.01$] which, again, were rated more arousing than were neutral stimuli (3.646) [$F(1,40) = 140.221$, $p < 0.001$].

Behavioral Procedures

In a dimly room, subjects were seated with 150 cm viewing distance from a computer screen. They were instructed to perform an arrow Eriksen Flanker Task. Each trial started with a 300–800 ms presentation of small black cross on the white computer screen. Then, an arrow Flanker Task which was superimposed on a picture was presented. Subjects were instructed to respond as accurately and quickly as possible. Responses were given with pressing the "F" key on the keyboard if the middle arrow pointed to the left, and pressing the "J" key if the middle arrow pointed to the right. The presentation of the arrow Flanker Task and the emotional picture was simultaneously terminated by a key press or after 1000 ms. Each response was followed by a 200 ms presentation of a blank screen (see **Figure 1**). Before the formal experiment, all subjects took part in pre-training with 12 practice trials during which they were familiarized with the procedure.

RESULTS

A repeated measures ANOVA of RTs and ERs was conducted with emotion (positive pictures, neutral pictures, and negative pictures), childhood maltreatment (high, low), and conflict type (congruent, incongruent) as factors. Before determining basic statistical analysis of ERs and RTs, we adopted one-Sample Kolmogorov–Smirnov test to analyze whether the data are suitable for normal distribution. PASW General Linear Model software Version 17 was adopted for statistical analyses (SPSS Inc., 2009). The Greenhouse-Geisser method was used to correct the degrees of freedom of the F-ratio in all these analyses. Simple effects analyses and pair-wise comparisons were conducted using Bonferroni-Holm correction method if significant main effects and interactions were detected.

The normal distribution of ERs significantly deviates. Thus the mean of the ERs was log transformed for the repeated measures ANOVA. We observed a significant main effect of conflict type [$F(1,77) = 25.985$, $p < 0.001$] (see **Table 1**). Incongruent conditions elicited more false responses than congruent conditions, disregarding of emotion. Furthermore, we observed a significant interaction between conflict type and emotion [$F(2,154) = 4.074$, $p < 0.05$]. The two-way interaction was manifested by the smallest differences between incongruent and congruent conditions during the negative pictures. To better present these results, we computed executive attention scores in ERs by calculating the difference between incongruent and congruent conditions. The repeated measures ANOVA on executive attention scores in ERs showed that negative pictures elicited smaller executive attention scores than neutral [$F(1,77) = 5.488$, $p < 0.05$] and positive pictures [$F(1,77) = 6.573$, $p < 0.05$], whereas neutral and positive pictures elicited similar executive attention scores [$F(1,77) = 0.182$, $p = 0.671$]. No other significant main effects or interaction effects were found for ERs. No other significant main effects or interaction effects were found for ERs.

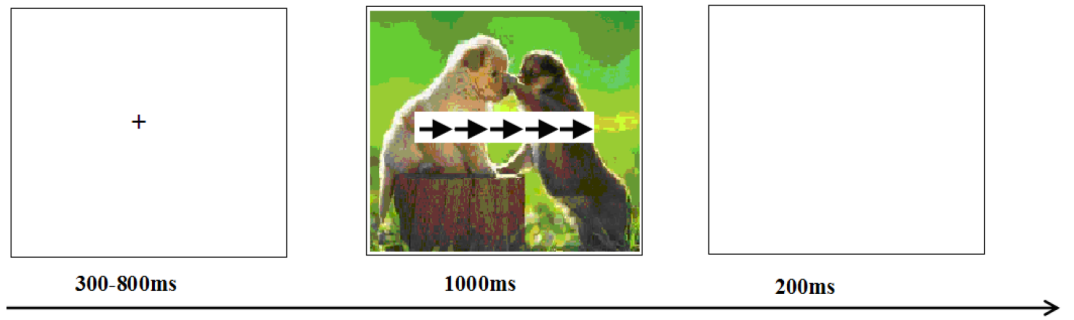

FIGURE 1 | The diagram of our experimental task.

TABLE 1 | During the presentation of negative, neutral and positive pictures, congruent and incongruent conditions elicited average reaction times (in Milliseconds) and error rates for subjects in low and high childhood maltreatment.

