Steroid Sulfatase Inhibitor DU-14 Protects Spatial Memory and Synaptic Plasticity from Disruption by Amyloid β Protein in Male Rats

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ABSTRACT

Alzheimer's disease (AD) is an age-related mental disorder characterized by progressive loss of memory and multiple cognitive impairments. The overproduction and aggregation of Amyloid β protein ($A\beta$) in the brain, especially in the hippocampus, are closely involved in the memory loss in the patients with AD. Accumulating evidence indicates that the $A\beta$ -induced imbalance of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) in the brain plays an important role in the AD pathogenesis and progression. The level of DHEA is elevated, while DHEAS is dramatically decreased in the AD brain. The present study tried to restore the balance between DHEA and DHEAS by using a non-steroidal sulfatase inhibitor DU-14, which increases endogenous DHEAS through preventing DHEAS converted back into DHEA. We found that: (1) DU-14 effectively attenuated the $A\beta_{1-42}$ -induced cognitive deficits in spatial learning and memory of rats in Morris water maze test; (2) DU-14 prevented $A\beta_{1-42}$ -induced decrease in the cholinergic theta rhythm of hippocampal local field potential (LFP) in the CA1 region; (3) DU-14 protected hippocampal synaptic plasticity against $A\beta_{1-42}$ -induced suppression of long term potentiation (LTP). These results provide evidence for the neuroprotective action of DU-14 against neurotoxic $A\beta$, suggesting that up-regulation of endogenous DHEAS by DU-14 could be beneficial to the alleviation of $A\beta$ -induced impairments in spatial memory and synaptic plasticity.

Keywords: DU-14; Amyloid β protein; Dehydroepiandrosterone sulfate; Spatial memory; Theta rhythm; Long term potentiation; Synaptic plasticity

INTRODUCTION

Alzheimer's disease (AD), the most common form of dementia, is characterized by memory loss and multiple cognitive impairments (Uzun et al., 2011). The estimated number of AD patients is approximately 35.6 million worldwide by the end of 2010, which is still rising with the world population aging (Wimo, 2010). It is frustrating that the precise etiology of AD is not completely clarified and nearly no therapy can stop or reverse the inexorable neurodegenerative process (Hölscher C, 2014). According to the amyloid hypothesis, the overproduction and aggregation of amyloid β protein ($A\beta$) in the brain, especially in the hippocampus and cortex, are closely involved in the memory loss of AD patients (Hardy and Selkoe, 2002). The neurotoxicity of $A\beta$ has been thought to be a major pathological mechanism underlying AD (Epelbaum et al., 2015; Yankner and Lu, 2009), which causes disruption of Ca^{2+} homeostasis (Lazzari et al., 2014; Mattson et al., 1993), cholinergic abnormalities (Leon and Marco-Contelles, 2011; Pakaski and Kalman, 2008), and dysfunction of learning and memory.

Neuroactive steroids refer to steroids which, regardless of their origin, are capable of regulating neural activities. Neuroactive steroids, either synthesized *de novo* in the nervous tissue or in the peripheral endocrine glands or as synthetic steroids, have exhibited multiple important modulatory effects on brain functions and diseases (Zheng, 2009). It is reported that the declining of some neuroactive steroids, such as dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) are closely associated with the increased risk of AD and reduced neuroprotection (El Bitar et al., 2014; Taylor et al., 2014; Wojtal et al., 2006). Some studies indicated that the administration of either DHEA or DHEAS could enhance

memory, but further studies confirmed that DHEAS is the actual active agent for memory enhancement (Johnson et al., 2000). DHEAS has many effects including enhancing ACh release in the brain (Rhodes et al., 1997), inhibiting A β -induced [Ca²⁺]_i elevation (Kato-Negishi and Kawahara, 2008) and protecting hippocampal neurons against glutamate-induced neuronal toxicity (Kaasik et al., 2001). Several recent clinical studies have shown that DHEAS are significantly decreased in the brains and serum of AD patients (Bonomo et al., 2009; Cho et al., 2006; Kim et al., 2003). Some research even found that DHEAS concentrations were negatively correlated with A β levels in some brain regions, such as striatum and cerebellum (Weill-Engerer et al., 2002). Different from the dramatical decrease in the DHEAS level, DHEA is significantly higher in the AD brains than age-matched controls, especially in the region of hippocampus (Brown et al., 2003; Naylor et al., 2008). DHEA produced in the brain does not help to protect brain from AD onset and progression (Kim et al., 2003). Some studies showed that high concentrations of DHEA is ineffective (Bologa et al., 1987) or even neurotoxic (Kimonides et al., 1999).

It seems that the imbalance of DHEA and DHEAS during their mutual transformation may play an important role in the AD pathological process (Luchetti et al., 2011). Steroid sulfatase, an enzyme involved in steroid metabolism, catalyzes the hydrolysis of steroid sulfates (e.g. DHEAS) into their unconjugated forms (e.g. DHEA) (Reed et al., 2005). Steroid sulfatase inhibitor can alter the metabolism of neuroactive steroids and prevent sulfated steroids from being converted back into their unsulfated forms (Maltais and Poirier, 2011). According to Rhodes's study, a single acute administration of p-O-(sulfamoyl)-N-tetradecanoyl tyramine (DU-14), a non-steroidal steroid sulfatase inhibitor,

produced a significant inhibition of steroid sulfatase activity in brain after 24 hours (Li et al., 1997). As well as elevating brain DHEAS levels, DU-14 can also enhance brain acetylcholine levels and reverse scopolamine-induced amnesia (Johnson et al., 2000; Li et al., 1997; Rhodes et al., 1997). However, none of these studies provide any evident that DU-14 can protect cognitive function and synaptic plasticity from AD-related or A β -induced disruption. Therefore, the present study was designed to investigate the probable neuroprotective roles of DU-14 against A β ₁₋₄₂-induced impairments in spatial memory, neuronal cooperative activity and hippocampal synaptic plasticity.

MATERIALS AND METHODS

Animals and drugs preparation

Adult male Sprague-Dawley rats (Research Animal Center of Shanxi Medical University, Taiyuan, China) weighing 180~200 g at the time of surgery served as subjects. Rats were kept in a standard animal room (25 $\pm$ 2 °C, 50 $\pm$ 5% humidity, 12 h light-dark cycle) individually with food and water available *ad libitum*. Animal handling and experimental procedures were performed according to the guidelines of the Shanxi Animal Research Ethics Committee. Efforts were made to minimize the number of animals used and their suffering. DU-14 (LKT Laboratory, USA) was dissolved in DMSO (50 μ g/ μ l). A β ₁₋₄₂ (Abcam, USA) solution was prepared according to the procedure described by Tan Tao et al (23 °C for 36 h, 5 μ g/ μ l) (Tan et al., 2011). The stock solutions were stored at -80 °C before using. After 5 days of acclimation, rats were randomly divided into four groups: vehicle (0.045% DMSO solution) + saline, DU-14 + saline, vehicle + A β ₁₋₄₂, and DU-14 + A β ₁₋₄₂. DU-14 and/or A β ₁₋₄₂ were

injected into bilateral hippocampi. Two weeks after injection, Morris water maze test and *in vivo* electrophysiological recording were performed. Figure 1 shows the experimental timeline (Fig.1). After finishing all experiments, the animals were painlessly killed with an overdose of urethane.

