Notch1 knockdown disturbed neural oscillations in the hippocampus of C57BL mice

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A B S T R A C T

Neural oscillations and their interactions are associated with the coordination of neural groups, which provide a mechanism underlying information processing of brain functions. Notch1 receptor is involved in the neurological and psychiatric disorders, such as neurodevelopmental deficits, cerebral ischemia, Alzheimer's disease and depression. Here, we investigated the dynamics of neural oscillations in hippocampus of Notch1+/- mice in urethane-anesthetized state. Notch1 knockdown altered the distribution of power in the hippocampal DG areas, reduced theta (3–8 Hz) power and enhanced low gamma (LG, 30–50 Hz) and high gamma (HG, 50–100 Hz) power. Moreover, theta-gamma phase-amplitude coupling in the hippocampal DG area was markedly impaired in the Notch1+/- mice. The data further showed that the expression of NR2B was decreased, and the expressions of GABAAR α1, GAD67 and parvalbumin were considerably increased after Notch1 knockdown. Taken together, our results suggest that Notch1 genetic deficiency significantly impaired the cross-frequency coupling of neural oscillations, and their interactions in the hippocampal DG region by means of disrupting the balance of excitatory and inhibitory receptors, which could be an underlying mechanism of cognitive impairment in neuropsychiatric disorders.

1. Introduction

It is currently thought that neural oscillations provide a coordinative mechanism for neuron activities. Furthermore, different frequencies of neural oscillations have various behavioral and cognitive correlates (Buzsaki and Draguhn, 2004). The hippocampus formation is an important part of neural networks that receives multimodal sensory inputs from other brain structures anatomically related the entorhinal cortex. Theta (3–14 Hz) and gamma (30–100 Hz) oscillations are two prominent rhythms expressed in the hippocampus (Yanovsky et al., 2014). Theta oscillations are generated by interaction of glutamatergic and gamma-aminobutyric acidergic (GABAergic) neurons (White et al., 2000; Leung and Shen, 2007). Generation of gamma activity is primarily dependent upon GABAergic interneurons and modulated by several neurotransmitter systems (Traub et al., 2004; Fukuda et al., 2006; Bartos et al., 2007). Abnormal performance at theta and gamma bands in the hippocampus was involved in the neurological and psychiatric disorders (Urcelay et al., 2008), such as depression, vascular dementia and schizophrenia (Uhlhaas et al., 2008; Zheng and Zhang, 2013, 2015; Shang et al., 2017a).

There are a number of studies, in which Notch signaling pathway is closely correlated with neurodevelopmental deficits (Luo and O'Leary, 2005), cerebral ischemia (Wang et al., 2009), Alzheimer's disease (Woo et al., 2009), depression (Wang et al., 2010) and so on. Notch signaling pathway is highly conserved through evolution, which can maintain the stability of neural stem cells (Weng et al., 2004), regulate the differentiation of nerve cells (Taniyama et al., 2001; Ge et al., 2002; Nagao et al., 2007), promote proliferation and renewal of neuron (Hitoshi et al., 2002) and involve in apoptosis (Yang et al., 2004). Mammalians have a Notch gene homologue, which are Notch1, Notch2 and Notch3. In the above homologue, Notch1 is expressed primarily in proliferating neural progenitor cells in the ventricular zone (VZ) of the embryonic brain (Lindsell et al., 1995), and continues to be expressed postnatally in specific regions (Tokunaga et al., 2004). Subgranular zone (SGZ) of the hippocampal dentate gyrus is one of two regions in which neurogenesis occurs of the adult rodent, nonhuman primate, and human brains (McDermott and Lantos, 1991; Eriksson et al., 1998; Jin et al., 2001; Sanai et al., 2004). A series of studies have suggested that Notch1 receptor expresses along the inner aspect of the DG area and modulate various stages of neurogenesis (Irvin et al., 2001; Androulakis et al., 2005).
et al., 2006; Johnson et al., 2009). Moreover, this receptor has been reported to be crucial for learning and memory process (Costa et al., 2003) and synaptic plasticity (Wang et al., 2004; Yang et al., 2016), which have a strong connection with cognitive ability. In one of our previous studies, we found that the Notch1 knockdown significantly attenuated bidirectional synaptic plasticity of PP-DG, and considerably impaired the ability of learning and memory in the behavioral experiment including Morris water maze and novel object recognition test. Furthermore, it was also found that voluntary wheel running evidently enhanced synaptic plasticity and improved learning and memory ability through the Notch1 signaling pathway (Zhang et al., 2017). However, little is known about the effect of theta and gamma oscillations on the hippocampus of Notch1 knockdown mice.