		Reaction times		Error rates	
		congruent	**incongruent**	**congruent**	**incongruent**
low childhood maltreatment	negative	499.691(7.012)	536.727(7.242)	2.8% (0.5%)	6.5% (0.9%)
	positive	499.133(6.896)	535.540(6.839)	2.4% (0.4%)	6.8% (1%)
high childhood maltreatment	neutral	496.381(6.622)	530.46(6.964)	2.5% (0.5%)	6.7% (1%)
	negative	496.924(8.067)	526.577(8.332)	3% (0.5%)	5.8% (1%)
	positive	490.956(7.934)	525.550(7.868)	2.8% (0.5%)	6.5% (1.1%)
	neutral	489.237(7.619)	525.066(8.012)	2.4% (0.5%)	6.2% (1.1%)

Values given in parenthesis were standard errors.

The normal distribution of RTs does not significantly deviate. The repeated measures ANOVA on RTs showed significant main effects of conflict type [$F(1,77) = 511.084$, $p < 0.001$] and emotion [$F(2,154) = 11.081$, $p < 0.001$]. Incongruent conditions elicited longer RTs than congruent conditions, disregarding of emotion. RTs were longer during negative [$F(1,77) = 19.107$, $p < 0.001$] and positive pictures [$F(1,77) = 13.698$, $p < 0.001$] than during neutral pictures, whereas RTs were similar between negative and positive pictures [$F(1,77) = 2.652$, $p = 0.101$]. More interesting, we observed a three-way interaction amongst emotion, conflict type, and childhood maltreatment [$F(2,154) = 5.021$, $p < 0.05$]. The breakdown of the three-way interaction showed that the interaction between conflict type and emotion was significant in subjects in high [$F(2,74) = 5.475$, $p < 0.01$], instead of low [$F(2,80) = 1.084$, $p = 0.333$], childhood maltreatment. For subjects in high childhood maltreatment, the smallest differences between incongruent and congruent conditions were observed during the negative pictures, whereas for subjects in low childhood maltreatment, similar differences between incongruent and congruent conditions were observed during negative, neutral, and positive pictures. To better present these results, we computed executive attention scores in RTs by calculating the difference between incongruent and congruent conditions. The repeated measures ANOVA on executive attention scores in RTs showed a significant two-way interaction between childhood maltreatment and emotion [$F(2,154) = 5.021$, $p < 0.05$]. The breakdown of the two-way interaction showed a significant emotion effect in subjects in high [$F(2,74) = 5.475$, $p < 0.01$], instead of low [$F(2,80) = 1.084$, $p = 0.333$], childhood maltreatment. For subjects in high childhood maltreatment, negative pictures elicited smaller executive attention scores than neutral [$F(1,37) = 10.037$, $p < 0.01$] and positive pictures [$F(1,37) = 7.272$, $p < 0.05$], whereas neutral and positive pictures elicited similar executive attention scores in RTs [$F(1,37) = 0.001$, $p = 0.977$]. By contrast, for subjects in low childhood maltreatment, executive attention scores in RTs were similar across three conditions (see **Table 1**). No other significant main effects or interaction effects were found for RTs.

DISCUSSION

Using an arrow Flanker Task, this study aims to investigate whether the impact of emotion on conflict resolution varies depending on childhood maltreatment. Regardless of emotion, incongruent conditions elicited slower RTs and lower response accuracy than congruent conditions, suggesting that the task used in the present study is effective in inducing attention executive control.

More interesting, our hypothesis that childhood maltreatment modulates the effect of emotion on conflict resolution in the measurement of executive attention scores in RTs was confirmed. Specifically, for subjects in high childhood maltreatment, negative pictures elicited smaller executive attention scores in RTs than positive and neutral pictures. By contrast, for subjects in low childhood maltreatment, similar executive attention scores

in RTs were yielded across three emotion conditions. When discussing the effect of negative emotion on cue utilization and the organization of behavior, Easterbrook (1959) argued that increased emotional arousal of negative stimuli may result in narrowing attention breadth and reducing interference of distracting or irrelevant information (Easterbrook, 1959). According to this argument, it is reasonable that negative emotion stimuli elicit increased emotion arousal for subjects in high childhood maltreatment, which narrows their attention to cues in the Flanker Task and thus speeds up their response in conflict resolution in the present study.

