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RESEARCH ARTICLE

U1 snRNA over-expression affects neural oscillations and short-term memory deficits in mice

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Abstract

Small nuclear RNAs (snRNAs) and other RNA spliceosomal components are involved in neurological and psychiatric disorders. U1 snRNA has recently been demonstrated to be altered in pathology in some neurodegenerative diseases, but whether it has a causative role is not clear. Here we have studied this by overexpressing U1 snRNA in mice and measured their hippocampal oscillatory patterns and brain functions. Novel object recognition test showed that the recognition index was significantly decreased in the U1 snRNA over-expression mice compared to that in the C57BL mice. U1 snRNA over-expression regulated not only the pattern of neural oscillations but also the expression of neuron excitatory and inhibitory proteins. Here we show that U1 snRNA over-expression contains the shrinkage distribution of theta-power, theta-phase lock synchronization, and theta and low-gamma cross-frequency coupling in the hippocampus. The alternations of neuron receptors by the U1 snRNA overexpression also modulated the decreasing of recognition index, the energy distribution of theta power spectrum with the reductions of theta phase synchronization and phase-amplitude coupling between theta and low-gamma. Linking these all together, our results suggest that U1 snRNA overexpression particularly causes a deficit in short-term memory. These findings make a bedrock of our research that U1 snRNA bridges the gap about the mechanism behind short-term memory based on the molecular and mesoscopic level.

Keywords U1 snRNA · Excitatory and inhibitory receptors · Local field potentials · Neural oscillations · Mice

Introduction

The U1 small nuclear RNA (snRNA) is one of the most abundant noncoding RNA in mammalian cells and the best known for its role in pre-messenger RNA (pre-mRNA) splicing events and translation with the help of several proteins (Mercer and Mattick 2013). Splicing of 90% of the human pre-mRNAs starts with the help of assembling of majority of spliceosome, such as U1 snRNPs, U2 snRNA, U2 snRNPs and U6 snRNPs. included with the priority of pre-mRNA splicing in the coordination of gene expression and protein formation. It also causes the protein mutation participating in the RNA-processing, and may affect pre-mRNA splicing and induce human disease (Cooper et al. 2009; Licatalosi and Darnell 2006). There are several of spliceosome disruptions and RNA processing impairments have been indicated in neurodegenerative disorders. A single U2 snRNA, stimulated neuron degeneration via malformation of pre mRNA splicing, causes dysfunction in cerebellum (Jia et al. 2012). SMN1 (survival of motor neuron 1) gene mutation influences the synthesis of UsnRNPs. Alteration in this gene induces spinal muscular atrophy (SMA) (Lefebvre et al. 1995; Brzustowicz et al. 1990). TDP-43 represents the common pathologic substrate relating with these neurodegenerative disorders. Pathologic TDP-43 was found in the affected central nervous system including hippocampus, neocortex, and spinal cord (Neumann et al. 2006; Sreedharan et al. 2008). In fact, it is well known that RNA processing abnormality is involved in the development of neurodegenerative diseases (Kim et al. 2013). All these messages actively suggest that changes in

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RNA processing work as a main incident for several neurodegenerative ataxias.

Moreover, according to a previous report it is also clear that discharge of insoluble protein assemblage is a trademark of neurodegenerative diseases (Bing 2013). In addition to this, it was also found that the multiplication of pathologic protein fragments is frequently occur in neurodegenerative disorders. Furthermore, U1-70K is a component of snRNP, implying that U1 snRNP modification present formerly in AD development (Bing 2014). Similarly, in another study U1 divergence show capacity towards intensifying the presence of cell cycle—reentry (CCR) in the AD neuronal degeneration (Bai 2018). Over expression of U1 snRNA could cause the loss in the function of U1 snRNA and resulted in PCPA, as well as the same downstream phenomena including the expression changes of genes specific to AD (Cheng et al. 2017b, c, 2018).

In the present study, we assumed that U1 snRNA was a new non-coding RNA that bridges splicing and the proteins in excitatory neurons, which was associated with short-term synaptic plasticity and memory via affecting the pattern of neural oscillations. It is well known that neural oscillations play an important role in spatial learning and memory processes (Buzsáki and Draguhn 2004). Different neural oscillation interacts with each other in different manner, which is explain with the help of several of measurement such as power spectra, synchronization and cross frequency coupling (Li et al. 2016). Recently, the synchronization of neural oscillation has been emerging evidences for supporting short-term memory along with an object recognition test. For example, oscillatory synchrony could be a base for phase-dependent neural coding with the phase amplitude coupling, playing an important role in short-term memory (Siegel et al. 2009; Holz et al. 2010). Every oscillations rhythm has its own specific role in the learning and memory. Theta oscillation performs as a strong depolarizing control on the hippocampus cells, which contain NMDA receptor. The temporal coupling of a theta oscillation convinced depolarization and the release of glutamate to NMDR, containing hippocampus cells from intra- and extra hippocampal fountain. This consecutive activity begins the operation, which starts a short-term annealing between pre-synaptic and post-synaptic hippocampal neuron and forms short-term memory (Vertes 2005). It is well known that neuron receptor proteins could help in the excitation and inhibition of neuron and thus affect neural oscillations. Overexpression of NMDR receptor subunit NR2B in the forebrain increased social recognition memory in the mice (Kline et al. 2012). Furthermore, NR2A is required for the improvement of working memory and cognition (McQuail et al. 2016). Including with excitatory receptor proteins, inhibitory receptor proteins also play an important role in the memory formation. GABA is known for the regulation of short-term memory formation and its action, which depends on the level of GABA expression during the learning process (Gibbs and Johnston 2005). Along with this, we certainly understand that the origin of theta and gamma modulation is linked with the GABAergic neurons (Traub et al. 2005; Fukuda et al. 2006; Bartos et al. 2007; Leung and Shen 2007). Along with this, theta and gamma oscillations alteration in the hippocampus was involved in the neurological disorders (Shang et al. 2017; Zheng and Zhang 2013).