Therefore, in the present study, a hypothesis was raised that Notch1 genetic deficiency disturbed neural oscillations and their interactions in the hippocampus through breaking the balance between excitatory and inhibitory proteins. This was done by recording the signals of LFPs from both Notch1 heterozygous deficient (Notch1+/-) mice and wildtype (WT) littersmate in urethane-anesthetized state, and further measuring theta and gamma neural activities not only power spectrum but also cross-frequency couplings in the hippocampal dentate gyrus (DG) area. In addition, biochemical detections were introduced to examine whether the balance between excitatory and inhibitory proteins was broken. Our results will help to understand the key role of Notch1 receptor in the hippocampal neural networks, which might provide a therapeutic target for the neurological and psychiatric disorders.

2. Results

2.1. Notch1 heterozygous deficiency did not impact the body weight

The Western blotting assay showed that the expression of Notch1 was much lower in the hippocampi of Notch1+/- mice compared to those of WT mice, as expected (Z = -8.748, P < .001, Fig. 1A). However, there was no significant difference of body weight between these two groups of mice (t66 = -0.606, P = .565, Fig. 1B).

2.2. Notch1 knockdown affected the expressions of excitatory and inhibitory receptors

In order to study the effect of Notch1 knockdown on the expressions of excitatory and inhibitory receptors, Western blotting assay was performed. NR2B, the representative protein of excitatory receptors, GABAARα1, the representative protein of inhibitory receptors, and GAD67, the protein catalyzing the production of gamma-aminobutyric acid from L-glutamic acid, were measured. It was found that the expression of NR2B was obviously decreased after Notch1 knockdown (Z = -8.763, P < .001, Fig. 2B). On the contrary, the expressions of both GABAARα1 and GAD67 were significantly increased after Notch1 knockdown (Z = -8.758, P < .001 for GABAARα1 and Z = -8.752, P < .001 for GAD67, Fig. 2C & D). Moreover, immunofluorescence staining showed the expressions of NR2B, GABAARα1, GAD67 and parvalbumin (PV) in the DG region of hippocampus. PV is a marker of PV interneurons, which continuously release GABA and are involved in gamma (30–80 Hz) oscillations. As shown in Fig. 2F–H, the expressions of GABAARα1, GAD67 and PV in DG region were stained with green fluorescence by Alexa 488-conjugated second antibody. It was found that fluorescence intensity and larger numbers of bright fluorescent particles (white arrows) were visibly enhanced. The fluorescence intensity of NR2B was weakened in the Notch1+/- group compared to that in the WT group (Fig. 2E). These immunofluorescence results were consistent with the results of western blot assay in Fig. 2A–D. These results suggest that the balance of excitatory and inhibitory neurotransmitters in mice is causally affected by the Notch1 knockdown.

2.3. Notch1 knockdown disturbed the power distributions

We obtained local field potentials (LFPs) from the hippocampal DG areas in both the WT group and the Notch1+/- group. HE staining was performed to verify the location of the LFPs recording site. According to the mouse brain in stereotaxic coordinates (Franklin and Paxinos, 2001) (Fig. 3A), the DG region of hippocampus (Fig. 3B) was identified and the location was consistent with our experiment.

There are two types of hippocampal theta rhythm, which have been recognized by their different pharmacology and behavioral properties (Kramis et al., 1975). Type II theta at 3–8 Hz is resistant to urethane but sensitive to atropine (Buzsáki, 2002). Accordingly, the period of typical type II theta activities was obviously discriminated under the urethane anesthetistic state. Fig. 4A represented raw LFP traces within 3 s (Fig. 4A), their time-frequency analysis within 200 s (Fig. 4B) and the mean power spectrum (Fig. 4C) in DG region in these two groups. From time-frequency analysis, there were more stable and brighter bands in the WT group compared to that in the Notch1+/- group, suggesting that there was a decrease of power at theta activities in the latter group (Fig. 4B). In addition to the mean power spectrum from 1 to 100 Hz, there was an increase of gamma power in DG area in the Notch1+/- group (Fig. 4C). In the present study, we divided the gamma band into two parts, which were low gamma (30–50 Hz) and high gamma (50–100 Hz). This is because LG and HG are involved in different cognitive functions (Zheng and Zhang, 2015). The statistical results provided further evidences that the distribution of power (Fig. 4D) was changed by Notch1 knockdown (F(5, 10) = 3.120, P = .059). Specifically, the power of theta frequency band was significantly attenuated in the Notch1+/- group compared to that in the WT group (F(1,
140 = 7.601, P < .05). Meanwhile, the power of LG activities ($F_{1,14} = 19.049, P < .001$) and HG activities ($F_{1,14} = 6.299, P < .05$) were markedly increased in the Notch1+/− group compared to those in the WT group.

