Final Report

OTKA PD 83581 research grant

Network oscillations in Alzheimer's Disease models

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Introduction

There is now considerable evidence that Alzheimer's disease (AD) represents synaptic failure, and in particular, that Aβ induced dysfunction of synaptic plasticity contributes to early memory loss that precedes neuronal degeneration. The accumulated evidence arising from both researches on basic memory mechanisms and recent advances in our understanding of molecular events leading to AD thus strongly suggests that Aβ interferes with the intracellular signaling cascade associated with memory formation, thereby resulting in impaired memory processes. We have shown that Aβ1-42 enhances NMDA receptor mediated neuronal firing, while ablates AMPA evoked activity in vivo. We and others also described LTP impairment upon Aβ1-42 application in vitro. However, despite the wealth of studies exploring the synaptotoxic mechanisms of Aβ1-42, the effect of Aβ1-42 on network function critical to memory formation is still poorly investigated.
Oscillatory network activity in neuronal networks is important for many higher-order cognitive processes in the brain, such as learning, memory and cognition. Hippocampal activity is the key event for consolidation of certain types of memory. Among the characteristic oscillations of the hippocampus, high frequency oscillations known as sharp wave/ripple complexes (SPW-Rs) represent one of the most synchronous network activity. These intense events are associated with synchronous discharge of a large population of neurons, resulting in a peak in the power spectrum at around 140-200 Hz. It is well known that hippocampal damage induces anterograde amnesia for episodic memories while leaving prior traces intact. This led to the two-stage memory theory that hippocampus serves as a temporary buffer for labile traces that are transferred to neocortex for more permanent storage (consolidation). A possible substrate for this is found during hippocampal ripple-sharp wave activity (brief 200 Hz oscillations) during sleep. Then hippocampal place neurons fire in the same sequence that they did during the previous waking experience of the rat.
Another prominent hippocampal oscillation is theta activity, which is usually between 3-12 Hz. Theta oscillation has been connected to higher cognitive functions, learning and memory. Theta oscillation may also coordinate the prefrontal cortex-hippocampal interaction. Pyramidal cells in the CA1 region have been shown to display a firing activity that is strongly connected to the ongoing theta oscillation. Certain memory traces have been hypothesized to be encoded by the firing of CA1 principal cells, which shows phase-preference and theta-dependent firing patterns.
Within the framework of the present grant we investigated the effect of Aβ1-42, a peptide having key role in Alzheimer's disease (AD), on SPW-Rs and theta coupled neuronal activity using in vitro and in vivo approaches. The results are presented under the specific tasks of the original workplan.

AIMS

The overall aim of this grant was to determine the effects of Abeta preparations on different modes of network oscillations in the hippocampus. To this end, we have used in vitro, slice technique and in vivo recordings.

Oscillations in vitro

- **Task 1**: How different Aβ conformations (aggregates) interact with theta and gamma oscillations, correlates of memory encoding, in hippocampal slices from wild-type mice?

Considerations for choosing Abeta preparations

Our preliminary results show that highly aggregated Abeta preparations do not impair LTP, a correlate of learning and memory and a widely used assay for synaptotoxicity. Therefore we decided to focus on the effects of oligomeric Abeta species, which are thought to underlie the cognitive symptoms seen in early AD. Every batch of the peptide solution was verified for biological activity by testing its effect on Schaffer-CA1 LTP (Figure 1).

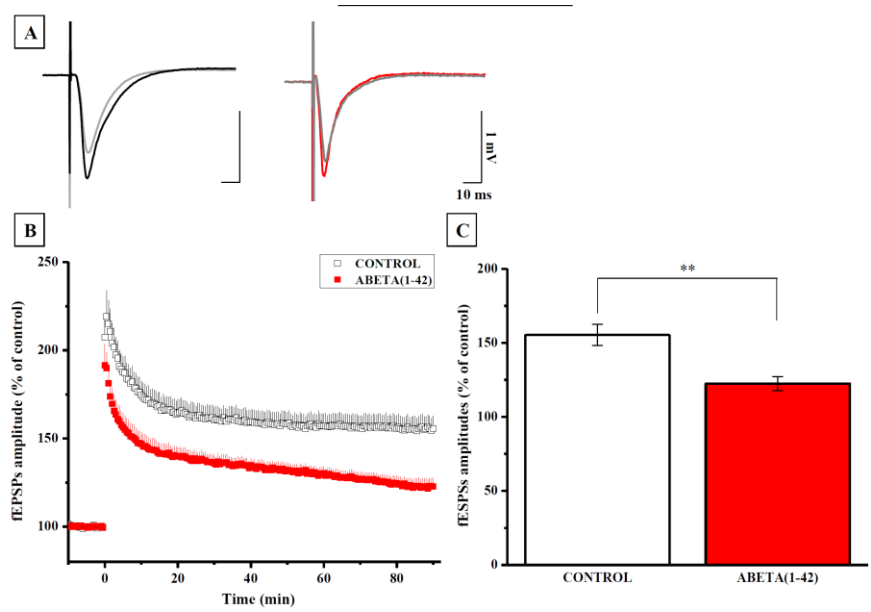

Figure 1: Every batch of Abeta preparation was verified for having LTP impairing effect. (A) exemplar fEPSPs before (gray) and after evoking LTP (black for control, red for Abeta treated slices). (B) Abeta preparations used for oscillation studies reliably impair LTP. (C) The level of fEPSP amplitude change 90 min after theta burst stimulation. ** ≤0.01; Student's t-test.

