Original Article
Correlations of encephalic neurotransmitters with contingent negative variation (CNV) in post-stroke depression patients

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Abstract: Background: To explore the mechanism of post-stroke depression by studying the correlations of contingent negative variation (CNV) with encephalic neurotransmitter levels via encephalofluctuograph technology (ET). Methods: Forty patients who met the screening criteria as the post stroke depression group. Another forty patients without post-stroke depression were selected as the control group. The encephalic neurotransmitter and CNV were detected in all groups. Results: Compared with the post-stroke group, the gamma-amino butyric acid (GABA) level was increased, but the levels of acetylcholine and 5-HT were decreased in post-stroke depression group; the amplitude of point B in the CNV was decreased, and the latency of point C was prolonged in post-stroke depression group. The 5-HT level was negatively correlated with the amplitude of point B (r = -0.33, P = 0.04). The acetylcholine level was positively correlated with the latency of point C (r = 0.44, P = 0.01) in post-stroke depression group. Conclusions: The 5-HT level was negatively correlated with the amplitude of point B in the post-stroke depression group, and the decreased 5-HT level may be the possible pathological basis in post-stroke depression.

Keywords: Post-stroke depression (PSD), neurotransmitters, encephalofluctuograph technology (ET), contingent negative variation (CNV)

Introduction

Post-stroke depression (PSD) is a common and frequently occurring disease, which is a severe complication of stroke. The morbidity is up to 20-79% in post-stroke depression patients. Approximately 25% of cases of depression occur during the acute phase after stroke, and 50% of depression cases occur 6 months after stroke [1]. Despondent feelings, lacking interest, self-accusation, self-guilt, sadness, and suicidal thoughts may occur in post-stroke depression patients [2]. The appearance of mental symptoms in patients can severely affect limb function recovery and completion of a rehabilitation program.

Post-stroke depression has been reported to closely correlate with changes in intracranial neurotransmitters after cerebral injury [3]. At present, the diagnosis and pathogenesis of post-stroke depression mainly depend on the scaling method and detection of neurotransmitters in the peripheral blood. For most of the patients with lalopathy and cognitive dysfunction, the doctor couldn’t collect medical history in detail by scaling method, and the screening method is affected by the subjectivity of the patients. Peripheral neurotransmitters are influenced by many factors during the process of transmission from the brain to the peripheral post stroke; therefore, the neurotransmitters level in peripheral cannot accurately represent encephalic neurotransmitter levels. Therefore it is difficult for the early diagnosis of post-stroke depression. Exploring the pathomechanism and studying objective and specific auxiliary examination of post-stroke depression is very important for the early diagnosis and intervention of post-stroke depression.

Encephalofluctuograph technology (ET) can be used to noninvasively analyze the changes in
ET and CNV in post stroke depression patients

brain neurotransmitters under native condition [4, 5]. The contingent negative variation (CNV) is an important indicator which reflects electrophysiological changes in cognitive processing and can be used as an effective and objective indicator on evaluating advanced functions of the brain; therefore, it can reflect human psychological activity [6-8]. Until now studies of the CNV in post-stroke depression patients was few. Therefore, we aimed to study the correlations of neurotransmitter levels in the brain combined ET with the CNV method. To explore the mechanism of post-stroke depression, and provide references for the early diagnosis and intervention of post-stroke depression.

Patients and methods

Subjects

Eighty patients with a first episode of ischemic stroke who were hospitalized in our department between June 2014 and April 2016 were recruited for this study. The post-stroke patients were divided into two groups: (i) a post-stroke depression group (n = 40), who suffered depression after stroke; (ii) a post-stroke group (n = 40), who did not suffer from depression after stroke. Depression was diagnosed based on Hamilton Depression Ranking Scale (HAMd). No significant difference was found in age, gender or degree of education between the post-stroke depression and post-stroke groups (Table 1). This research was approved by the Ethics Committee of The Third Affiliated Hospital of Zhejiang Chinese Medical University. All the participants were informed of the aim of the study and the relevance of their participation in the study. All of the participants provided written informed consent.