2.4. Notch1 knockdown attenuated theta-gamma phase-amplitude coupling in DG area

MVL algorithm as an indicator has been commonly used to measure phase-amplitude coupling (PAC) phenomenon. Specifically, the MVL value is identified that how the higher frequency (30–100 Hz) is modulated by the lower frequency (1–10 Hz). A typical PAC in DG was shown in Fig. 5A. It exhibited that there was a strong PAC between theta phase and gamma amplitude in the WT group, but the PAC phenomenon was almost disappeared in the Notch1+/− group. The data showed there were significant alterations in the hippocampus DG region (theta-LG: $t_{10.5} = 4.767, P < .001$; theta-HG: $t_{14} = 2.653, P < .01$, Fig. 5B). The results suggest that Notch1 knockdown damaged the interactions between theta and gamma in the hippocampal DG area.

3. Discussion

In the present study, we assessed the expressions of excitatory and inhibitory receptors in both Notch1+/− mice and WT mice. Specifically, our results show that the expression of NR2B is considerably decreased, and the expressions of either GABAAR α1, GAD67 or PV is significantly increased after Notch1 knockdown. By recording LFPs from the hippocampal DG area and performing the measurements of neural oscillations, the comparison was successfully implemented between the WT group and Notch1+/− groups. The data showed that Notch1 knockdown disturbed the power distribution, significantly
reduced the strength of cross-frequency PAC between theta and gamma rhythms in the hippocampal DG region.

In our study, we found that the expression of NR2B, a representative protein of excitatory receptors, was decreased in the Notch1+/− group, which could be supported by the work of Brai and colleagues. They reported that the expressions of both NR1 and NR2B were downregulated in Notch1 cKO mice compared to those of WT mice (Emanuele et al., 2015). On the other hand, our results revealed that GABAergic neurotransmitter was effectively increased after Notch1 knockdown. This viewpoint could also be verified by another study, suggesting that Notch/RBP-J signaling modulated synaptic transmission by increasing GABA transporter expression to reduce extracellular GABA (Liu et al., 2015). In addition, conditional knockdown of Notch1 and RBP-J could decrease GABA transporter expression and resulted in the enhancement of extracellular GABA (Liu et al., 2015). Even though Notch signaling pathway was reported to have great connections with excitatory and inhibitory neurotransmitter, as well as their receptors, the study might be the first one showing that the balance between excitatory receptors and inhibitory receptors was disturbed by Notch1 knockdown. Regrettably, we did not directly measure the NMDA and GABA neurotransmitters by using high performance liquid chromatography (HPLC). The improvement of further experimental design and techniques needs to be done.

There are many evidences that the NMDAR participates in the pathophysiology of several psychiatric diseases, such as schizophrenia, bipolar depression and drug addiction (Krystal et al., 2003; Paul and...
In our previous study, we found that the expression of either NR2B or Notch1 was reduced in a mouse model of chronic unpredictable mild stress (CUMS). Interestingly, nicotine significantly increased the expression of these two proteins, suggesting that there was a tight association between NMDAR and Notch1 receptor (Shang et al., 2017b). In addition, NMDAR antagonists are used to establish the model of schizophrenia over the last decade (Abi-Saab et al., 1998; Kittelberger et al., 2012), and their compounds are applicable to the treatment of bipolar depression (Mathew et al., 2012). The study for exploring potential mechanisms underlying these diseases showed that NMDAR antagonists dramatically increased the power of gamma oscillations in multiple cortical and subcortical structure in conscious rats, including the prefrontal cortex, hippocampus and striatum in either moving, sedated or anesthetized animals (Hakami et al., 2009).