Oscillations in the hippocampal slice preparations were evoked by 3 different compounds: DHPG, kainic acid and carbachol. These evoked activities shared similar power spectra, having robust compounds in the gamma (20-35 Hz) range.

- **Subtask 1**: Is there a difference in the effect of Aβ1-42 on the oscillations being either spontaneous or evoked by different compounds? Descriptive study.

Spontaneous oscillations were observed in a subset of slices. The power spectra of these activities were not significantly different from that of the evoked oscillations, but unfortunately this activity was proved to be temporary. We could record spontaneous network activity reliably for up to 1.5 h, therefore we decided not to test the effect of Abeta on these oscillations.

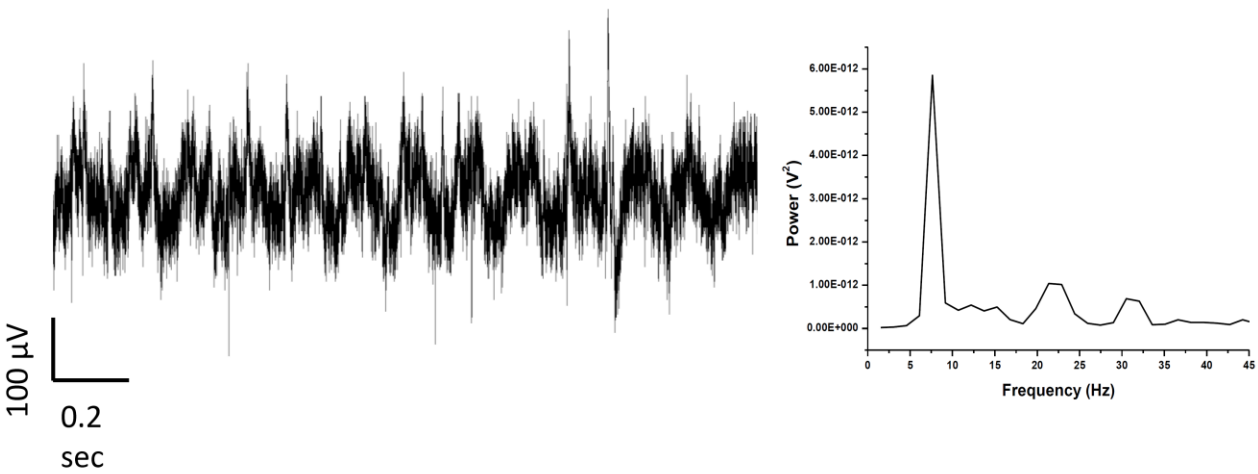

Figure 2: Transient spontaneous oscillation emerged in a subset of slices. The activity had a prominent peak in the power spectrum at around 6 Hz.