Bilateral intrahippocampal injection

The surgical operation for bilateral intrahippocampal injection was performed as previously described (Han et al., 2013). In brief, SD rats were anesthetized with chloral hydrate (0.3 g/kg, i.p.) and placed onto a stereotaxic apparatus (RWD, Shenzhen, China). The hair covering the skull surface was removed and an incision was made to expose the dorsal skull. Two small holes (0.4 mm) were drilled bilaterally in the skull for intrahippocampal injection. Dissolved drug (50 μ g DU-14 and/or 10 μ g A β ₁₋₄₂) solution (totally 3 μ l) was injected into the CA1 region of dorsal hippocampus with an injection rate of 0.15 μ l/min under the control of micropump (KDS310 Plus, KD Scientific Inc, USA). The coordinates of drug delivery site are: anterior posterior (AP), -3.0 mm; medial lateral (ML), $\pm$ 2.2 mm; dorsoventral (DV), -3.0 mm from bregma (Paxinos and Watson, 2006). Fig.2 shows a typical photomicrograph with two injection needle tracks. Two arrows in the figure indicated the injection sites in the brain slice, located at just the top of CA1 regions. The needle of micropump (D = 0.4 mm) was kept in the hippocampus for an additional 5 min in order to facilitate the diffusion of the drugs. In co-application group, A β ₁₋₄₂ was injected at least 20 minutes after DU-14 injection. After completion of the intrahippocampal injections, the burr holes were sealed with bone wax and skin was closed using silk surgical suture. Rats were monitored closely and kept warm until they fully recovered from anesthesia.

Morris water maze test

Morris water maze (MWM) test was designed and performed to evaluate the hippocampal-dependent spatial learning and memory ability of rats (Inostroza et al., 2011). After 3 day acclimatization, the rats were submitted to the Morris water maze as described before (Morris, 1984). The maze was a circular stainless steel pool with a diameter of 150 cm. The interior surface of the pool was painted flat black. The pool was filled with clear tap water at 23 ± 2 °C to avoid hypothermia, and was surrounded by curtains containing various prominent visual cues. The black escape platform (diameter, 14 cm) was positioned in the center of target quadrant and submerged 1.0~2.0 cm beneath the water surface.

Hidden platform test

The hidden platform test consisted of 5 consecutive training days and 4 trials per day with a 15 s intertrial interval. On each training day, a trial was started by introducing each rat to the water facing the pool wall and allowing it to swim freely to the escape platform. If the rat did not reach the platform within a period of 120 s, it will be guided to the platform gently. The rat was allowed to stay on the platform for 15 s. The swimming track of rat was monitored via a camera mounted overhead and the path distance and escape latency were analyzed by a behavior software system (Ethovision 3.0, Noldus Information Technology, Wageningen, the Netherlands).

Probe trial

Probe trials were performed on day 6 after finishing the acquisition task. The platform was removed from the pool and rats were allowed to swim freely for 120 s. The percentages

of time traveled in the target quadrant were measured to evaluate memory levels.

Visible platform test

After the last probe trial, a visible platform test was performed to examine the visual ability of rats. The visible platform was placed opposite the original position within the pool. A white cover rendered highly visible was attached to the platform to raise the surface above the water level (approximately 1.5 cm). The time arrived at the visible platform was recorded. The motor ability of rats was evaluated by comparing the swimming speed of rats during all trials.

In vivo hippocampal local field potential and theta rhythm recording

Prior researches have shown that sensory-induced hippocampal theta rhythm is closely associated with cognitive behavior. So, hippocampal theta rhythm of rats was recorded after behavioral test. The rats were anesthetized with urethane (1.3 g/Kg, i.p.) and mounted onto a stereotaxic apparatus (RWD, Shenzhen, China). Body temperature was monitored and maintained at 37 ± 0.5 °C by a feedback regulated heating pad (Temperature controller 69000, RWD, China). The skull was exposed and a small hole was drilled on the left skull for insert microelectrode. An epoxy insulation curing microelectrode (A-M system, 5 M Ω) was slowly lowered through the cortex and the upper layers of the hippocampus into the CA1 region (AP = -3.4 mm; ML = -2.5 mm) (Paxinos and Watson, 2006) with a micromanipulator (MO-10, Narishige, Japan). The signals of local field potential (LFP) from the hippocampus were sampled (500 Hz) and filtered (high pass, 0.1 Hz; low pass, 250 Hz) with a biological signal collecting and processing system (RX7 workstation, TDT, USA). After waveform became

stable, the spontaneous LFP was recorded for 2 min, and then theta rhythm was evoked by tail-pinch with a plastic clamp on the tail base (duration: 1 min; strength: about 10 grams gravity force) (Liu et al., 2013). For each successfully induced theta rhythm, we choose the period from 3rd to 6th min of the theta rhythm for statistical analysis. In the end, the recording site was visually confirmed.

***In vivo* hippocampal evoked excitatory postsynaptic potentials and LTP recording**

Considering the correlation between spatial memory and synaptic plasticity, we recorded evoked hippocampal field excitatory postsynaptic potential (fEPSPs) and its long term potentiation (LTP). After spontaneous local field potential and theta rhythm recording, a bound stimulating/recording electrode was inserted into the hippocampus through a small hole (diameter, 5.0 mm) drilled on the right skull. The tip of stimulation electrode was located at the Schaffer collateral/commissural pathway (AP = -4.2 mm, ML = +3.8 mm). The tip of recording electrode was located at the stratum radiatum in the CA1 region of hippocampus (AP = -3.4 mm, ML = +2.5 mm) (Paxinos and Watson, 2006). The precision of recording electrode placement in LTP recording was guaranteed by electrophysiological criteria, i. e. successful induction of evoked potential (Leung, 1980). When the recording electrode was accurately located in the stratum radiatum (AP = -3.4 mm, ML = +2.5 mm) of hippocampal CA1 region, a negative deflecting EPSP always appeared after delivering test stimulation. In test stimulation, fEPSPs were evoked at a frequency of 0.033 Hz. An input-output curve (stimulus intensity versus fEPSP amplitude) was plotted for each experiment. For the baseline fEPSP, the stimulus intensity was adjusted to induce 50% of the maximal fEPSP amplitude. The high-frequency stimulation (HFS) protocol to induce LTP consisted of 20 stimuli at 200