However, snRNA is not a mRNA, and not easy to be knocked down. Therefore, the overexpression might be a simple approach to see the effect of U1 snRNA inhibition, because it might be possible that excessive U1 snRNA interfere with the assembly of the other U1 component and causes memory loss and neurodegeneration. Accordingly, we hypothesized that U1 snRNA overexpression disturbs the recognition and memory and the pattern of neural oscillations in the hippocampus through breaking the balance between neurons excitatory and inhibitory proteins. This was done by recording the signals of LFPs from both U1 snRNA overexpressed and wild type CBL57 mice in urethane-anesthetized state and further measuring theta and gamma neural activities with not only power spectrum and synchronization but also cross-frequency couplings. In addition, biochemical detections were introduced to examine whether the balance between excitatory and inhibitory proteins was broken. Our data contribute to a better understanding about prominent role of U1 snRNA in the hippocampal neural networks leading to short-term memory, which provides a therapeutic target for the neurological and psychiatric disorders.

Materials and methods

Animals

Eight-week-old C57BL/6J mice were purchased from the Institute of Laboratory Animal Science, CAMS & PUMC. Then after they were reared in the Animal House of Medical School, Nankai University. The animals were fed with ad libitum access to food and water in a room under a 12 h light/dark cycle. The room temperature was kept at 24 ± 1 °C and humidity was kept at 50–60%. 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. Every effort has been made to minimize animal suffering and the number of animals.

Lentivirus preparation

In order to construct U1 snRNA over-expression lentiviruses, DNA fragments of U1 snRNA (including U1 snRNA native promoter) were amplified from genomic DNA obtained from the mice hippocampal tissue. The PCR products were subjected to electrophoresis in a 1% agarose gel, recovery of DNA fragments and cloned into the pLVX-shRNA1 vector using In-fusion® HD Cloning Kit (Clontech, USA). The lentivirus was prepared by using the Lenti-X™ HTX Packaging System (Clonth, USA), and the protocol was performed essentially as described by the manufacturer.

Lentivirus administration

After 3 days of adaptation with the artificial environment, we randomly divided the eight-week-old male C57BL/6 mice into two groups: control group (CON, n = 5) and U1 snRNA over-expression group (U1OE group, n = 5). The animals were anesthetized with 30% urethane (1.2 g/kg bodyweight, i.p., Sigma-Aldrich, St. Louis, MO, USA.) and then they were placed in a stereotaxic frame (SN-3, Narishige, Japan). In the U1OE group, 1 µl lentivirus was injected into lateral ventricles (LVs; 0.1 mm posterior to the bregma, 0.9 mm lateral to midline, 2.0 mm ventral below the dura) of the brain at a flow rate of 0.5 µl/min using a 10-µl Hamilton syringe (Hamilton Co., Reno, NV). The animals in the CON group were injected with empty lentivirus. After the injection, the needle was kept in brain in steady state for 5 min before it was slowly withdrawn.

Novel object recognition test

A gray-colored aluminum open-field arena was used for performing Novel object recognition behavioral test. The arena and objects were wiped with 70% ethanol between trials for the removal previous smells or any other previous indications. Each mouse was subjected to a 10 min acquisition trial, during which it was placed in the open-field arena in presence of two identical objects. We used a retention interval of 2 h for testing the short-term memory and learning. Mice were taken back into the arena and exposed to the familiar object and to a novel object for a further 10 min of interval. Total time spent exploring each of the two objects was recorded. A recognition index was known as the amount of time inspecting the familiar object or the novel object upon the total time used exploring both objects multiplied by 100 and was used to measure recognition and memory (TB/(TA + TB)) * 100, where A represents familiar object and B, novel object (McClean et al. 2011).

LFP data collection or electrophysiology recording

We anesthetized the mice with urethane (Sigma-Aldrich, St. Louis, MO, USA; 1.2 g/kg body weight; i.p.) before the collection of local field potential from the perforan- pathway (PP) to the dentate gyrus (DG) area. They were placed on a stereotaxic frame (Narishige, Japan). Mice skull were drilled by applying a dental drill for the stimulation of electrode and collecting the local field potentials signals. As it was reported in the mouse brain atlas that the bipolar stimulating electrode was smoothly embedded in angular bundle of the PP (3.8 mm posterior to bregma, 2.1 mm lateral to midline, 1.5 mm ventral below dura) and the recording electrode was placed in the granule cell layer of the DG (1.7 mm posterior to bregma, 1 mm lateral to midline, 1.5 mm ventral below the dura, (Franklin and Paxinos 2001). While the stimuli (range 0.3–0.5 mA, stimulus pulse with 0.2 ms, at 0.03 Hz) were delivered to the PP every 30 s to find the optimal stimulating intensity that could evoke a response of 70% of its maximum amplitude. LFP signals were collected at a sampling rate of 1000 Hz for 15 min.