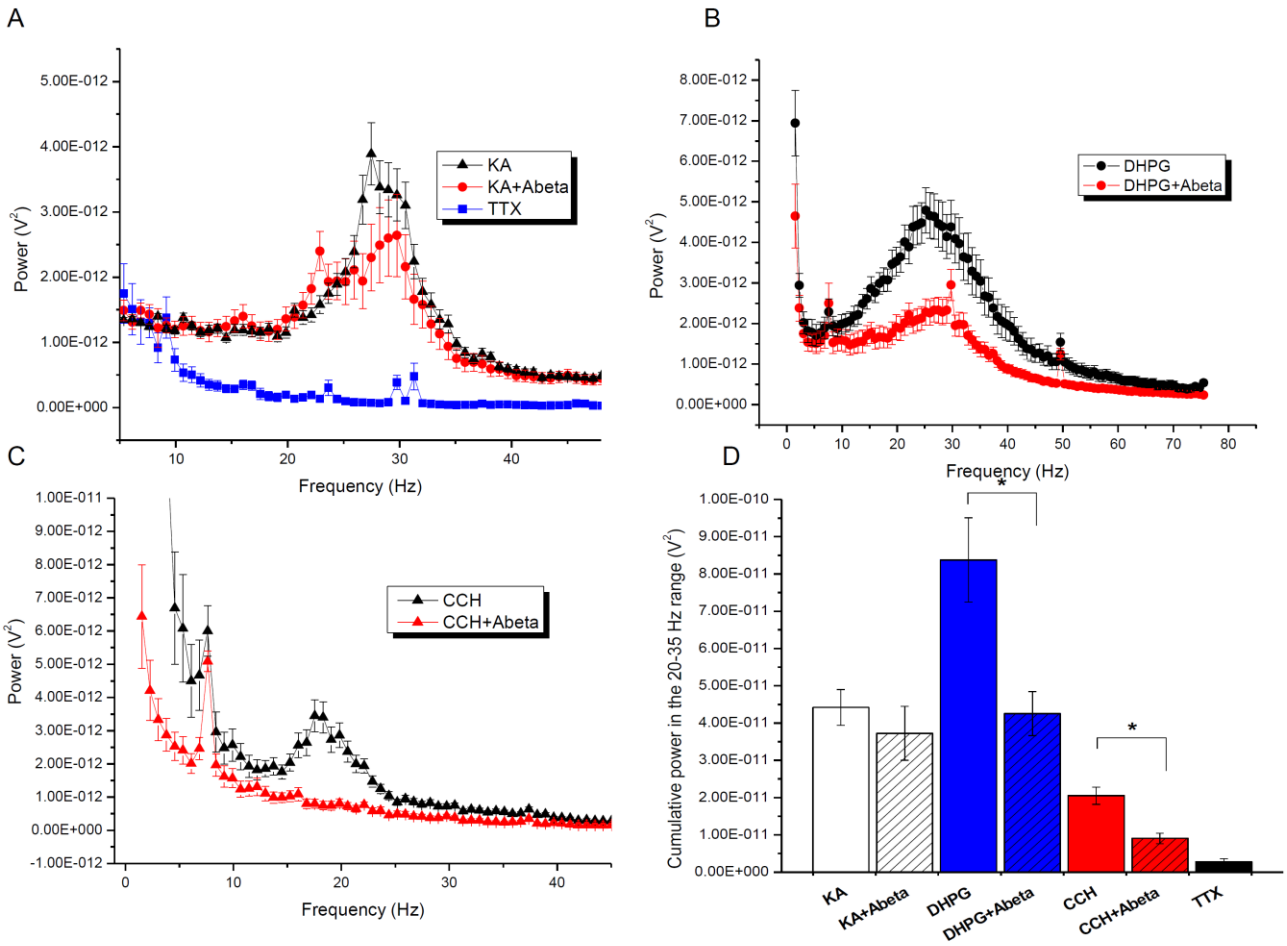

Figure 3: Power spectra of evoked oscillations before (black) and after Abeta application (red). Tetrodotoxin (TTX, blue) abolished oscillatory activity. (D) Cumulative power of the gamma range. *≤0.05, t-test

We have found that Abeta impairs DHPG and CCH evoked oscillations, but leaves KA-induced activity unchanged. These results suggest that Abeta interferes with mGluRs and cholinergic receptors.

- **Subtask 2**: Among the CA regions, CA1 or CA3 shows greater sensitivity to Aβ1-42? Descriptive study.

In theory, the multi-electrode array chip we used allows the simultaneous recording from CA1 and CA3. We did, however, rarely observed activity from both CA1 and CA3. This could be due to mechanical problems, since the slice could not be easily positioned to the electrodes in a way that both regions could be recorded. Preliminary results of a few slices suggest that the effect of Abeta does not differ in the two regions. The results we present here are from the CA1 region.

- **Task 2:** How different Aβ conformations (aggregates) interact with sharp wave oscillation, correlate of memory consolidation, in wild-type murine hippocampal slices? A descriptive study.

In a subset of slices, SPW-R like events emerged spontaneously. Sharp wave/ripple complexes could be detected immediately in the str. pyramidale and str. radiatum proximale after placing the slice into the recording chamber. Spontaneous oscillatory activity was detected in all of the recorded slices (n=11). The laminar profile and peak frequency of the SPW-Rs were similar to SPW-Rs recorded in vivo. The peak frequency of ripples was between 170-190 Hz, with a mean of 186±0.1 Hz. The occurrence of SPW-R events continuously decreased during the recording reaching 60% of the initial value after 4h of recording in the saline treated slices. Slices treated with Abeta showed an accelerated

decrease of SPW-R occurrence, reaching 50% after 4h of recording. Interestingly, the amplitude of SPW-R events increased during the first 1 h recording episode in the saline treated slices, but not in the Abeta treated slices.