Hz repeated three times at an intertrain interval of 30 s. The stimulus intensity was raised to induce 80% of the maximal fEPSP amplitude during HFS. Baseline fEPSPs were monitored for 30 min prior to HFS to ensure a steady basic response. After HFS, fEPSPs were recorded for at least 1 hour. The averaged value of baseline fEPSPs amplitude during 30 minutes was taken as 100%, and all recorded fEPSPs were normalized to this baseline value. A $\geq 30\%$ increase of fEPSP amplitude from the baseline was considered as significant LTP. Paired pulse facilitation (PPF) reflecting presynaptic release (Schulz et al., 1994) was also observed before HFS. Pairs of stimuli with an interval of 50 ms were used to evoke paired fEPSPs. The PPF ratio was calculated by dividing the second fEPSP amplitude by the first fEPSP amplitude. The body temperature was monitored at 37 ± 0.5 °C throughout the experiment. The signals from recording electrode were filtered (low pass: 1 kHz; sampling frequency: 40 kHz) and processed by a biological signal collecting and processing system (RM6240, Chengdu Instruments Ltd, China). The system triggered an electronic stimulator (Master-9 pulse stimulator, A.M.P.I, Israel) to generate constant current pulse stimulation through a stimulus isolation unit (ISO-Flex, A.M.P.I, Israel). All evoked responses were stored on computer for further off-line analysis.

Data analysis

All data were expressed as mean $\pm$ standard error (SEM). For the data from hidden platform test of MWM, the escape latency was analyzed using three-way repeated measures analysis of variance (ANOVA). Other data from MWM and the data of LFP, PPF and LTP were analyzed using two-way ANOVA. The power spectrums of LFP were analyzed by Matlab 7.7. All statistical analysis was performed by using the statistical software package

SPSS 13.0. The statistical significance level was defined as $P < 0.05$. Effect sizes were computed as η^2 for ANOVAs.

RESULTS

DU-14 attenuated $A\beta_{1-42}$ induced impairments in spatial learning and memory

Firstly, the learning ability of rats to acquire spatial information was evaluated by hidden platform test. Fig. 3A and B show the mean escape latencies in the hidden platform test in all four groups. Overall, with the increase of training days, the average escape latencies gradually decreased ($F_{(4,148)} = 106.69$; $P < 0.001$, $\eta^2 = 0.68$), indicating a learning process. But as expected, intrahippocampal injection of $A\beta_{1-42}$ seriously impaired the performance of rats in hidden platform tests. Three-way repeated measures ANOVA showed that there was a significant main effect of $A\beta_{1-42}$ injection ($F_{(1,38)} = 34.18$; $P < 0.001$, $\eta^2 = 0.18$) and DU-14 pretreatment ($F_{(1,38)} = 43.05$; $P < 0.001$, $\eta^2 = 0.23$) on the escape latency; there was also a significant interaction effects between $A\beta_{1-42}$ and DU-14 treatment ($F_{(1,38)} = 8.72$; $P < 0.05$, $\eta^2 = 0.05$). Further, Tukey's post hoc test revealed that the rats with $A\beta_{1-42}$ injection (vehicle + $A\beta_{1-42}$) learned more slowly in finding the hidden platform than control group (vehicle + saline) ($P < 0.001$, $n = 8-10$). DU-14 alone (DU-14 + saline) had no significant effect on the escape latency ($P > 0.05$, $n = 11$), but significantly reversed $A\beta_{1-42}$ -induced increase in the escape latency in DU-14+ $A\beta_{1-42}$ group ($n = 10$), compared with $A\beta_{1-42}$ only group ($P < 0.001$). Fig. 3C shows typical swimming tracks of rats on the 4th training day in the hidden platform tests.

Subsequently, the ability of spatial memory was tested by probe trials on day six (the platform was removed from the pool). As shown in the swimming tracks of Fig. 4A, A β_{1-42} alone injected rats (vehicle + A β_{1-42}) spent nearly equal time in each quadrant. By comparing the percentage of total time elapsed in the target quadrant of four groups (Fig. 4B), two-way ANOVA showed that both A β_{1-42} and DU-14 had significant main effects on the percentage of swimming time in the targeted quadrant (A β_{1-42} : $F_{(1,38)} = 11.222$, $P = 0.002$, $\eta^2 = 0.24$; DU-14: $F_{(1,38)} = 12.037$, $P = 0.001$, $\eta^2 = 0.26$; Fig. 4B). Tukey's post hoc test showed that A β_{1-42} injection produced a significant decrease in the percentage of swimming time in the targeted quadrant ($P < 0.001$, $n = 8-10$), while the impairment was attenuated by DU-14 pretreatment (A β_{1-42} by DU-14 treatment interaction: $F_{(1,38)} = 10.565$, $P = 0.003$).

To exclude possible influence of motor and vision ability on learning and memory, we compared the average swimming speeds of rats in all training days and the escape latency in visible platform test. As shown in Fig. 5, No significant difference was found among four groups for the swimming speed ($P > 0.05$, $\eta^2 = 0.00$, Fig. 5A) and escape latencies ($P > 0.05$, $\eta^2 = 0.02$, Fig. 5B).

DU-14 prevented A β_{1-42} -induced decrease in the power of theta rhythm in the hippocampal CA1 region

Hippocampal theta rhythm, usually appearing when rats are alert and active, was successfully induced by tail-pinch. Once induced, the theta rhythm could last for about 15 min. As shown in the representative raw LFP traces in Fig. 6A, hippocampal LFP showed a pattern of large and irregular activity before tail-pinch, which became more steady, sinusoidal-like

oscillatory activity after tail-pinch in most groups except vehicle + A β_{1-42} . The corresponding spectrograms (Fig. 6B) show the frequency distribution during 30 sec of LFP recording before (left) and after (right) tail-pinch. In control group (the 1st row), the frequency corresponding to the maximum power of LFP changed from ~2 Hz to 3~4 Hz, indicating that theta rhythm was successfully induced. However, in A β_{1-42} alone injected rats (2nd row), tail-pinch failed to induce theta rhythm. DU-14 alone (the 3rd row) did not affect theta rhythm induction, but DU-14 pretreatment restored theta rhythm in DU-14 + A β_{1-42} group (the 4th row).