The post-stroke depression group inclusion criteria were as follows: (i) first cerebrovascular event; (ii) depression between 2 and 4 weeks after the stroke; (iii) a definite diagnosis of a cerebrovascular disorder (including cerebral thrombosis and embolism and excluding cerebral and subarachnoid hemorrhage) by computed tomography (CT) or magnetic resonance imaging (MRI); (iv) depression diagnosis based on the third revised version of the Chinese Classification of Mental Disorders (CCMD-3); (v) HAMd score ≥ 8 points; (vi) Beck anxiety inventory (BAI) score < 45 points; and (vii) self-rating depression scale (SDS) score and depression severity degree index ≥ 0.5. The post-stroke depression group exclusion criteria were as follows: (i) disturbance of consciousness, aphasia, or cognitive dysfunction; (ii) severe organic diseases; (iii) affective disorders or correlative mental disorders before the onset of stroke.

Detection of neurotransmitters in the brain

The intracranial neurotransmitters detected by the ET analytical instrument (ML2001 encephalofluctuograph analyzer, Beijing Tongren Optoelectronics Technology Co., Ltd.). The patient was seated in a comfortable position and remained quiet with their eyes closed during ET detection. The electrical signals were recorded for 18 minutes via ET and were stored for EEG analysis. Then the EEG signals were analyzed to finally obtain a new informative frequency hidden in the brain waves, which typically falls in the range of 1-255 MHz. The waves were finally analyzed by ET, and thus, the signals corresponding to intracranial neurotransmitter activity were obtained. The results analyzed by ET were a super-slow shocking S system, including the S1-S255 series, which could reflect the activity of different intracranial neurotransmitters, with the S1, S2, S4, S5, S7, and S11 series corresponding to γ-aminobutyric acid (GABA), glutamate (Glu), 5-hydroxytryptamine (5-HT), acetylcholine (Ach), dopamine (DA), and norepinephrine (NE), respectively [5]. The distribution diagrams of the S spectrum detected by ET in the two groups were shown in Figures 1, 2.

Detection method for the contingent negative variation

The CNV of patients was detected by an EMG-evoked potentiometer (NIHON KOKIDEN COR-

<table>
<thead>
<tr>
<th>Table 1. Demographics of the participants</th>
<th>Post-stroke group (n = 40)</th>
<th>Post-stroke depression group (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables of interest</td>
<td>Age (years; mean ± S.D.)</td>
<td>58.60 ± 7.50</td>
</tr>
<tr>
<td></td>
<td>Education (years; mean ± S.D.)</td>
<td>9.50 ± 2.10</td>
</tr>
<tr>
<td></td>
<td>Sex (% female)</td>
<td>57.50</td>
</tr>
<tr>
<td></td>
<td>Course of stroke (months)</td>
<td>6.20 ± 2.30</td>
</tr>
<tr>
<td></td>
<td>Course of depression (weeks)</td>
<td>6.90 ± 1.10</td>
</tr>
</tbody>
</table>

*: Compared with the post-stroke group, P < 0.01.
Figure 1. Distribution diagram of the S spectrum detected by ET in the post-stroke group (The vertical axis represents the S spectrum in ET: S1, S2, S4, S5, S7, S11 and S13 series corresponding to γ-aminobutyric acid (GABA), glutamate (Glu), 5-hydroxytryptamine (5-HT), acetylcholine (Ach), dopamine (DA), norepinephrine (NE), and suppression, the abscissa axis showed the activity of the neurotransmitters in the post-stroke group which corresponding to the S spectrum).
ET and CNV in post stroke depression patients

Figure 2. Distribution diagram of the S spectrum detected by ET in the post-stroke depression group (The vertical axis represents the S spectrum in ET: S1, S2, S4, S5, S7, S11 and S13 series corresponding to γ-aminobutyric acid (GABA), glutamate (Glu), 5-hydroxytryptamine (5-HT), acetylcholine (Ach), dopamine (DA), norepinephrine (NE), and suppression, the abscissa axis showed the activity of the neurotransmitters in the post-stroke depression group which corresponding to the S spectrum).

Figure 3. Contingent negative variations (CNVs) in the post-stroke group. Cz: recording electrodes were placed on the central line; Fz: recording electrodes were placed on the forehead.