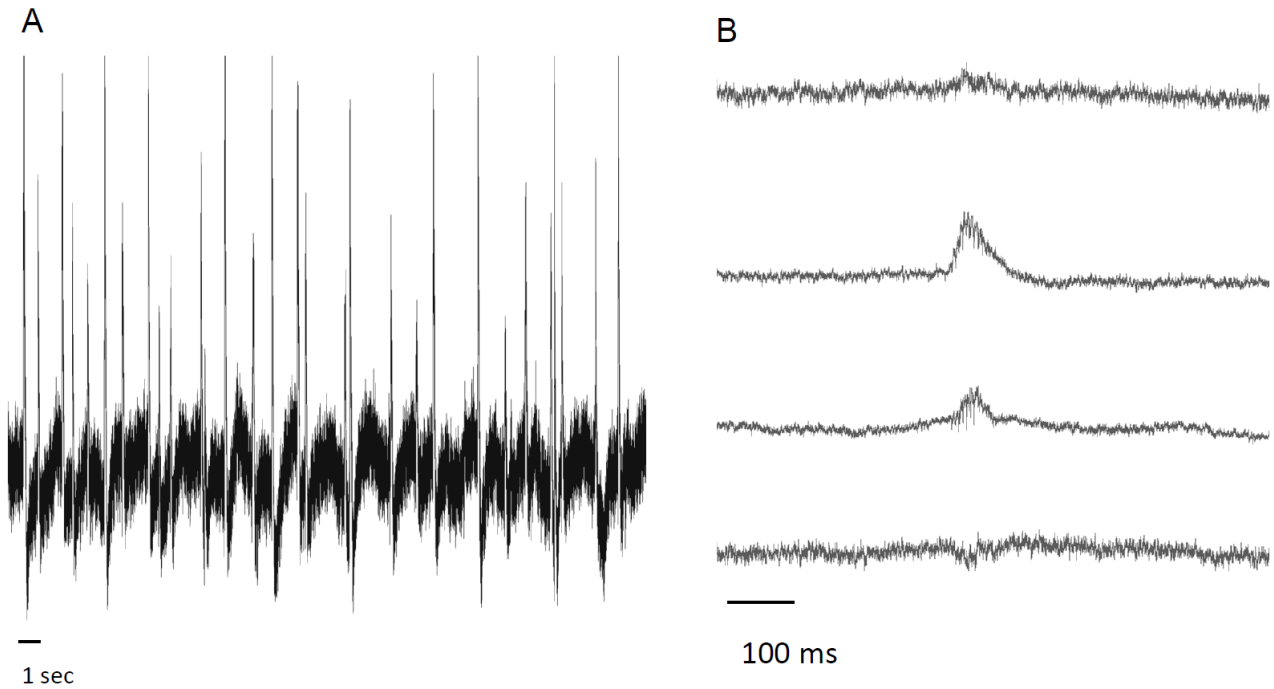

Figure 4: Spontaneous SPW events were observed in a subset of slices (A). The laminar profile of SPWs in vitro was similar to that of the in vivo SPWs (B).

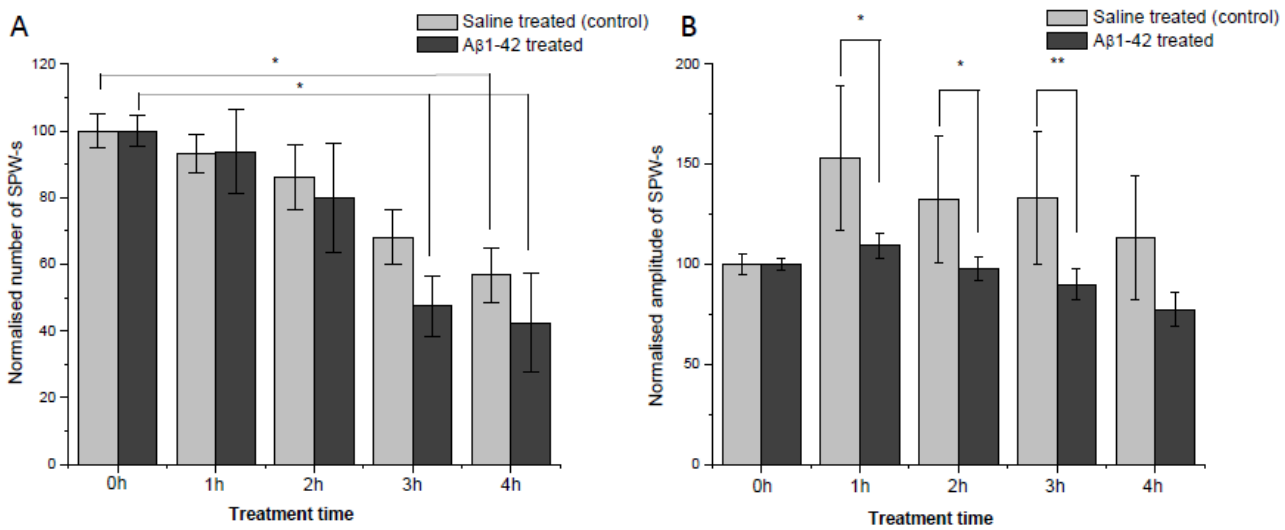

Figure 5: (A) The number of SPW-R events decreased continuously during the 4g long recording session. This decrease was accelerated in Abeta treated slices. (B) The amplitude of SPW-R events increased during the first hour of recording, and remained relatively stable for up to 4 h. In contrast, Abeta treated slices showed no such enhancement of SPW-R amplitude. *≤0.05, **≤0.01, t-test.