A recent study in rodents showed that ketamine, which was widely used as non-specific antagonists, could decrease theta power and coherence in the hippocampal CA1 region (Hinman et al., 2013). In the present study, it was found that Notch1 knockdown decreased the expression of NR2B subunit resulting in an increase power of gamma oscillations and a decrease power of theta oscillations in the hippocampus. These results were similar to that in the above literatures by the means of NMDAR antagonists.

Furthermore, brain rhythms of different frequencies can interact with each other in some specific ways. Another study reported that theta-gamma PAC was disrupted by NMDA receptor blockade in the hippocampi of behavioral rats (Caixeta et al., 2013). In this study, the theta-gamma PAC in DG area was considerably impaired in the Notch1+/− group compared to that in the WT group. It is well known that adult neurogenesis occurs in DG region and Notch1 is closely associated with neurogenesis. Therefore, it is reasonable to infer that Notch1 knockdown mainly impairs PAC in the DG area. These findings suggest that Notch1 receptor affects brain activity of the hippocampus partly through NR2B subunits.

In fact, abnormal protein expressions in neurological and psychiatric disorders are not only associated with excitatory proteins, but also inhibitory proteins. For example, abnormal prefrontal GABA function is significantly correlated to the gamma oscillation in schizophrenia (Chen et al., 2014). From the view of neuronal oscillations, it is widely accepted that the generation and modulation of theta and gamma oscillations are tightly connected to glutamatergic and GABAergic neurons (White et al., 2000; Traub et al., 2004; Fukuda et al., 2006; Bartos et al., 2007; Leung and Shen, 2007). Notch1 knockdown increased the expression of GAD67 in the hippocampus, which was one of several forms of glutamic acid decarboxylase, and responsible for catalyzing the production of GABA from glutamic acid. It further induced an increase of GABAARα1 subunit expression and a decrease of NR2B expression in the present study. These results suggest that the balance of excitatory and inhibitory neurotransmitters is disturbed by Notch1 knockdown. PV interneurons were reported to continuously release GABA and modulate several important process in neural system, such as neurogenesis (Ge et al., 2006; Song et al., 2013). The data showed that the expression of PV interneurons was increased in the hippocampus of Notch1 knockdown mice. The result could be corroborated by the data about GAD67 and GABAARα1 subunit expressions (Fig. 2) to some extent. We also found that the gamma power in the hippocampus was augmented with the increment of PV by Notch1 knockdown. It was consistent with previous reports, in which the synchronous recruitment of fast-spiking PV interneurons was correlated with the generation with gamma oscillations (Fuchs et al., 2007; Sohal et al., 2009). Another previous study showed that mice lacking NMDAR in PV cells exhibited enhanced cortical gamma rhythms (Carlen et al., 2012), suggesting that the excitatory receptor dysfunction of PV cell would enhance gamma activities in cortical network. Our results suggest that the balance of excitatory and inhibitory neurotransmitters is disturbed by Notch1 knockdown, which further affect the neural oscillations in the hippocampus.

In the present study, urethane was used as an anesthetic because it could moderate cardiovascular depression, maintain spinal reflexes, minimize effect on respiratory systems and make small change in multiple receptor systems (Field et al., 1993; Hará and Harris, 2002). Although anesthesia with urethane has some advantages, it is still potential experimental limitations of the study. Our data showed that Notch1 knockdown disturbed theta and gamma oscillations in the hippocampal DG area. Since the electrical signals were obtained from REM-like phases in the urethane state, it is conceivable that these changes in the oscillatory patterns could similarly occur in other anesthetics. The activity under the different level of isoflurane anesthesia in the rat hippocampus showed that there were two distinct states, in which there were several qualities reflecting the theta state during running and REM sleep, and large irregular activity state during awake resting and slow wave sleep (Lustig et al., 2016). We do understand that further studies are needed to evaluate if these oscillation changes of interactions also occurs under different states with different anesthetics.

As we known, entorhinal cortex (EC)-DG interactions are an important temporal network, and the hippocampus is very well connected to other brain areas via EC and thalamus and so on. More specifically, the hippocampus forms a principally unidirectional network with input from EC that connects with the DG and CA3 via the Perforant Path. Consequently, PP→DG is one of vital pathways inside of hippocampus. In addition, we also collected LFP signals from PP. The interaction between PP and DG was measured at either identical-frequency or cross-frequency, including spectra coherence (Supplementary Fig. 1A & 8), phase locking value (PLV, Supplementary Fig. 1C), generalized partial directed coherence (gPDC, Supplementary Fig. 1D) and PAC values (Supplementary Fig. 1E). It was found that there were significant impairments of the coupling in the Notch1+/− group (Supplementary Fig. 1).