RNA extraction, re-transcription and quantitative PCR (qPCR)

The level of U1 snRNA in the hippocampus was measured by qPCR. Total RNA was isolated from samples using the RNAiso Plus Reagent (TaKaRa, Japan) according to the manufacturer's instructions, separately. cDNA was synthesized by Mir-X™ miRNA First-Strand Synthesis Kit (Clontech, USA). 2 µl cDNA product was used to perform qPCR (Eppendorf, German) for each sample using U6 snRNA as internal control (Cheng et al. 2017a). The forward and reverse information of U1 snRNA is CTTACCTGGCAGGGGAGATACCA and mRQ 3′ primer (Clontech, USA), respectively.

Western blotting assay

The hippocampus was stored at − 80 °C and prepared lysate of tissue (Gao et al. 2015; Shang et al. 2017). An equal amount of protein loadings (30 µg) was electrophoresed in 10% SDS-PAGE gels. Then after, we transferred it onto polyvinylidene difluoride (PVDF) membranes (Millipore, USA) for blocking. Once the proteins were bind with membrane, Incubation of primary and secondary antibodies were performed. We used HRP substrate (Millipore, USA) and Tanon 5200 chemiluminescent imaging system (Tanon Science &Technology, China) for the identification of protein band intensities. Further, we

used β-actin for the internal control. Then after we did the quantitation analysis with help of Image J.

Antibodies

Rabbit polyclonal antibodies to NR2A (Abcam, 1:2000), NR2B (Abcam, 1:2000) and GAD67 (Abcam, 1:2000), mouse polyclonal antibody to $GABAAR_{\alpha 1}$ (Abcam, 1:2000). 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).

LFP data analysis

The LFPs data were analyzed offline using built-in and custom written MATLAB codes (Mathworks). In the present study, we searched light on three classical frequencies ranges of neural oscillations, which were theta (3–8 Hz), low-gamma (30–60 Hz) and high-gamma (60–100 Hz).

1. Power spectra analysis

Chronux routines (Bokil et al. 2010) were used based on the multitaper spectral estimation (Thomson 1982) for the calculation of absolute power spectra. A 40 s smoothing window with 50% overlap and the Slepian tapers field parameters were used for the calculations of power spectrum density. We normalized the absolute power for each frequency to the relative percentages throughout the total frequency band (1–100 Hz). Urethane-anaesthetized mice commonly display automatic cyclical transitions from Random Eye Movement (REM) to Non-random eye movement states (Pagliardini et al. 2013). In this study, we did the LFP analysis in the REM state, which can be recognized via theta oscillations (3–8 Hz).

2. Phase synchronization analysis

The phenomenon of phase synchronization was demonstrated in 1996 (Rosenblum et al. 1996). Phase locking value (PLV) is an extensively used approach to measure the strength of phase synchronization within rhythms between two brain regions. Phase synchronization is not only used for communication between two brain area but it is also used to implement the highest interactions between two or more brain areas (Fries 2001). Several of the studies used this index to express the status of memory (Holz et al. 2010; Gonzalez-Burgos and Lewis 2008). Therefore, to further understand the role of U1 snRNA in memory deficits, we examined synchronous neuronal activity among the three classical frequency band as defined in the PSD method section. The PLV algorithm details are as following.

Raw LFP data were filtered using eegfilt function from EEGLAB toolbox with a 1 Hz bandwidth (Arnaud Delorme and Scott Makeig 2004; Delorme et al. 2004), and computed instantaneous phase of each frequency by Hilbert transform. Finally, PLV was determined as

$$PLV = \left| \frac{1}{N} \sum_{t=1}^{N} \exp(i[\phi_{PP}(t) - \phi_{DG}(t)]) \right| \quad (1)$$

N stands for the length of the data. The PLVs values were within [0, 1], where 1 and 0 represented for full synchronization and no synchronization at all, respectively. In the processing, a time window of 300 s with an overlap of 50% was adopted.

3. Cross-frequency coupling analysis

Phase-amplitude coupling, where the phase of low-frequency band modulates the amplitude of high- frequency band, has been reported in continuous electrophysiological signals obtained in the brain at mesoscopic and macroscopic levels. In this study, we used the Modulation index to calculate the cross-frequency coupling given by A. tort (Cantolty and Knight 2010). His elementary purpose was that there was a discontinuous distribution of amplitude AHigh(t) conditioned on phase θLow(t) when PAC existed between them. The MI method has been developed based on Shannon Entropy of the fore going distribution and normalized by the largest value of Shannon Entropy when the distribution is uniform. The details of MI algorithm are as follows (Cheng et al. 2016)

Firstly, $\theta_{Low}(t)$ and $A_{High}(t)$ are extracted by Hilbert Transform. Secondly, each cycle of $\theta_{Low}(t)$ is equally divided into n intervals. Thirdly, the average of $A_{High}(t)$ can be calculated to every interval of $\theta_{Low}(t)$, which can be normalized by dividing it by the sum of all averages over all intervals to get the distribution of amplitude $A_{High}(t)$ conditioned on phase $\theta_{Low}(t)$.

$$P(j) = \frac{\langle A_{High}(t) \rangle_{\theta}(j)}{\sum_{k=1}^{n} \langle A_{High}(t) \rangle_{\theta}(k)} \quad (2)$$

Fourthly, the Shannon Entropy can be quantified as:

$$H(P) = -\sum_{j=1}^{n} P(j) * \log[P(j)] \quad (3)$$

Here, $\log(n)$ is the maximal possible value of $H(P)$. Therefore,$H(P)$ could be normalized to MI,

$$MI = \frac{\log(n) - H(P)}{\log(n)}, \quad MI \in [0, 1]$$

Statistics

All the statistical tests were processed via using SPSS 22 (IBM). We used two independent samples t-test for the group comparisons. While the correlation analysis was done by using Pearson's correlation analysis. Mean ± S.E.M and p values are used to show the status of data.