Fig. 7A-D shows typical power spectrums of LFP in four groups after Fourier transformation, demonstrating the change of the power of theta rhythm. The average peak frequency (F_{peak} , Fig. 7E) and peak power (P_{peak} , Fig. 7F) of theta rhythm were quantified and compared among groups. Two-way ANOVA showed that neither A β_{1-42} nor DU-14 had significant effect on the F_{peak} of theta rhythm (A β_{1-42} : $F_{(1,71)} = 2.673$, $P = 0.107$, $\eta^2 = 0.04$; DU-14: $F_{(1,71)} = 1.774$, $P = 0.187$, $\eta^2 = 0.03$; A β_{1-42} by DU-14 interaction: $F_{(1,71)} = 0.089$, $P = 0.765$, $\eta^2 = 0.00$; Fig. 7E). These results indicated that A β_{1-42} and DU-14 did not affect the frequency of theta rhythm. However, A β_{1-42} and DU-14 treatments had significant main and interaction effects on the P_{peak} of theta rhythm (A β_{1-42} : $F_{(1,71)} = 24.416$, $P < 0.001$, $\eta^2 = 0.26$; DU-14: $F_{(1,71)} = 4.223$, $P = 0.044$, $\eta^2 = 0.06$; A β_{1-42} by DU-14 interaction: $F_{(1,71)} = 6.523$, $P = 0.013$, $\eta^2 = 0.09$; Fig. 7F). Tukey's post hoc test showed that A β_{1-42} injection significantly decreased the P_{peak} of theta rhythm ($P < 0.001$, $n = 18$), which was prevented by DU-14 pretreatment ($P < 0.01$, $n = 18$).

DU-14 protected hippocampal synaptic plasticity from deficits induced by A β_{1-42}

After a stable recording of baseline fEPSPs for 30 min, LTP was induced by HFS. As shown in the Fig. 8A, immediately after HFS delivery, the amplitude of fEPSP in control group increased from 100% to $187 \pm 7.13\%$, remaining at $145 \pm 3.90\%$ 60 min after HFS, indicating a successful LTP induction and maintaining. Fig. 8B shows typical fEPSP traces from the different groups. Two-way ANOVA showed that both $A\beta_{1-42}$ and DU-14 had significant main effects and interaction effects on the induction ($A\beta_{1-42}$: $F_{(1,23)} = 33.379$, $P < 0.001$, $\eta^2 = 0.63$; DU-14: $F_{(1,23)} = 10.844$, $P = 0.004$, $\eta^2 = 0.35$; $A\beta_{1-42}$ by DU-14 interaction: $F_{(1,23)} = 12.494$, $P = 0.002$, $\eta^2 = 0.39$; Fig. 8C) and maintenance ($A\beta_{1-42}$: $F_{(1,23)} = 7.849$, $P = 0.011$, $\eta^2 = 0.28$; DU-14: $F_{(1,23)} = 5.011$, $P = 0.037$, $\eta^2 = 0.20$; $A\beta_{1-42}$ by DU-14 interaction: $F_{(1,23)} = 4.741$, $P = 0.042$, $\eta^2 = 0.19$; Fig. 8D) of LTP. Tukey's post hoc tests showed that DU-14 alone had no significant effect on LTP ($P > 0.05$, $n = 6$); $A\beta_{1-42}$ significantly impaired LTP induction ($P < 0.001$, $n = 6$) and maintenance ($P < 0.001$, $n = 6$), while DU-14 pretreatment reversed the deficits effectively ($P < 0.01$, $n = 6$).

To clarify whether presynaptic mechanism was involved in the effects of $A\beta_{1-42}$ and DU-14 on the synaptic plasticity, the PPF in the hippocampal CA1 region was recorded before HFS (Fig. 9A). After paired-pulse was applied to the Schaffer collaterals, the PPF in CA1 stratum radiatum always appeared, with an obvious increase in the amplitude for the second fEPSP. Two-way ANOVA showed that both $A\beta_{1-42}$ and DU-14 had no significant main effects on the paired-fEPSPs ratio ($fEPSP_2/fEPSP_1$) ($P > 0.05$, $\eta^2 = 0.02$; Fig. 9B), indicating that $A\beta_{1-42}$ and DU-14 did not change the presynaptic transmitter release.

DISCUSSION

Accumulating evidence has demonstrated that cognitive behavior such as spatial learning and memory is strongly dependent on the normal functional activity of the hippocampus in the brain (Inostroza et al., 2011; O'Keefe, 1993). The cholinergic-related theta rhythm and long-term synaptic plasticity in the hippocampus are closely associated with the normal cognitive activities in animals (Duzel et al., 2010; Jutras and Buffalo, 2010), while the impairments in the theta rhythm and LTP are related to the memory deficits and pathological changes in AD (Goutagny and Krantic, 2013; Rubio et al., 2012). Most recent evidences indicate that soluble A β oligomers rather than plaques are the major cause of AD-related impairments in synaptic plasticity, neuronal network activity and cognitive function (Mucke and Selkoe, 2012; Walsh et al., 2002; Wisniewski and Go09i, 2015). In the present study, we also found that intrahippocampal injection of full length of A β ₁₋₄₂ oligomers not only impaired spatial learning and memory in MWM tests, but also strongly suppressed tail-pinch induced cholinergic theta rhythm and HFS induced LTP in the hippocampus of A β -treatment animals, further clarifying the association between the cognitive deficits and the hippocampal electrical activity disorder. The behavioral and electrophysiological tests in the present study were performed two weeks after a single A β injection into the hippocampus, which is mainly based on the following considerations: 1) to observe the acute effects of A β in the hippocampus; and 2) to limit multiple injection induced brain damage. Many studies have reported a long-lasting damage effect of A β single injection. For example, abnormal spatial memory was observed one week following a single i.c.v. administration of A β (Liu et al., 2008); a delayed and prolonged (2-3 weeks) deficit in memory function was induced by A β injection into rat brain (Jia et al., 2013). Our previous studies also indicates that A β -induced long-lasting effects on

behavioral and electrophysiological activity may be involved in the alteration of some intracellular signal molecules such as Ca^{2+} , Caspase-3, STAT3 and long term synaptic plasticity (Han et al., 2013; Wang et al., 2014; Wu et al., 2015).