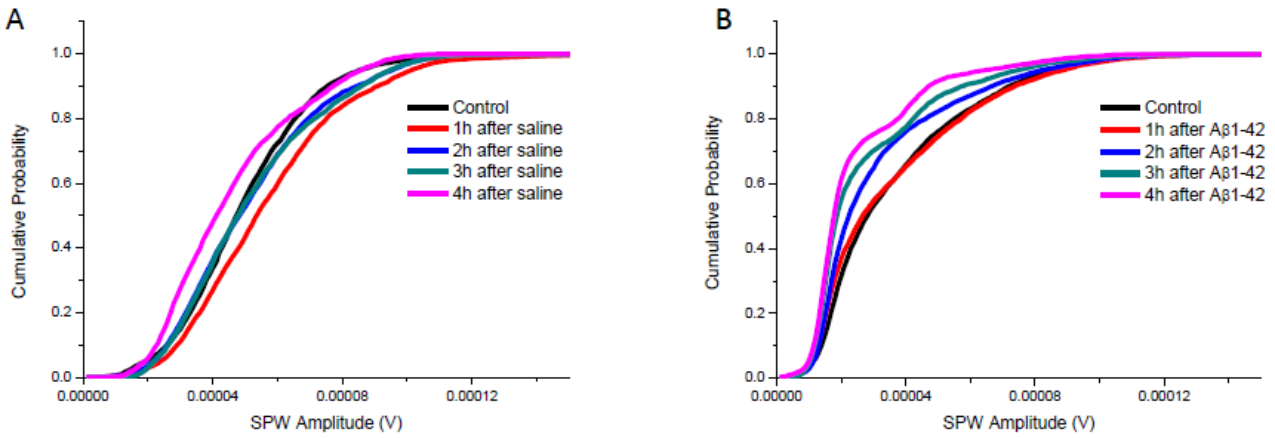

Figure 6: Cumulative probabilities of SPW amplitudes in saline treated (A) and in Abeta treated (B) slices. The initial increase and the following decline of SPW amplitudes in A is evident, while Abeta treated slices do not show the initial surge of excitation.

Oscillations in vivo

- Task 3: How different Aβ conformations (aggregates) interact with the firing patterns of hippocampal cells during specific LFP oscillation periods in anaesthetized rats?

The effects of Abeta on theta-coupled firing activity in vivo

For in vivo recordings, we have used urethane anaesthetized rats. Single units and LFP were recorded from the CA1 using a carbon-fiber microelectrode, which was equipped with microcapillaries for compound ejection. Theta activity was evoked by a brief tail pinch. Figure 7 shows a typical theta activity period after tail-pinch. Only well separable spikes were included into the analysis. Having recorded 4-5 theta epochs in each recording session, Aβ1-42 was applied. We have analyzed the frequency distribution of theta oscillations before and after Aβ1-42 application, and found no significant difference (mean frequency 3.7±0.5 Hz before, and 3.8±0.4 Hz after; n=6; see figure 8 for representative data).

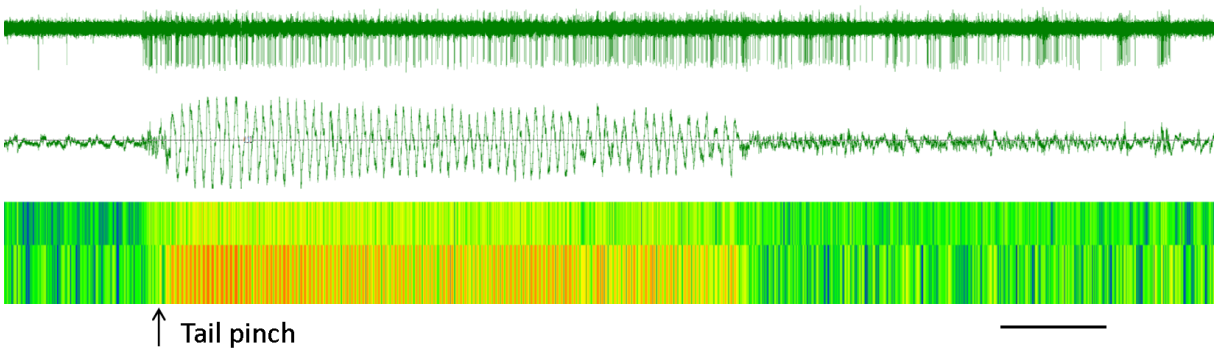

Figure 7: Theta activity evoked by a brief tail-pinch. The upmost trace shows single-unit activity filtered between 300-3000 Hz. The middle trace is the raw recording (0.1-1 kHz). The bottom trace is the sonogram showing prominent theta activity after tail-pinch. Calibration bar is 5 sec.

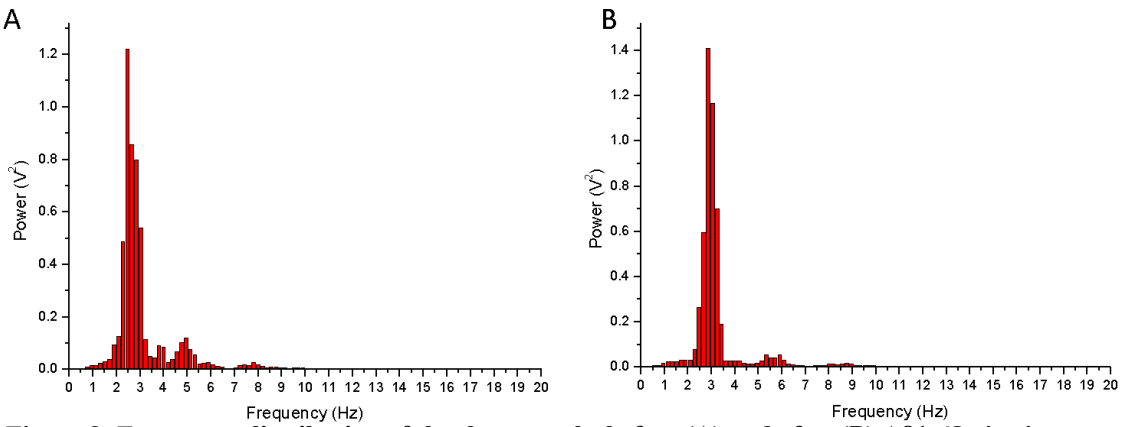

Figure 8: Frequency distribution of the theta epochs before (A) and after (B) Aβ1-42 ejection.