Results

U1 snRNA overexpression impaired the short-term memory in U1OE mice

Quantitative PCR showed that the level of U1 snRNA in the hippocampus was higher in the U1OE group compared to that in the CON group ($p < 0.01$, Fig. 1a). In order to evaluate the short-term memory, Novel object test was performed. Two independent samples t-test showed that the recognition index was lower in the U1OE group than that in the CON group ($p < 0.05$, Fig. 1b).

U1 snRNA overexpression decreased theta power distribution in the hippocampus

Figure 1c and d show an example of original traces of neural activities. There was a dense and brighter energy band in the theta region in a normal mouse than that in an UIOE animal (Fig. 1e and f), implying that there was a reduced of power spectrum density in the zone of theta dominant frequency band (3–4 Hz). In addition to energy band of power spectra, the corresponding power spectral distribution was plotted on a logarithmic scale in both DG and PP regions. Figure 2a showed the mean power spectrum in DG area either in the CON gblack line) or in the U1OE group (red dash line). The relative mean power of theta rhythm was significantly attenuated in the U1OE group compared to that in the CON group ($p < 0.05$, Fig. 2c). However, there was no statistical difference of the

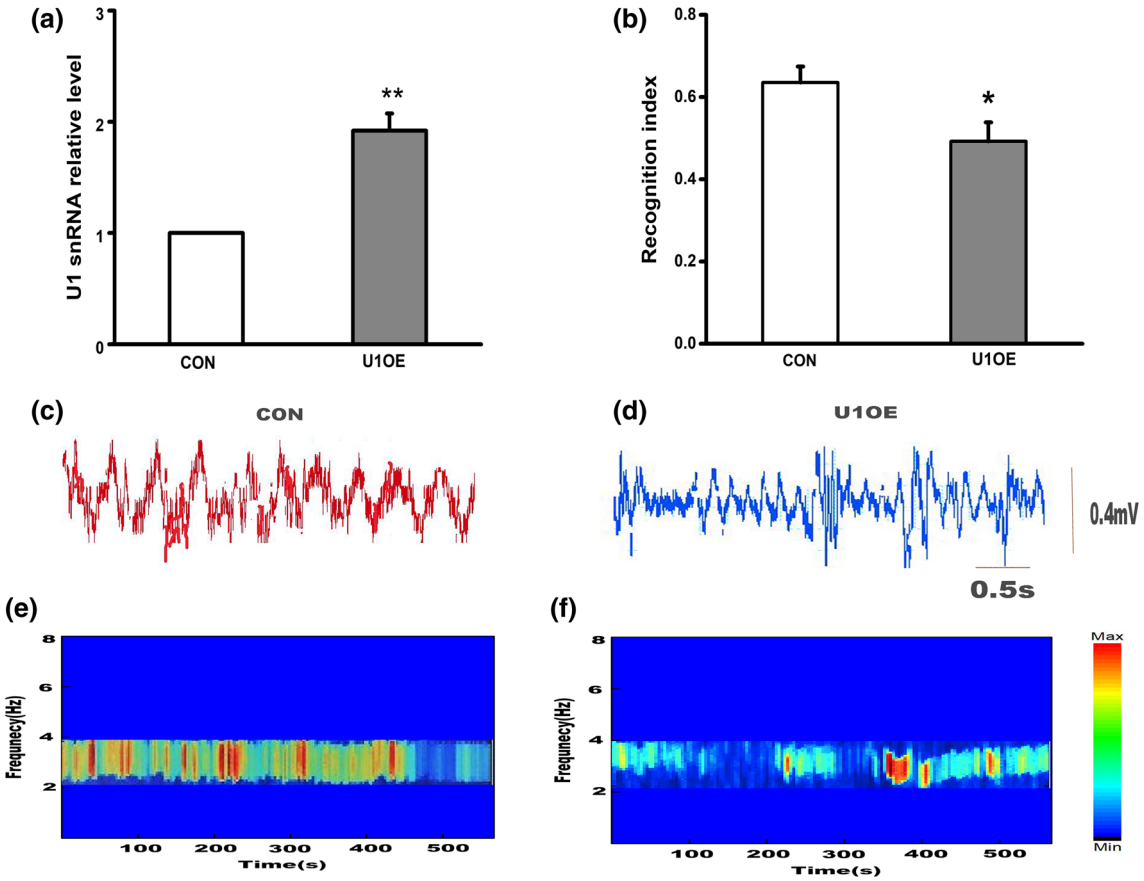

Fig. 1 The expressions of U1 snRNA, representative excitatory and inhibitory proteins in hippocampi. **a** A comparison of U1 snRNA relative amount between U1 snRNA over-expressed mice and C57BL mice. **b** A comparison of recognition index between U1 snRNA over-expressed mice and C57BL mice. **c** and **d** Representative examples of raw signals (5 s) in the given pathway in the CON group (**c**) and the U1OE (**d**). **e** and **f** Time–frequency analysis (500 s) from 1 to 8 Hz. The color bar was the power in μV2 from the view of uniform scales for time–frequency spectrograms. The redder and bright color represent the high power. Data are expressed as mean ± SEM. *$p < 0.05$ and **$p < 0.01$ comparison between the CON group and the U1OE group. (Color figure online)

