Title: Differential Effect of Amyloid Beta1-40 on Short-Term and Long-Term Plasticity in Dentate Gyrus of a Rat Model of Alzheimer's Disease

Authors: Javad Fahanik-Babaei\textsuperscript{1}, Tourandokht Baluchnejadmojarad\textsuperscript{1,2,}\textsuperscript{*}, Mehrdad Roghani\textsuperscript{3}

\textsuperscript{1} Physiology Research Center, Iran University of Medical Sciences, Tehran, Iran.

\textsuperscript{2} Department of Physiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.

\textsuperscript{3} Neurophysiology Research Center, Shahed University, Tehran, Iran.

* Corresponding author:

Tourandokht Baluchnejadmojarad, Prof.

Department of Physiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.

Tel: +98-21-88058709, Fax: +98-21-88058709, e-mail: tmojarad@yahoo.com

To appear in: Basic and Clinical Neuroscience

Received date: 2018/05/26

Revised date: 2019/05/17

Accepted date: 2018/07/24
ABSTRACT

Introduction: Synaptic plasticity is inappropriately affected in neurodegenerative diseases including Alzheimer’s disease (AD). In this study, we examined the effect of intrahippocampal amyloid beta (Aβ1-40) on dentate gyrus long-term potentiation (LTP) and presynaptic short-term plasticity in a rat model of AD.

Methods: The experimental groups in this research study included control with no treatment, sham-operated receiving the vehicle (normal saline), and Aβ-lesioned. For modeling AD, aggregated Aβ1-40 (10 μg/2 μl on each side) was injected into the hippocampal CA1. Three weeks later, population spike (PS) amplitude and slope ratios were determined at different inter-pulse intervals (IPI) of 10, 20, 30, and 50 ms as a valid indicator of short-term presynaptic facilitation and/or depression. In addition, PS amplitude and slope was taken as an index of long-term synaptic plasticity after application of high frequency stimulation (HFS) to induce LTP in medial perforant-dentate gyrus pathway.

Results: No significant differences were noted amongst the experimental groups regarding fEPSP slope and paired-pulse indices as indicators of short-term plasticity. In contrast, fEPSP slope and PS amplitude significantly decreased following application of HFS in amyloid beta-injected group. In addition, there was no significant difference between the control and sham-operated groups regarding the mentioned parameters.

Conclusion: Findings of this study clearly demonstrated that microinjection of amyloid beta1-40 into CA1 could impair LTP in dentate gyrus but could not modify short-term plasticity.

Keywords: Alzheimer’s disease, Amyloid beta, Synaptic plasticity
1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease in the elderly with high incidence in the human society that at its late stages finally leads to dementia and death (Crous-Bou, Minguillon, Gramunt, & Molinuevo, 2017; Lane, Hardy, & Schott, 2018). Brain extracellular deposition of amyloid β (Aβ) peptide plaques is the key pathologic hallmark of patients with AD (Magalingam, Radhakrishnan, Ping, & Haleagrahara, 2018). Aβ due to its neurotoxic properties is responsible for neuronal degeneration and synaptic loss in AD (Reiss, Arain, Stecker, Siegart, & Kasselman, 2018; Vargas, Cerpa, Munoz, Zanlungo, & Alvarez, 2018). Accumulating evidences in experimental models of AD and in affected patients strongly indicate that Aβ alone even before development of plaques could cause neuronal injury and death (Parihar & Brewer, 2010). Synaptic plasticity is the capability of synaptic structures to alter the efficacy and/or strength of synaptic transmission. In this respect, alterations of synaptic efficacy are suggested to play a key role in learning and memory processes (Esmaeili Tazangi, Moosavi, Shabani, & Haghani, 2015). Earlier investigators have shown two kinds of synaptic plasticity, i.e. a short-term plasticity (STP) using paired-pulse stimulation protocol (Moghaddam, Baluchnejadmojarad, Roghani, Goshadrou, & Ronaghi, 2013; Ohno et al., 2011) at appropriate inter-stimulus intervals (ISIs) that reflects the release probability of the presynaptic neurons with a spontaneous decay of conductance level, and a long-term plasticity or potentiation (LTP) using a tetanic stimulation protocol (high frequency stimulation, HFS) that is responsible for the memory formation in humans (Ohno et al., 2011). Regarding STP, it can occur as paired-pulse facilitation (PPF) or paired-pulse depression (PPD) and their generation in the hippocampus is strongly related to the location of stimulation, stimulus strength, and the time between the two successive stimuli (Ohno et al., 2011). PPF is due to inward calcium current through presynaptic structures (Nicoll & Malenka, 1999; Staubli, 1992) and PPD is attributed to desensitization of AMPA receptors in addition to modulation of GABAergic system (G. L. Li, Vigh, & von Gersdorff, 2007; Ohno-Shosaku et al., 2011; Unichenko, Myakhar, & Kirischuk, 2012). Experimental studies have demonstrated that loss of cognitive performance in AD are somewhat due to alterations of presynaptic functions and disability of synapse to act normally (Fernandez-Fernandez, Rosenbrock, & Kroker, 2015; Lee et al., 2012; Yang, Wang, Wang, Justice, & Zheng, 2009). In moderate deterioration of cognitive ability, the number of active synapses in the hippocampus
decreases with counterbalancing through increasing the dimension of synapses. Finally, with the progression of the disease, the number of synapses relative to neuronal population decreases and this imbalance is well related to memory loss (Giralt et al., 2017; Kawano et al., 2017; Vilella et al., 2017). However, the detailed pathogenic mechanisms that are responsible for the occurrence of these changes are not well-defined. Hippocampal long-term potentiation (LTP) is a well-defined kind of synaptic plasticity that its deficit could lead to memory decline (Babri et al., 2014; Freir, Costello, & Herron, 2003). Until now, the effect of amyloid beta on short-term presynaptic facilitation and/or depression has not been well-delineated considering different forms of amyloid beta (with varying degrees of neurotoxicity) and different sites of injection (i.e. intracerebral or intracerebroventricular), this study was designed to examine the effect of intrahippocampal amyloid beta (Aβ1-40) on dentate gyrus long-term potentiation and presynaptic short-term plasticity in a rat model of AD using HFS and paired pulse stimulation protocols.