Figure 4. Contingent negative variations (CNVs) in the post-stroke depression group. Cz: recording electrodes were placed on the central line; Fz: recording electrodes were placed on the forehead.

PORATION, MEB-9200K). Patients were kept in a waking state with fixed attention, relaxed systemic muscles, wearing headphones and eye mask, and in a supine position in a quiet shielded room. According to the international 10-20 electrode system, recording electrodes were placed on the central line (Cz); reference electrodes were placed on the ear lobes (A1 and A2) of the patients, and the electrode impedance was < 5 KW. Sound and light stimulation was applied to record the reflection of the brain. Patients were told to concentrate and indicate when they heard a sound in the headset, and they were told to immediately push a button if they saw a red light, then relax their body. All trials were repeated 20 times. Warning after auditory stimuli were used to stimulate S1 and visual stimulation administered 1-2 seconds after the S1 command was used to stimulate S2. After receiving S1 stimulation, a series of negative-phase deflection potentials were recorded on the scalp. When patients pushed the button in response to S2 stimulation, the potential changed to the positive phase; later, the potential returned to baseline. The point at which the potential diverged from the baseline was set as point A; the highest potential of the negative phase deflection was recorded as point B, and the point where the potential returned to baseline was set as point C. The CNVs changes in the two groups were shown in Figures 3, 4.

Statistical analysis

The data were analyzed using SPSS 18.0 software. The data are presented as the mean ± standard deviation (SD). Descriptive statistics
ET and CNV in post stroke depression patients

Descriptive statistics were performed to determine whether the data were normally distributed. Normally distributed data were analyzed with one-way analysis of variance (ANOVA) and independent sample t test, and non-normally distributed data with non-parametric test. Correlation analysis was performed using Pearson correlation analysis. P ≤ 0.05 was considered statistically significant.

Results

Neurotransmitters associated with the S spectrum in the two groups

Descriptive statistics were performed to determine whether the data were normally distributed. The data were normally distributed. Comparisons between groups were performed with independent sample t test, and the differences among the groups are shown in Figure 5. Compared with the post-stroke group, the GABA level was increased, while the levels of glutamate, acetylcholine, and 5-HT were decreased in the post-stroke depression group (P ≤ 0.05; Figure 5).

Changes in the CNV in the two groups

Descriptive statistics were performed to determine whether the data were normally distributed. The data were normally distributed. Comparisons between groups were performed with independent sample t test, and the differences among the groups are shown in Figure 6. Compared with the post-stroke group, the latency of point C prolonged (P ≤ 0.01; Figure 6A), while the amplitude of point B in the CNV decreased in the post-stroke depression group (P ≤ 0.05; Figure 6B).

Correlation analysis of neurotransmitter levels with the CNV

Descriptive statistics were performed to determine whether the data were normally distributed. The data were normally distributed. Correlation analysis was performed using Pe-
ET and CNV in post stroke depression patients

arson correlation analysis. The 5-HT level was negatively correlated with the amplitude of point B \( (r = -0.327, P = 0.04) \), and the acetylcholine level was positively correlated with the latency of point C \( (r = 0.435, P = 0.005) \) in the post-stroke depression group (Table 2).

### Table 2. Correlation analysis of neurotransmitters with the CNV

<table>
<thead>
<tr>
<th>S spectrum</th>
<th>Latency of point A</th>
<th>Amplitude of point B</th>
<th>Latency of point C</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>r-value 0.13</td>
<td>-0.13</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td>P-value 0.94</td>
<td>0.42</td>
<td>0.82</td>
</tr>
<tr>
<td>S2</td>
<td>r-value -0.23</td>
<td>-0.16</td>
<td>-0.03</td>
</tr>
<tr>
<td></td>
<td>P-value 0.15</td>
<td>0.31</td>
<td>0.84</td>
</tr>
<tr>
<td>S4</td>
<td>r-value 0.07</td>
<td>-0.33</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>P-value 0.68</td>
<td>0.04</td>
<td>0.87</td>
</tr>
<tr>
<td>S5</td>
<td>r-value 0.44</td>
<td>-0.18</td>
<td>-0.25</td>
</tr>
<tr>
<td></td>
<td>P-value 0.01</td>
<td>0.27</td>
<td>0.12</td>
</tr>
</tbody>
</table>

S1 corresponding to γ-aminobutyric acid, S2 corresponding to glutamate, S4 corresponding to 5-HT, S5 corresponding to acetylcholine.