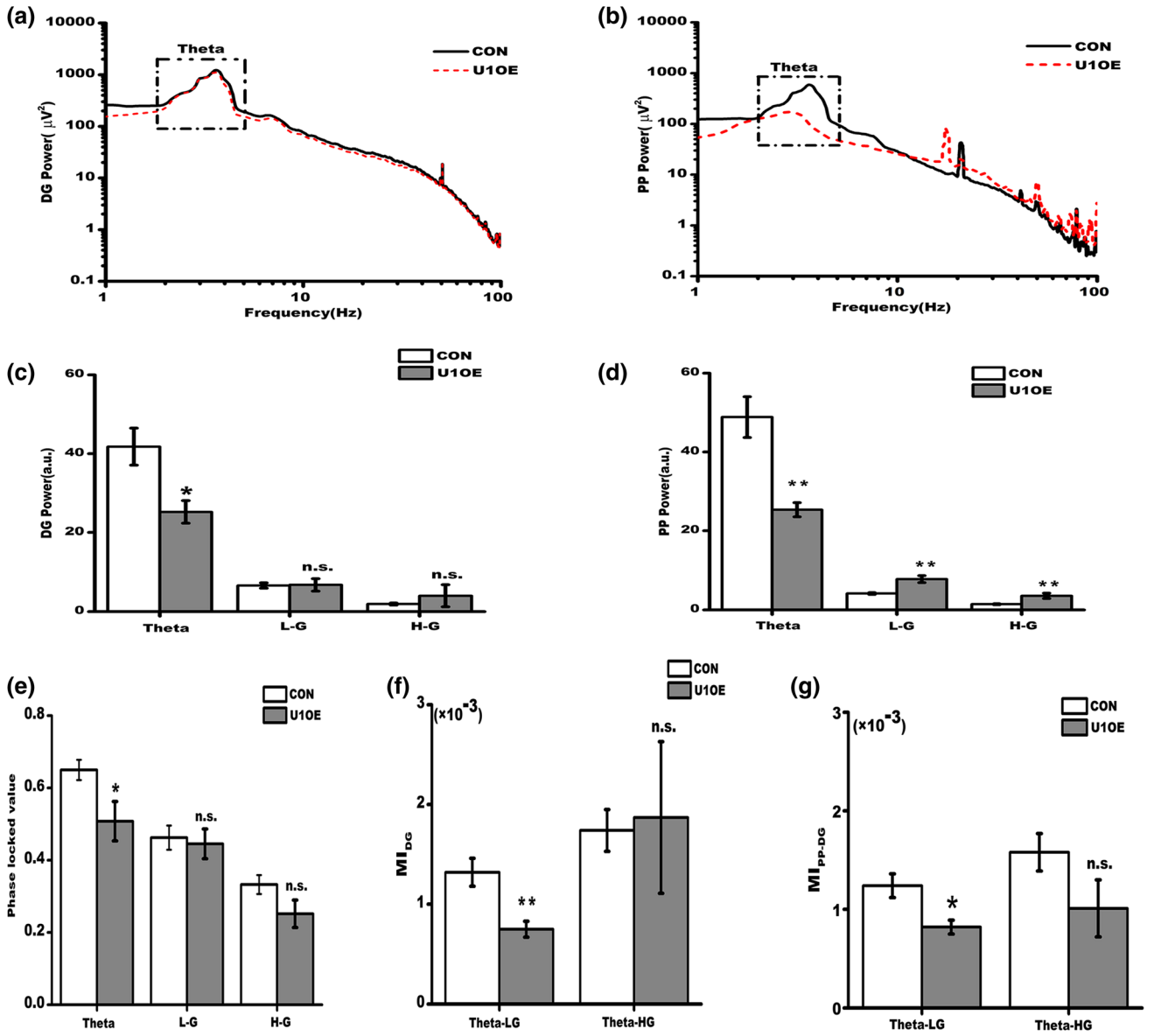

Fig. 2 Power spectral analysis, PLV and PAC measurements. **a** and **b** 1–100 Hz average power spectrum associated with in the PP-DG pathway of the CON group (black lines) and the U1OE group (red dot lines). **c** and **d** The relative mean of power distribution at theta (3–8 Hz), LG (30–60 Hz) and HG (60–100 Hz) frequency bands in these two groups. **e** The Phase- lock synchronization of theta rhythm in the PP-DG pathway. **f** and **g** The Phase-amplitude coupling between DG and PP. Data are expressed as mean ± SEM. $*p < 0.05$ and $**p < 0.01$ comparison between the CON group and the U1OE group. (Color figure online)

low-gamma or high-gamma power in DG region between these two groups (Fig. 2c). Along with this, it can also be seen that there is an average power spectrum in PP area either in the CON group (black line) or in the U1OE group (red dash line) (Fig. 2b). The relative mean power of theta was much smaller in the U1OE group than that in the CON group ($p < 0.01$), while either low-gamma or high-gamma power was higher in the U1OE group than that in the CON group ($p < 0.01$, Fig. 2d).

U1 snRNA overexpression significantly reduced theta synchronization and phase-amplitude coupling

Figure 2e showed that the value of theta-PLV was lower in the U1OE group than that in the CON group (CON: 64.9% vs. U1OE = 50.7%, $p < 0.05$), while there are no significant differences of PLV between these two groups either in low-gamma or high-gamma frequency bands. Moreover, Pearson correlation analysis showed that a positive correlation between theta PLV and excitatory synaptic protein

(NR2B: r = 0.727, $p < 0.05$, Fig. 4a), while there was a negative correlation between theta PLV and synaptic inhibitory protein (GABAARα1: r = − 0.832, $p < 0.01$, Fig. 4b).