To sum up, Notch1 deficiency dramatically attenuates neural activities in the hippocampus through disrupting the balance of excitatory and inhibitory receptors, which could be a potential underlying mechanism for psychiatric disorders. In other words, our results possibly

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**Fig. 5.** The Phase-amplitude coupling in the hippocampal DG area. (A) Phase-amplitude coupling (PAC) examples between low frequency rhythm (1–10Hz) and gamma rhythm (30–100Hz) in the WT group (left) and the Notch1−/− group (right). (B) The statistical results of both theta-LG and theta-HG PAC measured by MVL. **P < .01 and ***P < .001 represent significant differences between the WT group and the Notch1+/− group.
provide an evidence for the critical role of Notch1 receptor in the hippocampus for expression of normal neural oscillations and interactions.

4. Methods

4.1. Subjects

Seven ten-week-old male Notch1 heterozygous deficient (Notch1+/−) mice and nine WT littermates were purchased from Institute of Laboratory Animal Science, CAMS & PUMC, and reared in the Animal House of Medical School, Nankai University. All experimental mice were in a C57BL6/J hybrid background. The Notch1+/− mice were generated by deleting a large portion of the Notch1 gene using a positive/negative targeting vector. These mice have been backcrossed to C57BL/6 J mice for at least 10 generation. A total knockout Notch1+/− strain was not used in our experiments because the knockout Notch1 animals were die at embryonic day 11 (Conlon et al., 1995). Both the temperature and humidity were kept at 24 ± 2 °C and 50–60%, respectively. Food and water were freely available under a 12 h light/dark cycle. All animal experiments were approved by the Animal Research Ethics Committee, Nankai University (20160004) and performed in accordance with the Animal Management Rules of the Ministry of Health of the People’s Republic of China. Body weight measurement was performed before electrophysiology recording.

4.2. LFP data collection/electrophysiology recording

Mice were anesthetized with urethane (Sigma-Aldrich, St. Louis, MO, USA; 1.2 g/kg body weight; i.p.) and positioned on a stereotaxic frame (Narishige, Japan). One electrode was implanted into the DG (1.7 mm posterior to the bregma, 1 mm lateral to midline, 1.5 mm ventral below the dura) according to the mouse brain atlas (Franklin and Paxinos, 2001), and then the signals of local field potentials (LFPs) were acquired at a sampling rate of 1000 Hz for 20 min. The left and right hemispheres were randomly used for recording signals. In order to verify the location of the LFP recording site, cells of DG region was damaged by a 2 mA direct current performed through the electrode for 20 s after LFP recordings. The electrode placement could be confirmed by hematoxylin/eosin staining, which was described below.

4.3. Hematoxylin/eosin staining

After LFP recording, mice were sacrificed and the brains were immediately removed. Then, they were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4) at 4 °C for 24 h. After that, the brains were dehydrated in a gradient by 15% and 30% sucrose solution. Subsequently, the brains were embedded in OCT compound (Tissue-Tek, Miles) at −20 °C and then sliced in 20 μm thick coronary slices for hematoxylin and eosin (HE) staining. Finally, the sections were photographed on a Leica microscope (Wetzlar, Germany).

4.4. Immunofluorescence

The immunofluorescence staining was conducted with some modification (Yang et al., 2017). Briefly, brains were sliced in 20 μm thick coronary slices for immunofluorescence staining. The slices were washed with PBS for 5 min twice followed by 15 min permeabilization in 0.3% Triton X. Then, they were washed with PBS for 5 min twice and blocked with 10% normal goat serum (NGS) for 2 h at room temperature. After that, different primary antibodies were used to incubate the slices overnight at 4 °C. Finally, the slices were washed by PBS (3 × 10 min) and incubated with Alexa 488-conjugated anti-rabbit IgG, Alexa 488-conjugated anti-mouse IgG and Alexa 488-conjugated anti-donkey IgG for 1 h at room temperature. Nuclei were stained with DAPI. The fluorescent images were obtained from a laser scanning confocal microscope (Olympus FV1000, Japan).