It is reported that $\text{A}\beta$, by inducing oxidative stress, decreased availability of reduced glutathione (GSH) (Hodgson et al., 2013; Mandal et al., 2015), an co-factor required for the function of hydroxysteroid sulphotransferase (Sinha et al., 2010). The function of hydroxysteroid sulphotransferase is to convert DHEA into DHEAS. Therefore, $\text{A}\beta$ -induced GSH suppression will greatly decrease the production of DHEAS by lowering enzymatic activity of hydroxysteroid sulphotransferase. The imbalance of DHEA and DHEAS levels in AD brain may further accelerate $\text{A}\beta$ aggregation and AD progress. Based on this hypothesis, we proposed that steroid sulfatase inhibitor DU-14, by inhibiting enzymatic hydrolysis of DHEAS and increasing DHEAS level in the brain, could be helpful in alleviating pathological damage and functional disorder of AD. Indeed, the present data provides the first demonstration that DU-14 effectively reversed $\text{A}\beta_{1-42}$ induced deficits, suggesting an effective neuroprotective action of DU-14 against toxic $\text{A}\beta$. DU-14, as a non-steroid sulfatase inhibitor, can also affect other steroidal conjugates such as PREG and PREGS. However, a large number of studies have shown that the neuroprotective effects of DU-14 is mainly mediated by preventing sulfated steroids (e.g. DHEAS) from being converted back into unsulfated forms (e.g. DHEA) (Maltais and Poirier, 2011; Reed et al., 2005), and increasing the level of DHEAS in the brain (Johnson et al., 1997; Johnson et al., 2000; Rhodes et al., 1997). Especially, the imbalance between DHEA and DHEAS has been confirmed in the patients with AD, with an increased level of DHEA and a decreased level of DHEAS in the

hippocampus (Kim et al., 2003; Luchetti et al., 2011). The increased DHEA is probably the consequence of A β -induced ROS production (Brown et al., 2003), because application of A β *in vitro* increased the levels of ROS and DHEA in human glioma cells (Brown et al., 2000). Nonetheless, the increased DHEA does not help to protect the brain from AD onset and progression. On the contrary, there is a negative correlation between serum DHEAS levels and age (Sinha et al., 2010); the DHEAS levels were also negatively correlated with A β in some brain region; the decrease of the DHEAS is more remarkable in AD patients compared with that in normal subjects (Cho et al., 2006). These studies clearly indicate that the imbalance of DHEA and DHEAS in the brain may play a major role in the AD pathogenesis and progression. Therefore, the main intention of the present study is to restore the balance of DHEA and DHEAS by specifically inhibiting steroid sulfatase using DU-14.

In the present study, we first confirmed that DU-14 effectively rescued spatial learning and memory of rats from A β ₁₋₄₂ induced deficits in the Morris water maze. The escape latency and the percentage of swimming time of the rats in DU-14 plus A β group were similar to the values in vehicle treated saline injection group. According to the results of MWM test, two important electrophysiological parameters closely associated with cognitive behavior, i. e. hippocampal theta rhythm and hippocampal LTP, were further investigated. Recent studies have reported the alteration of theta rhythm activity in AD patients and in animal models of AD (Al-Jumeily et al., 2015; Goutagny and Krantic, 2013; Rubio et al., 2012; Scott et al., 2012; Vorobyov et al., 2015). The disruption of hippocampal theta rhythm, especially type 2 theta rhythm, has been well known to be related to the cholinergic neuron damage and cognitive deficits in AD (Colom et al., 2010; Navarrete et al., 2012). In the local field

potential experiment, we evoked cholinergic type 2 theta rhythms through somatosensory stimulation with tail-pinch. We found that intrahippocampal injection of A β ₁₋₄₂ oligomers induced a significant decrease in peak power of theta rhythm, suggesting that A β ₁₋₄₂ impaired the activities of cholinergic neurons in the hippocampus. This result was similar to our another recent study (Liu et al., 2013). Interestingly, intrahippocampal injection of DU-14 effectively alleviated A β ₁₋₄₂-induced theta rhythm damage, implying that DU-14 could protect hippocampal cholinergic neuronal activities from neurotoxicity of A β ₁₋₄₂. Considering the close association between spatial memory and hippocampal synaptic plasticity, we further observed the effects of DU-14 on the hippocampal LTP, a persistent increase in synaptic efficacy after HFS. We found that A β ₁₋₄₂ suppressed LTP, while the pretreatment with DU-14 effectively prevented A β ₁₋₄₂ induced impairment of LTP. The protective effects of DU-14 on hippocampal cholinergic theta rhythm and synaptic plasticity strongly support the behavioral improvements seen in the MWM test.

The mechanistic pathway that underlying the behavioral and electrophysiological effects of DU-14 is closely associated with the increased DHEAS. According to previous studies, up-regulation of DHEAS in the brain could: 1) block A β ₁₋₄₂ induced intracellular calcium overload (Kato-Negishi and Kawahara, 2008); 2) increase the production of growth factors (Luppi et al., 2009) and enhance brain cholinergic function (Dong and Zheng, 2012); 3) play an immunomodulatory role on NK functional activity in physiological aging and AD (Solerte et al., 1999); 4) have an antiglucocorticoid activity and neuroprotective effects, while its levels decreased in AD (Murialdo et al., 2001); 5) prevent the cells from entering late apoptosis and necrosis and stimulate neurite outgrowth per se (El Bitar et al., 2014); 6) protect

hippocampal neurons against glutamate through elevating a kappaB-dependent transcription factor activity (Leskiewicz et al., 2008; Mao and Barger, 1998). Of course, a further investigation on the effects of DU-14 and its mechanisms in AD transgenic animals or AD patients would be urgently required in the future. In addition, it also needs to declare that only male rats were used in the present study to limit the consequences of menstrual cycle. So, the data may be relevant to males closely and can not be assumed to be relevant to females directly.

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References

- Al-Jumeily, D., Iram, S., Vialatte, F.B., Fergus, P., Hussain, A., 2015. A novel method of early diagnosis of Alzheimer's disease based on EEG signals. *ScientificWorldJournal* 2015, 931387.
- Bologa, L., Sharma, J., Roberts, E., 1987. Dehydroepiandrosterone and its sulfated derivative reduce neuronal death and enhance astrocytic differentiation in brain cell cultures. *J Neurosci Res* 17, 225-234.
- Bonomo, S.M., Rigamonti, A.E., Giunta, M., Galimberti, D., Guaita, A., Gagliano, M.G., Muller, E.E., Cella, S.G., 2009. Menopausal transition: a possible risk factor for brain pathologic events. *Neurobiol Aging* 30, 71-80.
- Brown, R.C., Cascio, C., Papadopoulos, V., 2000. Pathways of neurosteroid biosynthesis in cell lines from human brain: regulation of dehydroepiandrosterone formation by oxidative stress and beta-amyloid peptide. *J Neurochem* 74, 847-859.
- Brown, R.C., Han, Z., Cascio, C., Papadopoulos, V., 2003. Oxidative stress-mediated DHEA formation in Alzheimer's disease pathology. *Neurobiol Aging* 24, 57-65.
- Cho, S.H., Jung, B.H., Lee, W.Y., Chung, B.C., 2006. Rapid column-switching liquid chromatography/mass spectrometric assay for DHEA-sulfate in the plasma of patients with Alzheimer's disease. *Biomed Chromatogr* 20, 1093-1097.
- Colom, L.V., Castaneda, M.T., Banuelos, C., Puras, G., Garcia-Hernandez, A., Hernandez, S., Mounsey, S., Benavidez, J., Lehker, C., 2010. Medial septal beta-amyloid 1-40 injections alter septo-hippocampal anatomy and function. *Neurobiol Aging* 31, 46-57.