- Subtask 1: What is the effect of Aβ1-42 on the theta-coupled firing patterns of various CA1 cells? Descriptive study

The recorded spikes were presumably from pyramidal cells based on the morphology of action potential (half-width and average firing rate). Around 60% of the cells showed firing preference to any phase of the ongoing oscillation during theta activity. Most probably some NMDA leaked from the capillary attached to the carbon-fiber, which excited the recorded neurons, therefore they were unable to follow the periodic excitation-inhibition population activity (lack of phase preference during theta). However, at those cells which exhibited a clear phase preference, applying Abeta impaired this coordinated activity (Figure 9). Control units having received saline kept their phase preference. We have shown previously that NMDA evoked firing rate increases after Abeta ejection. We confirmed this finding in a few recordings to show that Abeta really reached the recorded neuron (figure 9). The spontaneous firing rate (not evoked with NMDA ejection) did not change. Autocorrelation of the sorted spikes showed firing activity that followed the frequency of the ongoing oscillation. This pattern has changed drastically following Aβ1-42 ejection (see fig 9).

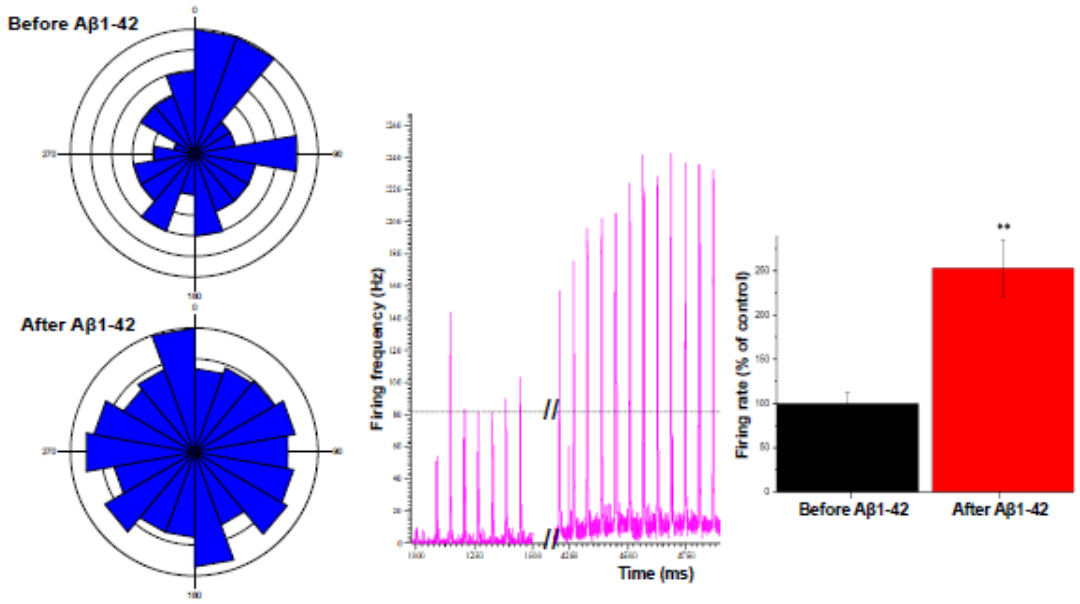

Figure 9: (A) Circular diagrams showing phase locked firing during theta of a principal cell before (upper) and after (lower) Abeta ejection. NMDA evoked firing rate increased after Abeta application (purple diagram and columns) ** ≤0.01; t-test

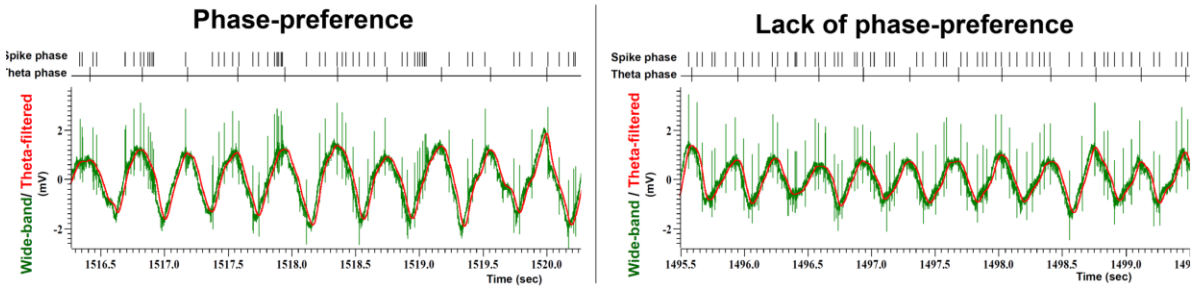

Figure 10: Representative wide-band recordings showing a phase-locked firing activity (right panel), which is impaired by Abeta (left panel).