4.5. Western blotting assay

Three random mouse hippocampi were separated after the sacrifice and stored at −80 °C for the preparation of tissue lysates. The preparation of tissue lysates and Western blotting assay were described in our previous studies (Gao et al., 2015; Shang et al., 2017a,b). Briefly, equal weight protein loadings (30 μg) were electrophoresed in 10% SDS-PAGE gels and then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, USA). After blocking, incubating with primary antibodies and secondary antibodies, protein band intensities of membranes were detected with HRP substrate (Millipore, USA) by using the Tanon 5200 chemiluminescent imaging system (Tanon Science & Technology, China). β-actin was used as an internal control and the quantitation analysis was performed by Image J. The number of repetitions of Western blots is 4 at least.

4.6. Antibodies

Rabbit polyclonal antibodies to Notch1 (1:2000), NR2B (1:2000 for Western blotting and 1:500 for immunofluorescence staining) and GAD67 (1:2000 for Western blotting and 1:500 for immunofluorescence staining), mouse polyclonal antibody to GABAARα1 (1:1000 for Western blotting and 1:500 for immunofluorescence staining), as well as donkey polyclonal antibody to Parvalbumin (1:500) were obtained from Abcam (Cambridge, UK). Rabbit polyclonal antibody to β-actin (1:5000) were purchased from Sangon Biotech (Shanghai, China). The anti-rabbit and anti-mouse secondary peroxidase-conjugated antibodies (1:5000) were bought from Promega (Promega Co, USA). Alexa 488-conjugated anti-rabbit IgG, Alexa 488-conjugated anti-mouse IgG and Alexa 488-conjugated anti-donkey IgG were purchased from Invitrogen (New York, USA).

4.7. LFP data analysis

All the LFP data were analyzed offline using built-in and custom-written MATLAB codes (Mathworks). In the present study, there were three rhythms to be focused on which were theta band (3–8 Hz), low gamma band (LG, 30–50 Hz) and high gamma band (HG, 50–100 Hz).

4.7.1. Power spectra analysis

The absolute power spectra were calculated on 1–100 Hz by Chronux routines (Mitra and Bokil, 2008), based on the Multitaper Spectral Estimation (Thomson, 1982). The parameters of processing were as follows: 40 s smoothing window with 50% overlap and the Slepian tapers field as [5, 9]. For each rhythm, the absolute power for each frequency was normalized to relative percentages across the total frequency band (1–100 Hz). Time-frequency power decomposition was also obtained by Multitaper time-frequency spectrum using 20 s sliding windows with 10 s time steps. Urethane-anesthetized mice normally show spontaneous cyclical transitions from REM-like to non-REM-like states (Pagliardini et al., 2013). In this study, all LFP analysis was performed in the REM-like state, which were characterized by theta oscillations (3–8 Hz).

4.7.2. Cross-frequency coupling analysis

Phase-amplitude coupling (PAC) is a kind of neural oscillation cross-coupling mode, which shows the amplitude of fast oscillations time-locked to the trough of slow oscillations. In the study, the mean vector length (MVL) method was employed to compute the PAC of filtered LFP signals in the hippocampal DG area (Canolty et al., 2006). As previously described (Xu et al., 2013a,b, 2015), we first obtained the slow oscillation phase series and the fast oscillation amplitude series extracted by Hilbert transform. Then, constructed a complex time series and computed the length of average vector. This index denotes how much the
empirical phase-amplitude distribution deviates from the uniform distribution, which characterizes the strength of PAC. The procedure was performed for a data length of 40 s with 20 s time steps. Usually, the MVL values were normalized for obtaining more robust and accurate estimate through a surrogate data approach (Canolty et al., 2006; Ozkurt and Schüzctzer, 2011). Here surrogate data were generated by shuffling the high amplitude time series with 50 surrogate times.

4.8. Statistics

Statistical analysis was performed using SPSS 22 (IBM). One-Sample Kolmogorov-Smirnov Test was used as the normality of each group data. For group comparisons of Gaussian distributed data, we used two independent samples t-test or adjust-t test. Otherwise, Mann-Whitney U test was employed. The differences of power distribution were tested using multivariable general linear model between two groups. Data are shown as Mean ± S.E.M.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpb.2018.01.019.

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Author contributions

Q.L., X.Z. and T.Z. designed the experiment; X.Z. & C.Y. conducted the animal experiments; Q.L. and N.C. performed data analysis; Q.L., X.Z., & T.Z. wrote the manuscript.

Ethical statement

The submitted manuscript was involved the animal research. All procedures were in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

The submitted manuscript is based on a research study which was subjected to a full review and approved by the Committee for Animal Care at Nankai University, PR China.

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