Dong, Y., Zheng, P., 2012. Dehydroepiandrosterone sulphate: action and mechanism in the brain. *J Neuroendocrinol* 24, 215-224.

Duzel, E., Penny, W.D., Burgess, N., 2010. Brain oscillations and memory. *Curr Opin Neurobiol* 20, 143-149.

El Bitar, F., Meunier, J., Villard, V., Almeras, M., Krishnan, K., Covey, D.F., Maurice, T., Akwa, Y., 2014. Neuroprotection by the synthetic neurosteroid enantiomers ent-PREGS and ent-DHEAS against Abeta₂₅₋₃₅ peptide-induced toxicity *in vitro* and *in vivo* in mice. *Psychopharmacology (Berl)* 231, 3293-3312.

Epelbaum, S., Youssef, I., Lacor, P.N., Chaurand, P., Duplus, E., Brugg, B., Duyckaerts, C., Delatour, B., 2015. Acute amnesic encephalopathy in amyloid- β oligomer-injected mice is due to their widespread diffusion *in vivo*. *Neurobiol Aging* 36, 2043-2052.

Goutagny, R., Krantic, S., 2013. Hippocampal oscillatory activity in Alzheimer's disease: toward the identification of early biomarkers? *Aging Dis* 4, 134-140.

Hölscher C, C., 2014. New drug treatments show neuroprotective effects in Alzheimer's and Parkinson's diseases. *Neural Regeneration Research* 9, 1870-1873.

Han, W.N., Holscher, C., Yuan, L., Yang, W., Wang, X.H., Wu, M.N., Qi, J.S., 2013. Liraglutide protects against amyloid-beta protein-induced impairment of spatial learning and memory in rats. *Neurobiol Aging* 34, 576-588.

Hardy, J., Selkoe, D.J., 2002. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297, 353-356.

Hodgson, N., Trivedi, M., Muratore, C., Li, S., Deth, R., 2013. Soluble oligomers of amyloid-beta cause changes in redox state, DNA methylation, and gene transcription by inhibiting EAAT3 mediated cysteine uptake. *J Alzheimers Dis* 36, 197-209.

Inostroza, M., Cid, E., Brotons-Mas, J., Gal, B., Aivar, P., Uzcategui, Y.G., Sandi, C., Menendez de la Prida, L., 2011. Hippocampal-dependent spatial memory in the water maze is preserved in an experimental model of temporal lobe epilepsy in rats. *PLoS One* 6, e22372.

Jia, J., Kang, L., Li, S., Geng, D., Fan, P., Wang, L., Cui, H., 2013. Amelioratory effects of testosterone treatment on cognitive performance deficits induced by soluble Abeta₁₋₄₂ oligomers injected into the hippocampus. *Horm Behav* 64, 477-486.

Johnson, D.A., Rhodes, M.E., Boni, R.L., Li, P.K., 1997. Chronic steroid sulfatase inhibition by (p-O-sulfamoyl)-N-tetradecanoyl tyramine increases dehydroepiandrosterone sulfate in whole brain. *Life Sci* 61, PL 355-359.

Johnson, D.A., Wu, T., Li, P., Maher, T.J., 2000. The effect of steroid sulfatase inhibition on learning and spatial memory. *Brain Res* 865, 286-290.

Jutras, M.J., Buffalo, E.A., 2010. Synchronous neural activity and memory formation. *Curr Opin Neurobiol* 20, 150-155.

Kaasik, A., Kalda, A., Jaako, K., Zharkovsky, A., 2001. Dehydroepiandrosterone sulphate prevents oxygen-glucose deprivation-induced injury in cerebellar granule cell culture. *Neuroscience* 102, 427-432.

Kato-Negishi, M., Kawahara, M., 2008. Neurosteroids block the increase in intracellular calcium level induced by Alzheimer's beta-amyloid protein in long-term cultured rat hippocampal neurons. *Neuropsychiatr Dis Treat* 4, 209-218.

Kim, S.B., Hill, M., Kwak, Y.T., Hampl, R., Jo, D.H., Morfin, R., 2003. Neurosteroids: Cerebrospinal fluid levels for Alzheimer's disease and vascular dementia diagnostics. *J Clin Endocrinol Metab* 88,

5199-5206.