For the effect of emotion on conflict resolution accuracy in the measurement of executive attention scores in ERs, our findings showed that negative pictures elicited smaller executive attention scores in ERs than neutral pictures for subjects in both high and low childhood maltreatment. This suggests that subjects in both low and high childhood maltreatment showed the enhancement of the accuracy of conflict resolution in negative emotional stimuli. On the one hand, this finding is consistent with previous research (e.g., Finucane and Power, 2010), suggesting that during negative emotional experience subjects were better able to inhibit irrelevant information resulting in accurate response to a target. On the other hand, this finding indicated that childhood maltreatment did not modulate the effect of emotion on conflict resolution accuracy using the Flanker Task. This maybe because of a "ceiling effect," that is, the conflict resolution task in the present study is relatively easy for all participants so that it is lack of discrimination validity to produce a significant difference in conflict resolution accuracy.

In contrast with the impact of negative stimuli on conflict resolution, we did not observe the impact of positive stimuli produced on conflict resolution. That is, executive attention scores in ERs and RTs were similar between positive and neutral stimuli in subjects in both high and low childhood maltreatment. According to the motivational intensity theory of affective states (Harmon-Jones et al., 2012, 2013a,b), one possible explanation for these results is that compared to negative pictures, positive and neutral pictures used in this study elicited similar but low level motivational intensity, which did not produce significant influence on narrowing cognitive scope.

LIMITATIONS AND FUTURE DIRECTIONS

A number of important limitations of the present study and future directions should be mentioned. First, negative and positive pictures used in the present study differed not only on valence but also on arousal level. As a result, it is unclear whether the accelerated conflict resolution during the presentation of negative emotional stimuli in subjects in high childhood maltreatment is driven by valence or arousal level. This issue is worthy of further investigation by including positive and negative emotional stimuli of equally low arousal level and equally high arousal level in future studies. Second, the sample size is relatively small. The number of subjects in high childhood maltreatment is 41 and the number of subjects in low childhood maltreatment is 38. The findings of the present study need to be replicated in a larger sample. Third, subjects in this study are healthy. Even the subjects in high childhood maltreatment did not meet criteria for clinical diagnosis of psychiatric population. Hence, the present results need to be replicated in psychiatric population in future studies. Fourth, as there are known modulatory effects of cultural (Butler et al., 2007; Matsumoto et al., 2008; Soto et al., 2011) and age (Meng et al., 2015; Yuan et al., 2015) in the studies of emotional processing, our findings are limited to only Chinese subjects of a small age range. Future studies recruiting larger age range samples of subjects from Chinese and other nations will broaden and increase confidence in the present findings.

THEORETICAL AND PRACTICAL IMPLICATIONS

Despite several limitations, the present study has important theoretical and practical implications for the future studies. Subjects in high childhood maltreatment showed faster response times in conflict resolution in negative emotion situation, which may imply their oversensitivity to negative stimuli in threatening situations. And the oversensitivity to threat further facilitates both the development and maintenance of emotional disorders (Li et al., 2008; Yuan et al., 2009, 2014, 2015; Meng et al., 2015, 2016). The present finding may partly account for why individuals suffering from childhood maltreatment are vulnerable to psychopathology.

Furthermore, if oversensitivity to negative stimuli in threatening situations of subjects in high childhood maltreatment is ultimately shown to contribute to their psychopathology, this observation may provide new therapeutic insights. For instance, new therapies might specifically target underlying abnormalities in sensitivity to negative stimuli as a means of affecting psychopathology.

In summary, the present study demonstrated that the impact of negative emotional stimuli on conflict resolution varied depending on childhood maltreatment. Specifically, subjects in high, instead of low, childhood maltreatment showed an enhanced speed of conflict resolution during the presentation of negative emotional stimuli.

ETHICS STATEMENT

The experimental procedure was in accordance with the ethical principles of the 1964 Declaration of Helsinki (World Medical Organization, 1996). The experimental procedure were approved by the IRB of the School of Psychology in Southwest university.

AUTHOR CONTRIBUTIONS

XM and WL conducted the experiments and analyzed the data. XM, WL, SG, and LZ proposed the concept of the measurements. HL helped in the experimental design. XM and HL supervised the project, and conducted the theoretical investigations leading to presented simulations. All authors discussed and contributed to the manuscript.

FUNDING

This study was financed by the National Natural Science Foundation of China (NSFC31700947 and 31600886), the research projects of the social science and humanity in Henan Province (2017-ZZJH-380), the Key Program of the Higher Education Institutions of Henan Province (17AJ90002), the 13th 5-Year education science planning projects of Henan Province (2017-JKGHYB-0134), and the Youth Foundation of Nanyang Normal Uninversity (QN-2019-046).

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