- Subtask 2: How Aβ1-42 alters sharp-wave coupled firing of CA1 cells? Descriptive study

We recorded SPW-R events from the CA1 of anaesthetized rats. We realized that the sorting of extracellularly recorded single spikes is challenging during high frequency ripple activity. The large and fast deviations in the LFP mask action potentials, which renders the reliable spike sorting almost impossible. A possible solution for this problem is the use of either an intracellular electrode, or a combined dual electrode having a high and a low impedance part for recording single-units and LFP respectively. Unfortunately we have no access to such methodology.

Summary and conclusions

Within the framework of the present grant, we have successfully set up an in vitro model system for investigating various hippocampal oscillations, which could serve as a platform for future studies. We think that combining these studies with behavioral tests will offer a strong preclinical tool for studying the mechanisms of memory decline.

In collaboration with the Department of Medical Chemistry we have developed a protocol for reliable preparation of oligomeric Abeta species. This preparation was characterized by Western-blots, dynamic light scattering and transmission electronmicroscopy.

We determined the effects of synaptotoxic Abeta oligomers on induced hippocampal oscillations. Our findings indicate a strong functional interference between Abeta oligomers and mGluR pathways and nicotinic receptors. Indeed, there is strong evidence that Abeta peptides inhibit alpha7 nAcHRs (D'Andrea and Nagele, 2006). Moreover, Abeta was also shown to affect mGluR function (Um et al., 2013).

Hippocampal SPW activity is thought to play key roles in the consolidation of memory traces (Schwindel and McNaughton, 2011). One of the most prominent symptoms of Alzheimer's disease is the early occurrence of impaired memory, which might stem from the inhibited consolidation process. We have shown that Abeta decreases the occurrence and the amplitude of SPW events in vitro. This result is in contrast to a recent report, which shows that there is no difference in the SPW events generated spontaneously in slices between AD modelling transgenic mice and wild-type counterparts (Hermann et al., 2009; Zhang et al., 2013). This contradiction might be due to the differences in the model systems we and the authors have used. We applied a characterized oligomeric Abeta solution, while the other study have investigated a transgenic animal which expresses a wide range of Abeta species.

The temporal coordinated firing activity of hippocampal cells are crucial is memory encoding and consolidation. Network oscillations provide a framework for the coherent function of the neuronal network. Using anaesthetized animals we provided evidence that Abeta inhibits the precise phase-locked firing mode of CA1 pyramidal cells. Similar results were obtained using AD modelling transgenic mice, where the activity of the so called place-cells are impaired as the plaque load increased in the hippocampus (Cacucci et al., 2008). Also, exogenous Abeta was shown to impair the theta-locked firing pattern of septal cells (Villette et al., 2010), but to our knowledge, this is the first report that shows a direct impact of Abeta oligomers on CA1 theta-coupled firing.

Results beyond the original scope of the grant:

We have identified a possible mechanism by which Abeta oligomers induce synaptotoxicity and network dysfunction. We have found that Abeta impairs glutamate recycling in the synapse, which leads to glutamate spillover and activation of the extrasynaptic NMDA receptors. An inhibitor of glutamate transporters, TBOA mimics the effects of Abeta. Both compounds impairs LTP, massively enhances spontaneous spiking activity in hippocampal slices without affecting evoked fEPSPs. Ifenprodil, an antagonist of NR2B subunit of the NMDARs found predominantly at the extrasynapse, prevented Abeta induced LTP damage.

Interestingly, if Abeta or TBOA was applied following LTP induction, then these compounds failed to damage LTP or induce spiking activity enhancement. We hypothesize that extrasynaptic NR2B subunits are recruited to the synaptic compartment after LTP induction, decreasing the extrasynaptic/synaptic NMDAR ratio. There are a few data available in the literature showing that NR2D, another perisynaptic NMDAR subunit is translocated into the synaptic domain after LTP induction, but this has not been described from NR2B. Identification of the molecular players of this pathway might lead to new targets against AD and other neurodegenerative condition associated with Glu spillover.

Together these findings suggest that Abeta impairs glutamate recycling, which most probably leads to Glu-spillover and activation of perisynaptic NR2B receptors. Based on these results we propose that eliminating the excess extracellular Glu might be protective against Abeta and in turn against AD. An attractive way for "mopping up" glutamate from the perisynaptic space is the use of glutamate scavenger enzymes, GOT ad GPT. Indeed, our preliminary results are promising. GPT rescues Abeta induced LTP damage. We have submitted a grant application to OTKA for following up this hypothesis.

Tasks that we were unable to perform during the grant period

- Task 4: How network oscillations are altered in slices from Aβ1-42 overexpressing mice?

In the original grant application, we planned to investigate the oscillations of AD modelling transgenic animals (APPSwe/PS1) using hippocampal slices. We have started to perform these experiments, but we are still at the beginning of this task. Unfortunately, we were unable to gather additional financial sources for purchasing the necessary number of mice.
We believe that this shortcoming is not limiting our results, and hope that the reviewers will appreciate the additional results we gathered during the period of this grant. We plan to perform task 4 as soon as we receive funding. Additional grant proposals had been submitted that would cover the expenses.