- Kimionides, V.G., Spillantini, M.G., Sofroniew, M.V., Fawcett, J.W., Herbert, J., 1999. Dehydroepiandrosterone antagonizes the neurotoxic effects of corticosterone and translocation of stress-activated protein kinase 3 in hippocampal primary cultures. *Neuroscience* 89, 429-436.
- Lazzari, C., Kipanyula, M.J., Agostini, M., Pozzan, T., Fasolato, C., 2014. A β 42 oligomers selectively disrupt neuronal calcium release. *Neurobiol Aging* 36, 877-885.
- Leon, R., Marco-Contelles, J., 2011. A step further towards multitarget drugs for Alzheimer and neuronal vascular diseases: targeting the cholinergic system, amyloid-beta aggregation and Ca²⁺ dyshomeostasis. *Curr Med Chem* 18, 552-576.
- Leskiewicz, M., Jantas, D., Budziszewska, B., Lason, W., 2008. Excitatory neurosteroids attenuate apoptotic and excitotoxic cell death in primary cortical neurons. *J Physiol Pharmacol* 59, 457-475.
- Leung, L.S., 1980. Behavior-dependent evoked potentials in the hippocampal CA1 region of the rat. I. Correlation with behavior and EEG. *Brain Res* 198, 95-117.
- Li, P.K., Rhodes, M.E., Burke, A.M., Johnson, D.A., 1997. Memory enhancement mediated by the steroid sulfatase inhibitor (p-O-sulfamoyl)-N-tetradecanoyl tyramine. *Life Sci* 60, PL45-51.
- Liu, R.Y., Gu, R., Qi, X.L., Zhang, T., Zhao, Y., He, Y., Pei, J.J., Guan, Z.Z., 2008. Decreased nicotinic receptors and cognitive deficit in rats intracerebroventricularly injected with beta-amyloid peptide(1-42) and fed a high-cholesterol diet. *J Neurosci Res* 86, 183-193.
- Liu, X.J., Yuan, L., Yang, D., Han, W.N., Li, Q.S., Yang, W., Liu, Q.S., Qi, J.S., 2013. Melatonin protects against amyloid-beta-induced impairments of hippocampal LTP and spatial learning in rats. *Synapse* 67, 626-636.
- Luchetti, S., Huitinga, I., Swaab, D.F., 2011. Neurosteroid and GABA-A receptor alterations in Alzheimer's disease, Parkinson's disease and multiple sclerosis. *Neuroscience* 191, 6-21.
- Luppi, C., Fioravanti, M., Bertolini, B., Inguscio, M., Grugnetti, A., Guerriero, F., Rovelli, C., Cantoni, F., Guagnano, P., Marazzi, E., Rolfo, E., Ghianda, D., Levante, D., Guerrini, C., Bonacasa, R., Solerte, S.B., 2009. Growth factors decrease in subjects with mild to moderate Alzheimer's disease (AD): potential correction with dehydroepiandrosterone-sulphate (DHEAS). *Arch Gerontol Geriatr* 49 Suppl 1, 173-184.
- Maltais, R., Poirier, D., 2011. Steroid sulfatase inhibitors: a review covering the promising 2000-2010 decade. *Steroids* 76, 929-948.
- Mandal, P.K., Saharan, S., Tripathi, M., Murari, G., 2015. Brain Glutathione Levels - A Novel Biomarker for Mild Cognitive Impairment and Alzheimer's Disease. *Biol Psychiatry* 78, 702-710.
- Mao, X., Barger, S.W., 1998. Neuroprotection by dehydroepiandrosterone-sulfate: role of an NFkappaB-like factor. *Neuroreport* 9, 759-763.
- Mattson, M.P., Barger, S.W., Cheng, B., Lieberburg, I., Smith-Swintosky, V.L., Rydel, R.E., 1993. beta-Amyloid precursor protein metabolites and loss of neuronal Ca²⁺ homeostasis in Alzheimer's disease. *Trends Neurosci* 16, 409-414.
- Morris, R., 1984. Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* 11, 47-60.
- Mucke, L., Selkoe, D.J., 2012. Neurotoxicity of amyloid beta-protein: synaptic and network dysfunction. *Cold Spring Harb Perspect Med* 2, a006338.
- Murialdo, G., Barreca, A., Nobili, F., Rollero, A., Timossi, G., Gianelli, M.V., Copello, F., Rodriguez, G., Polleri, A., 2001. Relationships between cortisol, dehydroepiandrosterone sulphate and insulin-like growth factor-I system in dementia. *J Endocrinol Invest* 24, 139-146.

- Navarrete, M., Perea, G., Fernandez de Sevilla, D., Gomez-Gonzalo, M., Nunez, A., Martin, E.D., Araque, A., 2012. Astrocytes mediate *in vivo* cholinergic-induced synaptic plasticity. *PLoS Biol* 10, e1001259.
- Naylor, J.C., Hulette, C.M., Steffens, D.C., Shampine, L.J., Ervin, J.F., Payne, V.M., Massing, M.W., Kilts, J.D., Strauss, J.L., Calhoun, P.S., Calnaido, R.P., Blazer, D.G., Lieberman, J.A., Madison, R.D., Marx, C.E., 2008. Cerebrospinal fluid dehydroepiandrosterone levels are correlated with brain dehydroepiandrosterone levels, elevated in Alzheimer's disease, and related to neuropathological disease stage. *J Clin Endocrinol Metab* 93, 3173-3178.
- O'Keefe, J., 1993. Hippocampus, theta, and spatial memory. *Curr Opin Neurobiol* 3, 917-924.
- Pakaski, M., Kalman, J., 2008. Interactions between the amyloid and cholinergic mechanisms in Alzheimer's disease. *Neurochem Int* 53, 103-111.
- Paxinos, G., Watson, C., 2006. The rat brain in stereotaxic coordinates: hard cover edition. Access Online via Elsevier.
- Reed, M.J., Purohit, A., Woo, L.W., Newman, S.P., Potter, B.V., 2005. Steroid sulfatase: molecular biology, regulation, and inhibition. *Endocr Rev* 26, 171-202.
- Rhodes, M.E., Li, P.K., Burke, A.M., Johnson, D.A., 1997. Enhanced plasma DHEAS, brain acetylcholine and memory mediated by steroid sulfatase inhibition. *Brain Res* 773, 28-32.
- Rubio, S.E., Vega-Flores, G., Martinez, A., Bosch, C., Perez-Mediavilla, A., del Rio, J., Gruart, A., Delgado-Garcia, J.M., Soriano, E., Pascual, M., 2012. Accelerated aging of the GABAergic septohippocampal pathway and decreased hippocampal rhythms in a mouse model of Alzheimer's disease. *FASEB J* 26, 4458-4467.
- Schulz, P.E., Cook, E.P., Johnston, D., 1994. Changes in paired-pulse facilitation suggest presynaptic involvement in long-term potentiation. *J Neurosci* 14, 5325-5337.
- Scott, L., Feng, J., Kiss, T., Needle, E., Atchison, K., Kawabe, T.T., Milici, A.J., Hajos-Korcsok, E., Riddell, D., Hajos, M., 2012. Age-dependent disruption in hippocampal theta oscillation in amyloid-beta overproducing transgenic mice. *Neurobiol Aging* 33, 1481 e1413-1423.
- Sinha, M., Saha, A., Basu, S., Pal, K., Chakrabarti, S., 2010. Aging and antioxidants modulate rat brain levels of homocysteine and dehydroepiandrosterone sulphate (DHEA-S): implications in the pathogenesis of Alzheimer's disease. *Neurosci Lett* 483, 123-126.
- Solerte, S.B., Fioravanti, M., Schifino, N., Cuzzoni, G., Fontana, I., Vignati, G., Govoni, S., Ferrari, E., 1999. Dehydroepiandrosterone sulfate decreases the interleukin-2-mediated overactivity of the natural killer cell compartment in senile dementia of the Alzheimer type. *Dement Geriatr Cogn Disord* 10, 21-27.
- Tan, T., Zhang, B.L., Tian, X., 2011. [Chronic effects of oligomeric A β ₁₋₄₂ on hippocampal synaptic plasticity *in vivo*]. *Sheng LI Xue Bao* 63, 225-232.
- Taylor, M.K., Stone, M., Laurent, H.K., Rauh, M.J., Granger, D.A., 2014. Neuroprotective-neurotrophic effect of endogenous dehydroepiandrosterone sulfate during intense stress exposure. *Steroids* 87, 54-58.
- Uzun, S., Kozumplik, O., Folnegovic-Smalc, V., 2011. Alzheimer's dementia: current data review. *Coll Antropol* 35, 1333-1337.
- Vorobyov, V., Kaptsov, V., Gordon, R., Makarova, E., Podolski, I., Sengpiel, F., 2015. Neuroprotective effects of hydrated fullerene C60: cortical and hippocampal EEG interplay in an amyloid-infused rat model of Alzheimer's disease. *J Alzheimers Dis* 45, 217-233.
- Walsh, D.M., Klyubin, I., Fadeeva, J.V., Cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J., Selkoe,