In the hippocampal DG region, the PAC phenomenon between theta and low-gamma was significantly weaker in the U1OE group than that in the CON group (CON = 1.32×10^{-3} vs. U1OE = 0.75×10^{-3}, $p < 0.01$, Fig. 2f). Furthermore, the value of PAC between PP and DG was considerably smaller in the U1OE group than that in the CON group CON = 1.24×10^{-3} vs. U1OE = 0.82×10^{-3}, $p < 0.05$, Fig. 2g). However, there were no significant differences of PAC between theta and high-gamma either in the hippocampal DG region or between PP and DG areas (Fig. 2f and g).

U1 snRNA overexpression affected the excitatory and inhibitory protein expression level

In order to support the molecular evidence for the role of U1 snRNA overexpression in synaptic plasticity and memory, we performed Western blot assay with the neuron excitatory proteins (NR2A, NR2B) and inhibitory proteins (GAD67 and $GABAAR_{\alpha 1}$) as shown in Fig. 3a. Western blot assay showed that the expression level of excitatory proteins was lower in the U1OE group than that in the CON group (NR2A & NR2B: $p < 0.001$, Fig. 3b and c). However, the expression of inhibitory proteins was higher in the U1OE group than that in the CON group ($GABAAR_{\alpha 1}$ and GAD67: $p < 0.001$, Fig. 3d and e).

Recognition memory and DG theta power correlates with synaptic proteins

Pearson correlation analysis showed that there was an evidently positive correlation between the recognition index and the expression levels of excitatory synaptic protein (NR2B: r = 0.813, $p < 0.01$, Fig. 3f), and a clearly negative correlation between the recognition index and the expression levels of inhibitory synaptic protein (GABAARα1: r = − 0.87, $p < 0.01$, Fig. 3g). Similarly, there was a positive correlation between DG theta power and excitatory synaptic protein (NR2B: r = 0.794, $p < 0.01$ Fig. 4c). Furthermore, there was a negative correlation between DG theta power and inhibitory synaptic protein (GABAAR: r = − 0.736, $p < 0.05$, Fig. 4d).

Discussion

In this study, we assessed the recognition memory and the pattern of neural oscillations followed by the expression of excitatory and inhibitory receptors in the both U1 snRNA over-expression mice and C57BL mice. We found that U1 snRNA over-expressed mice (U1OE) showed impairment in the recognition memory. Furthermore, our data showed that U1 snRNA over-expression disturbed the power distribution, significantly reduced theta synchronization and the strength of cross-frequency PAC between theta and low-gamma rhythms either in the hippocampal DG region or in the PP-DG pathway. In addition, the expression of both NR2A and NR2B was significantly reduced, and the expression of both GAD67 and GABAARα1 was considerably enhanced after U1 snRNA over-expressed. Based on the measurements of neural oscillations, the comparison was successfully performed between the U1OE group and the CON groups.

Our result showed that the ability of learning and memory was impaired in the U1OE group, which was consistent with the following works (Clarke et al. 2010; Taglialatela et al. 2009). Typical short-term memory experiments differ from the time separating training and retention time for testing from 5 min to a few hours (Vogel-Ciernia and Wood 2014). We used 2 h. retention interval time and mice were exposed to familiar objects to the new object after the interval of 10 min for testing short-term memory, which was consistent with a previous work (McClean et al. 2011). Similarly, our result show that the recognition index is significantly reduced in the U1OE group compared to that in the CON group, suggesting that U1 snRNA over-expression may induce the impairment of synaptic plasticity and short-term memory.

Including with the behavioral test results, the data showed that U1 snRNA over-expression changed the pattern of neural oscillations. There is another important view of synaptic plasticity that is known as a mesoscopic view based on neural oscillation. Several previous studies reported that the theta rhythm was directly involved in memory formation and functions of the hippocampus in the rats by disturbing the theta rhythm and caused in decline in memory (Vertes 2005; O'Keefe and Burgess 1999; Hasselmo 2002, 2005; Verbitsky 2004).

In this study, the power at theta frequency band was lower in the U1OE group than that in the CON group, suggesting that U1 snRNA over-expression was closely associated with the impairments of synaptic plasticity and memory. As we know that neural oscillations are one of the core mechanism for any type of memory, which is associated with the theta phase synchronization (Clouter et al. 2017; Hanslmayr et al. 2016; Parish et al. 2018), suggesting that theta rhythm plays an important role in the memory formation and functions. Our results show less theta-phase synchronization in U1 snRNA over-expression mice, implying that U1 snRNA is possibly associated with theta synchronization in the hippocampus. Additionally, high gamma power is modulated by theta phase and, it plays a