Publications supported by the present grant

Full Papers (first and last authorship is marked with bold):

1. Horváth J, Szögi T, Müller G, **Szegedi V**. The anxiolytic buspirone shifts coping strategy in novel environmental context of mice with different anxious phenotype. Behav Brain Res. 2013 Aug 1;250:32-8.

2. Tóth ME, Szegedi V, Varga E, Juhász G, Horváth J, Borbély E, Csibrány B, Alföldi R, Lénárt N, Penke B, Sántha M. Overexpression of Hsp27 ameliorates symptoms of Alzheimer's disease in APP/PS1 mice. Cell Stress Chaperones. 2013 Nov;18(6):759-71.

3. Lénárt N, Szegedi V, Juhász G, Kasztner A, Horváth J, Bereczki E, Tóth ME, Penke B, Sántha M. Increased tau phosphorylation and impaired presynaptic function in hypertriglyceridemic ApoB-100 transgenic mice. PLoS One. 2012;7(9):e46007

4. Fülöp L, Mándity IM, Juhász G, Szegedi V, Hetényi A, Wéber E, Bozsó Z, Simon D, Benkő M, Király Z, Martinek TA. A foldamer-dendrimer conjugate neutralizes synaptotoxic β-amyloid oligomers. PLoS One. 2012;7(7):e39485.

5. Barkóczi B, Juhász G, Averkin RG, Vörös I, Vertes P, Penke B, **Szegedi V**. GluA1 phosphorylation alters evoked firing pattern in vivo. Neural Plast. 2012;2012:286215. doi: 10.1155/2012/286215.

6. **Szegedi V**, Juhász G, Zhang X, Barkóczi B, Qi H, Madeira A, Kapus G, Svenningsson P, Spedding M, Penke B. Tianeptine potentiates AMPA receptors by activating CaMKII and PKA via the p38, p42/44 MAPK and JNK pathways. Neurochem Int. 2011 Dec;59(8):1109-22

Cumulative impact factor: 18.794

Papers in preparation

2 papers, describing the effect of Abeta on SPW-R activity in vitro and on theta-coupled firing activity in vivo

Materials and methods in brief

Animal care and handling

The mice (CFLP, Animal Breeding Facility, University of Szeged) are kept and the experiments are conducted in conformity with Council Directive 86/609/EEC, the Hungarian Act of Animal Care and Experimentation (1998, XXVIII).

Hippocampal slice electrophysiology

Using standard procedures, 350 µm thick transverse hippocampal slices are prepared from the brain of 3 months old mice using a McIlwain tissue chopper (Campden Instruments, Loughborough, UK). Slices are incubated in standard artificial cerebrospinal fluid (ACSF) at ambient temperature for 60 min, which is constantly gassed with 95% O_2–5% CO_2. ACSF contains (in mM): NaCl, 130; KCl, 3.5; $CaCl_2$, 2; $MgCl_2$, 2; NaH_2PO_4, 0.96; $NaHCO_3$, 24; D-glucose, 10 (pH 7.4). Individual slices are transferred to a 3D-MEA chip with 60 tip-shaped and 60-µm-high electrodes spaced by 100 µm (Ayanda Biosystems, S.A., Lausanne, Switzerland). The slice is continuously perfused with oxygenated ACSF (3 ml/min at 34 °C) during the whole recording session. Data are recorded by a standard, commercially available MEA setup (Multi Channel Systems MCS GmbH, Reutlingen, Germany).

Theta activity in anaesthetized rats

Extracellular single-unit recordings were made in urethane anesthetized male Wistar rats weighing between 300–380 g. The head of the animal was mounted in a stereotaxic frame, the skull was opened above the hippocampus (a-p:–3.8 mm from bregma; lat: ±2 mm either side from the midline), and the dura mater was carefully removed. Single unit activity was extracellularly recorded by means of a low impedance (< 1 MΩ) 7 µm carbon fiber-containing microelectrode from the hippocampus between the depths of 2 to 4 mm, and drugs were delivered from the surrounding outer barrels. The action potentials were amplified by a model 1700 differential amplifier (A-M Systems, Sequim, WA) and monitored with an oscilloscope. Filter bandpass frequencies were 0.1 to 10000 Hz. The amplified signals were sampled and digitalized at 50 kHz frequency. Spikes were sorted using the Spike2 software package (Cambridge Electronic Design Limited, Cambridge, UK). Collection of experimental data was performed by a multifunction instrument control and data acquisition board (CED, micro3, 1401) installed in a personal computer. A multibarrel electrode affixed to the recording electrode was used for the iontophoretic ejection of 100 mM NMDA Na in 100 mM NaCl (pH 8). NMDA was ejected at negative iontophoretic current ranging from 2 to 100 nA for 5 sec. Retaining current of opposite direction between 2–21 nA was used. Aβ1-42 (50 µM) was ejected at -386 nA for 60 sec.

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Zhang, X., Herrmann, U., Weyer, S.W., Both, M., Muller, U.C., Korte, M., Draguhn, A., 2013. Hippocampal network oscillations in APP/APLP2-deficient mice. PloS one. 8**,** e61198.