Discussion

CNV analysis is a neuropsychological technique which can reflect the level of cerebral cortex awakening, alertness and anticipation in response to a warning command sequence [9]. The CNV relies on the close correlation of two stimuli with psychological activity, including anticipation, attention, awakening, memory, motivation, preparation, and decision-making [10]. The CNV can objectively reflect the progress of even slight mental activity. The CNV analysis can effectively eliminate the patient’s subjective and poor cooperation in the scaling method, and even mild psychological change can be reflected in the brain electrical activity of CNV. To date, CNV analysis has been widely used as an objective evaluation tool for mental disease in clinical practice [11-13].

ET is a non-invasive approach to brain function detection to indirectly measure the levels of brain neurotransmitters, which mainly depend on analyzing the ultra-slow cortical oscillations of EEG. It can reflect the level of neurotransmitters in the brain by scanning dominant frequency and analyzing information in consistent with neurochemical oscillation, which being hidden in the ultra-slow frequency [14, 15]. It is an encephalofluctuograph (EFG) analysis technique, which has been reported to the diagnosis of Parkinson’s disease, primary insomnia, and job-related burnout [16-18].

Although the clinical manifestation of post-stroke depression is very similar to that of endogenous depression, its mechanism remains elusive [19]. Most scholars consider that the pathogenesis of post-stroke depression is based on the stroke and is the result of multiple neurobiological mechanisms [20, 21]. Previous reports have shown that post-stroke depression is closely correlated with changes in intracerebral neurotransmitters after stroke, mainly 5-HT and acetylcholine [22, 23]. 5-HT is closely correlated with human behavior, and its deficiency is an important factor for depression [24]. In our study, we found that the values of S4, which reflected the 5-HT level, and S5 which reflected the acetylcholine level, were decreased in the post-stroke depression patients compared with those in the post-stroke control patients. 5-HT can elicit a reward response and plays an important role in regulating and controlling emotional balance. The abnormal level of 5-HT in the central nervous system is closely correlated with many mental diseases [24, 25]. The 5-HT level decreased after stroke, and adrenocorticotropic increased in pituitary secretions, which induced the increasing of cortisol in peripheral secretions. The increased secretion of cortisol can induce the production of tryptophan pyrrole in the liver, which can degrade tryptophan in the blood. The decreased level of tryptophan results in a deficiency of 5-HT, which may induce symptoms of depression, including suicidal thoughts, obsessive-compulsive disorder, chronic pain, and somniphathy [26]. Our results showed that the 5-HT level was negatively correlated with the degree of depression, which coincided with previous reports [27, 28]. The encephalic 5-HT soma is located in the brainstem, and its axons travel through the hypothalamus, basal ganglia, surrounding corpus callosum and corona radiata, and deep white matter and then gradually branch to the frontal lobes. This loop circuit is closely related to emotional regulation. Lesions in anatomical sites such as the temporal lobe, frontal lobe and basal ganglia exhibit close correlations with post-stroke depression [29]. Large infarctions in the prefrontal cortex pathway, especially in the basal ganglia area including the caudate nucleus, globus pallidus,
ET and CNV in post stroke depression patients

and capsule knee, were frequently complicated by post-stroke depression. Areas of the prefrontal cortex pathway involved in ischemic stroke were significantly correlated with post-stroke depression [30, 31]. Wiartl et al hypothesized that damage to any part of the frontal lobe pathway, basal leaf, and ventral brainstem could induce depression [32], and multiple studies have confirmed this hypothesis [33, 34]; however this loop is a projection loop of the 5-HT neural circuit.