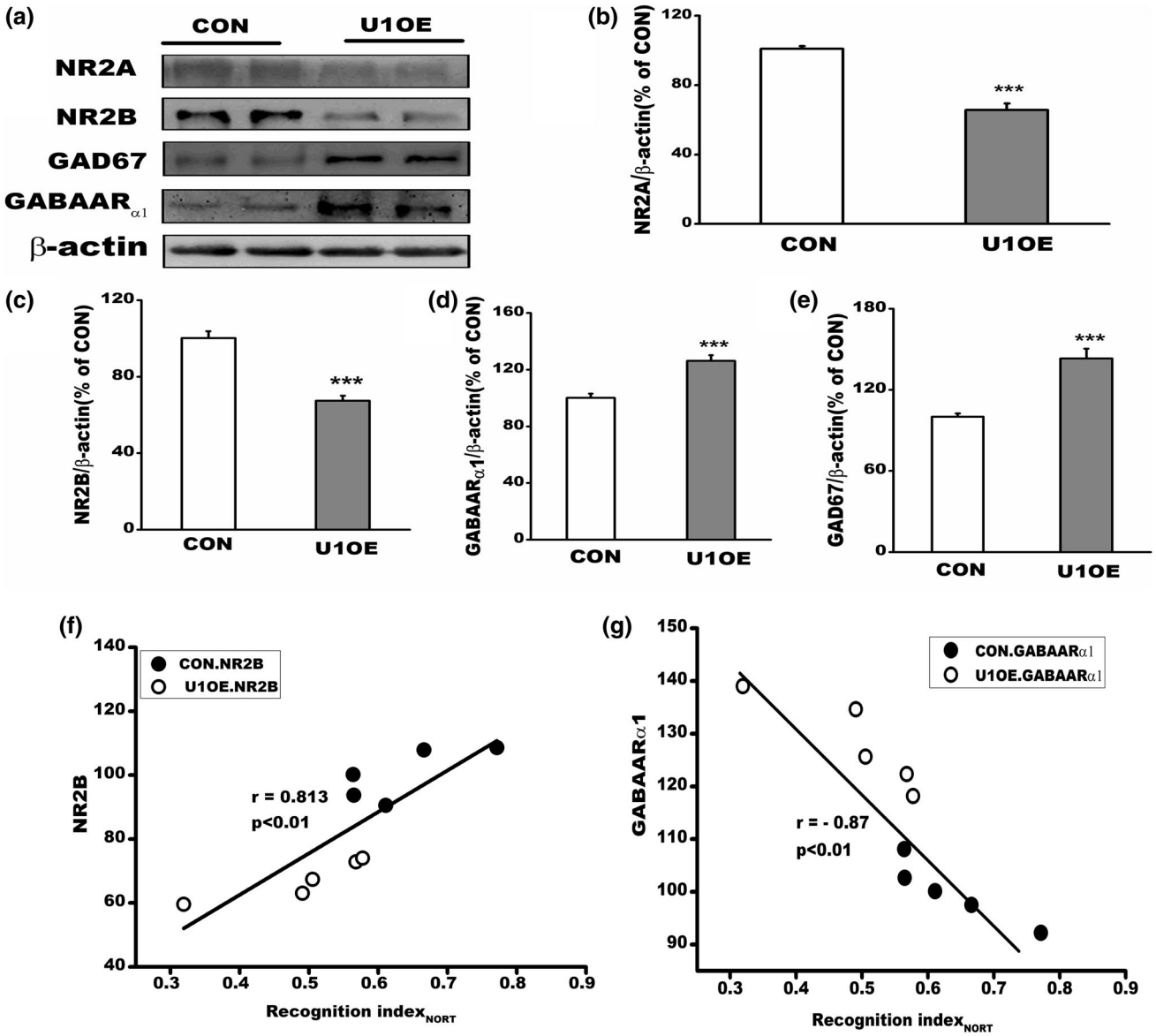

Fig. 3 **a** The representative immune reactive bands of NR2A, NR2B, GABAARα1, GAD67 and β-actin of the CON and U1OE mice. **b** A comparison of the optical density ratio of NR2A/β-actin between CON group and U1OE group. **c** A comparison of the optical density ratio of NR2B/β-actin between the U1OE group and the CON group. **d** A comparison of the optical density ratio of GABAARα1/β-actin between the U1OE group and the CON group. **e** A comparison of the optical density ratio of GAD67/β-actin between the U1OE group and the CON group. **f** Correlations between the level of neurons excitatory proteins (NR2B) and recognition index of NORT. **g** Correlations between neuron inhibitory proteins (GABBARα1) and recognition index of NORT. Data are expressed as mean ± SEM. ***$p < 0.001$ comparison between the CON group and the U1OE group

significant role in the fluctuation of synaptic plasticity. It was absorbed in the human neocortex that high gamma power caused the phase lock at theta oscillations (Canolty et al. 2006). Moreover, high-frequency tetanisation enhanced the gamma oscillations when the performant path was activated (Bikbaev and Manahan-Vaughan 2008). Our data showed that the strength of cross-frequency coupling between theta and low-gamma rhythms was much weaker in the U1OE group with comparison to the CON group. It suggests that U1 snRNA over-expression significantly disturbs the energy distribution, thereby further induces the reduction of phase synchronization along with the decreased strength of cross-frequency coupling between theta phase and low-gamma amplitude.