- D.J., 2002. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature* 416, 535-539.
- Wang, Z.J., Han, W.N., Yang, G.Z., Yuan, L., Liu, X.J., Li, Q.S., Qi, J.S., 2014. The neuroprotection of Rattin against amyloid beta peptide in spatial memory and synaptic plasticity of rats. *Hippocampus* 24, 44-53.
- Weill-Engerer, S., David, J.P., Sazdovitch, V., Liere, P., Eychenne, B., Planos, A., Schumacher, M., Delacourte, A., Baulieu, E.E., Akwa, Y., 2002. Neurosteroid quantification in human brain regions: comparison between Alzheimer's and nondemented patients. *J Clin Endocrinol Metab* 87, 5138-5143.
- Wimo, A., Prince, M., 2010. World Alzheimer Report 2010: the global economic impact of dementia. Alzheimer's Disease International.
- Wisniewski, T., Go09i, F., 2015. Immunotherapeutic Approaches for Alzheimer's Disease. *Neuron* 85, 1162-1176.
- Wojtal, K., Trojnar, M.K., Czuczwar, S.J., 2006. Endogenous neuroprotective factors: neurosteroids. *Pharmacol Rep* 58, 335-340.
- Wu, M.N., Zhou, L.W., Wang, Z.J., Han, W.N., Zhang, J., Liu, X.J., Tong, J.Q., Qi, J.S., 2015. Colivelin ameliorates amyloid beta peptide-induced impairments in spatial memory, synaptic plasticity, and calcium homeostasis in rats. *Hippocampus* 25, 363-372.
- Yankner, B.A., Lu, T., 2009. Amyloid beta-protein toxicity and the pathogenesis of Alzheimer disease. *J Biol Chem* 284, 4755-4759.
- Zheng, P., 2009. Neuroactive steroid regulation of neurotransmitter release in the CNS: action, mechanism and possible significance. *Prog Neurobiol* 89, 134-152.

Figure legends:

Fig. 1 Experimental timeline for intrahippocampal injection, Morris water maze test and *in vivo* electrophysiological recording.

After 5 days of acclimatization, rats were subjected to intrahippocampal A β_{1-42} or/and DU-14 injection. Two weeks later, Morris water maze test and *in vivo* electrophysiological recording (*in vivo* hippocampal LFP and LTP recording) were performed.

Fig.2 A photomicrograph showing the injection sites in the hippocampus of rats.

Two arrows in the figure indicated the injection sites in bilateral hippocampi, just the top of CA1 regions. The coordinates of drug delivery sites are: anterior posterior (AP), -3.0 mm; medial lateral (ML), ± 2.2 mm; dorsoventral (DV), -3.0 mm from bregma.

Fig. 3 DU-14 protected against A β_{1-42} induced deficit in spatial learning ability of rats.

(A) and (B) show the escape latency of rats to find the hidden platform over five consecutive training days. The escape latency was significantly increased in vehicle-treated A β_{1-42} injection animals compared with that in vehicle treated-saline injection group ($***P < 0.001$), while the escape latency in DU-14 treated A β_{1-42} injection animals was significantly lower than that in vehicle treated A β_{1-42} injection animals ($***P < 0.001$) during training days 2-5. Each point represents the mean $\pm$ SEM of four trials per day. (C) Typical swimming tracks of rats in the hidden platform tests on the 4th training day.

Fig. 4 DU-14 protected against A β_{1-42} induced impairment in spatial memory of rats.

(A) Representative original swimming traces of rats in probe trails within 120 sec. (B) Histograms showing the percentage of swimming time in target quadrant. Each column was the mean $\pm$ SEM. Clearly, A β_{1-42} injection induced decrease in the swimming time in the target quadrant was significantly reversed by DU-14 ($***P < 0.001$).

Fig. 5 The motor ability and the vision of rats were not affected by A β_{1-42} or DU-14.

(A) Histograms showing no significant difference in the average swimming speed among groups during all training days ($P > 0.05$). (B) Histogram showing no statistical differences in

the escape time of rats among groups in visible platform tests ($P > 0.05$). Each column indicates the mean $\pm$ SEM.

Fig. 6 Raw signals of LFP recorded in the hippocampal CA1 region and their spectrograms.

(A) The typical raw LFP traces recorded from the hippocampal CA1 region in urethane anesthetized rats. The slow and irregular activities in LFP turned into more steady oscillatory activity after tail-pinch. Scale Bar: 1sec. (B) Spectrograms of LFP signals in the different groups. The frequencies corresponding to maximum power increased from 2 Hz to 3~4 Hz in most groups after tail-pinch (right column). Color bars on the right are power (dB) scales.

Fig. 7 The effects of DU-14 and $A\beta_{1-42}$ on the peak frequency and peak power of theta rhythm in the CA1 region of the hippocampus.

(A-D), Typical power spectrums of LFP in different groups, showing an obvious increase in the power of theta rhythm after tail-pinch in all groups except vehicle+ $A\beta_{1-42}$ group. (E), Histograms showing the average frequency of theta rhythm in all four groups. Neither DU-14 nor $A\beta_{1-42}$ had any effect on the average frequency of theta rhythm ($P > 0.05$). (F), Histograms showing the average power of theta rhythm in these groups. The $A\beta_{1-42}$ injection-induced decrease ($***P < 0.001$) in the power of theta rhythm was significantly reversed in the co-application of DU-14+ $A\beta_{1-42}$ ($**P < 0.01$). Each column shows the mean $\pm$ SEM.

Fig. 8 The effects of DU-14 and $A\beta_{1-42}$ on the *in vivo* hippocampal LTP in the CA1 region.

(A) Plots showing the time course of fEPSPs before and after HFS. Successful induction and maintenance of LTP were found in all groups except $A\beta_{1-42}$ only group. (B) Typical fEPSP traces recorded from different time and different groups. (C and D) Histograms showing the average fEPSPs in each group at the first 4 min (C) and the last 10 min (D) after HFS. $**P < 0.01$; $***P < 0.001$. Each column shows the mean $\pm$ SEM of fEPSPs amplitude.

Fig. 9 Neither $A\beta_{1-42}$ nor DU-14 changed the PPF ratio in the hippocampal CA1 region.

(A) A representative paired-pulse stimulation-induced fEPSP trace, showing an obvious increase in the amplitude of second fEPSP. (B) Histograms showing neither $A\beta_{1-42}$ nor DU-14

affected the paired-pulse facilitation ($fEPSP_2/fEPSP_1$) in the hippocampal CA1 region ($P > 0.05$). Error bars indicated SEM.

A **B**

A **B**