Hiruma et al reported that the decreasing amplitude of point B represented the main change in CNV nerve electrophysiology in affective disorder patients [35]. The decreased amplitude or the absence of point B represented changes in the CNV of depressive disorder patients and that PINV delays were diagnostic indicators of depression [36]. We found that the amplitude of point B was decreased in post-stroke depression patients compared with post-stroke patients, and the level of 5-HT was negatively correlated with the amplitude of point B, which coincided with the finding that a 5-HT agonist could reduce the amplitude of point B [37]. Dendritic depolarization of nerve cells occurs extensively on the top surface of the prefrontal cortex, which changes the CNV. These CNV changes are regulated by the basal ganglia, thalamus, midbrain reticular formation, hippocampus, amygdala, cingulate cortex and striatum [6]. Therefore, a common neural anatomical mechanism in depression involves neural projections of the 5-HT loop and is the origin of the CNV. These correlations coincide with common pathological mechanisms. This phenomenon may be caused by ATP exhaustion in the infarction area after stroke, which resulting dysfunction of various ion channels. Previous study has proved that various subtypes of calcium ion channels can regulate the neurotransmitter release on presynaptic membrane. Abnormal calcium ion channels induced decreasing calcium fluxes in the presynaptic membrane, which may ultimately decrease the amount of 5-HT released from the presynaptic membrane into the synaptic cleft. Therefore, we speculate that the decreased level of 5-HT in the synaptic cleft may induce post-stroke depression. 5-HT reuptake inhibitor treatment can increase the level of 5-HT in the synaptic cleft. And clinical effect of 5-HT reuptake inhibitor drugs treatment for depression also supports our hypothesis. T type calcium channel can increase the reflection sensitivity of neural dendrites to the weak current signal, which may obviously influence on the synaptic subliminal integration. Regarding the decreased ATP capacity, if the ion concentration of sodium is abnormal, the synaptic subliminal integration will be affected. On the macro performance, when receiving external stimuli, the neuronal action potential will be depressed or unable to elicit neuronal excitation, which will result the decreasing of the amplitude of point B. The above hypothesis still need further study to prove.

Acetylcholine participates in the regulation of learning, memory, emotion, sleep, and pain. The cholinergic system, which includes acetylcholine receptors participates in the depression [38]. Antagonism of acetylcholine receptors can enhance emotional stability [39]. Acetylcholine somas are mainly distributed in the cortex, basal ganglia, striatum, and hypothalamus. Their anatomical distribution overlaps with the emotion regulation loop area, and damage to this area has been reported to be closely correlated with post-stroke depression. CNV analysis involves the application of a combined warning sequence, and the latency of point A is the point where a negative deflection to the baseline occurs after the brain receives the warning stimulus. The delayed latency of point A in post-stroke depression patients indicated that even though many resources are devoted to the warning response, the expectation of the cognitive task is slower than that of post-stroke patients. The irrational psychological response and extended reaction time indicate that the attention of post-stroke depression patients is not as easily focused as that of the post-stroke patients. Due to the feeling of depression and lack of interest in novelty and excitement, the lack of an effective response to external stimuli by the central nervous system may be characterized by the latency which causes the delay in point A. The regulatory function of acetylcholine as the primary slow wave component has been previously demonstrated. The effect of acetylcholine on the CNV mainly occurs before the S2 warning stimulus; this period is represented by the length of the delay of point A. We found that the acetylcholine level was positively correlated with latency of point A, which coincided with findings from
ET and CNV in post stroke depression patients

previous studies. No significant difference was found in the latency of point A in post-stroke depression patients compared with that in post-stroke patients.

Conclusions

Importantly, our research applied ET, which can be used to noninvasively and accurately determine brain neurotransmitter information under physiological conditions, and the CNV, which can be used to accurately detect depression. We analyzed correlations of neurotransmitters levels using the CNV to explore the mechanism of post-stroke depression and found that the level of 5-HT was negatively correlated with the amplitude of point B in post-stroke depression patients, which may be responsible for the pathogenesis of post-stroke depression. Our results provide references for early diagnosis and rehabilitation in clinical practice. However, the trends of the neurotransmitter levels merely represented the whole brain neurotransmitter trends, which cannot represent the trends of particular brain regions. Therefore, further studies on the neurophysiologic mechanism of the correlation between neurotransmitter levels and the CNV in each brain region should be conducted in the future.

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Disclosure of conflict of interest

None.

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