Apart from the mesoscopic view of synaptic plasticity, there is another important view of synaptic plasticity that is known as a molecular view based on the expression level of excitatory and inhibitory neuron receptor proteins. The formation and modulation of theta and gamma oscillations are firmly connected to glutamatergic and GABAergic

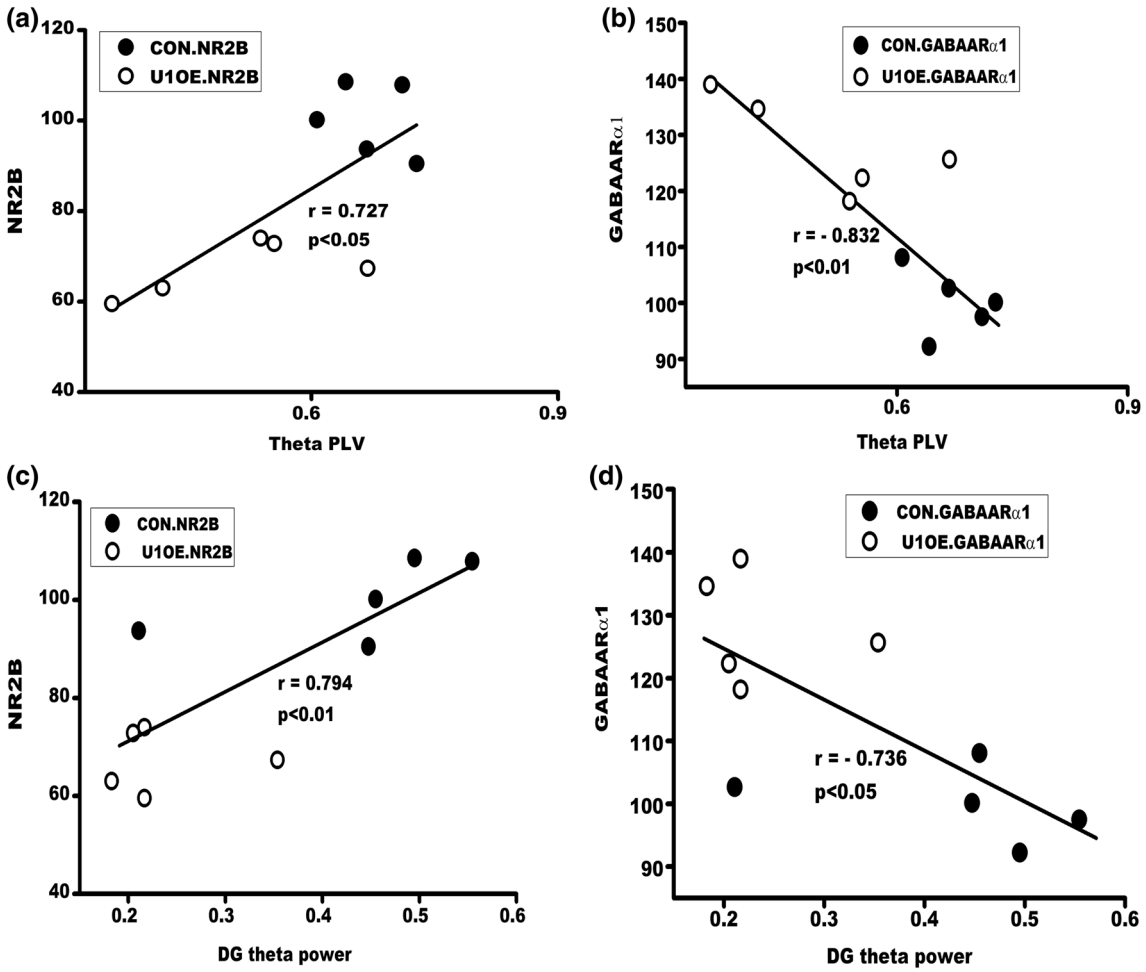

Fig. 4 The correlations analysis of theta synchronization (theta PLV) with the expression level of excitatory and inhibitory neuron receptors proteins as well with the theta power spectra. **a** and **b** The correlations between theta PLV and neurons excitatory proteins (NR2B) as well correlation between recognition index and neuron inhibitory proteins (GABBARα1). **c** The representative of correlations between neuron excitatory proteins (NR2B) and theta power spectra. **d** The representative of correlations between neurons inhibitory proteins (GABBARα1) and theta power spectra. Correlation index(r) and significant values (*p*) are annotated in the figure

neurons (White et al. 2000; Traub et al. 2005; Fukuda et al. 2006; Leung and Shen 2007; Bartos et al. 2007; Lazarewicz et al. 2009). GABA is the main inhibitory neurotransmitter in the central nervous system (Pálvölgyi et al. 2018). The maximum and minimum doses of GABA were noticed on short-term memory loss after the injection of GABAα (Gibbs and Johnston 2005). Accordingly, our results showed that there was a high amount of GAD67 and GABAARα1 included with the low amount of NR2A and NR2B in the U1OE group compared to that in the CON group, suggesting that U1 snRNA over-expression possibly induced the impairment of short-term synaptic plasticity and memory by disturbing the level of excitatory and inhibitory proteins. Therefore, U1 snRNA can be consider as a bridge between mRNA splicing and neuron receptor proteins, which possibly alter the neural oscillations and influences the synaptic plasticity and memory.

Conclusions

U1 snRNA over-expression intensely attenuates neural activities in the hippocampus through disrupting the balance of excitatory and inhibitory neurons receptors proteins, which is a potential underlying mechanism for neurological disorders. In other words, the results feasibly provide an evidence for the critical role of U1 snRNA in the hippocampus for the abnormal pattern of neural oscillations and interactions leading to deficits in short-term memory. U1-based approach has been becoming a modern technique to correct splice defects, which cause either recessive or dominant diseases (Glaus et al. 2011). Based on these previous study and our new findings, we can use splice defect and U1 snRNA over-expression as an early solution in the form of gene therapy against the Alzheimer in clinical studies.

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Author's contribution TZ designed the study; EK performed numerical experiments; YS and ZC performed the animal experiments; EK and TZ wrote the manuscript.

Compliance with ethical standards

Conflict of interest There is not a conflict of interest for all authors.

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