2. Methods
2.1. Animals

Male albino Wistar rats were obtained from laboratory animal breeding center of Iran University of Medical Sciences (IUMS), Tehran, Iran (age: 11-13 weeks; body weight: 250-290 g). The rats were housed in Plexiglas cages with woodchip bedding in groups of 3-4 per cage at standard room temperature (21-23°C) and a humidity of 40-50% under 12 h light-dark cycle (the light period started on 07:00 a.m.). Food and water were freely provided. All practical interventions regarding animals and their care were done in compliance with guidelines stipulated by National Institutes of Health of USA for the care and use of experimental animals and those of Iran University of Medical Sciences (IUMS; Tehran, Iran).

2.2. Materials

Amyloid β peptide fragment 1-40 (Aβ1-40) was purchased from Sigma (USA).

2.3. Aβ (1-40) preparation
Amyloid beta peptide (Ab1-40) was dissolved in normal saline at a concentration of 2 mg/ml and was stored at -20°C. Aggregation of beta-amyloid was done by in vitro incubation at 37°C for 72 h.

2.4. Experimental procedure

The rats (n=18) were randomly allocated and grouped into 3 experimental groups including control, sham, and Aβ1-40. Sham animals received an injection of an equivalent volume of normal saline. On the day of surgery, animals (n = 6 per group) were anesthetized with an i.p. injection of ketamine-HCl (100 mg/kg) and xylazine (10 mg/kg). The rats were fixed in a stereotaxic apparatus and according to Paxinos’ brain atlas, the scalp was incised at midline and small burr holes were made at appropriate sites, bilaterally, (AP -3.8, ML ± 2.2, DV -2.7). Then, Aβ (1-40) solution (10 μg/2μl) was bilaterally injected into dorsal hippocampus over 5 min by a Hamilton microsyringe. Sham-operated rats received vehicle solution. The skin was then sutured and the animals were maintained to recover in a warm box before returning to their home cages.

2.5. Electrophysiological study

Three weeks following intracerebral microinjection of Aβ or vehicle, rats were deeply anesthetized with urethane (1.7-1.8 g/kg b.w., i.p.) and their heads were placed in a stereotaxic frame. A homeothermic device was used to sustain body temperature at 36.5°C. Then, the cranium was exposed and two burr holes were drilled for insertion of stimulating and recording electrodes. A bipolar stainless steel stimulating electrode with a diameter of 0.125 mm (A-M Systems, USA) was placed in the medial perforant pathway (4.2 mm lateral to the lambda, 3.2 mm ventrally) and a stainless steel recording electrode was placed in the dentate gyrus with the maximum response (3.8 mm posterior and 2.2 mm lateral to the bregma). Evoked field potentials were recorded from dentate granule cells after stimulation of medial perforant pathway. Recording of field potentials began at least 15 minutes following insertion of the stimulating and recording electrodes. Applied stimuli were biphasic square waves (a width of 200 ms). Extracellular field potentials were amplified 1000x, digitized at 10 kHz, and filtered at a band of 0.1 Hz-10 kHz with the aid of a differential amplifier. Signals were passed through A/D interface (Science Beam Co., Iran) to a computer and data were analyzed using e-probe.
software. Stimulation intensity was adjusted at a level to evoke 40% of the maximal response (field excitatory post-synaptic potential (fEPSP) and population spike (PS)). In addition, PS amplitude was measured as the average of the potential difference between the peak of the first positive wave and the peak of the first negative deflection and the potential difference between the peak of the second positive wave and the peak of the first negative deflection. Meanwhile, fEPSP slope as an index of synaptic efficacy was determined as the maximum slope between initial point of fEPSP and the first positive wave.

2.5.1. Input/output functions

Input-output (I/O) functions were obtained by graded variation of the stimulus intensity (100-1100 µA) for assessment of synaptic efficacy before LTP induction. fEPSP and PS were triggered in dentate gyrus using 0.1 Hz stimulation and five evoked responses were averaged at each current intensity.

2.5.2. Paired-pulse response

After recording for 40 min, paired pulse depression/facilitation was determined. The response to paired-pulse stimulation was subsequently recorded, delivered at 40% of maximal stimulus intensity with an inter-pulse intervals (IPI) of 10, 20, 30, and 50 ms. For each IPI points, 10 consecutive evoked responses were averaged. The population spike amplitude ratio [ratio of second population spike amplitude to first population spike amplitude; PS2/PS1%, paired pulse index (PPI)] and the fEPSP slope ratio [second fEPSP slope/first fEPSP slope at percent; fEPSP2/fEPSP1%] were determined at various inter-stimulus intervals.

2.5.3. Long-term potentiation (LTP)

After stable baseline recording for at least 30 min, LTP was induced by delivery of high-frequency stimulation (HFS; 10 trains of 15 pulses at 200 Hz separated by 10 s) and after the tetanic stimuli, the baseline stimulation was resumed and recording continued for at least 90 min and 5 consecutive evoked responses were averaged at stimulus intervals of 10 s.

2.6. Data analysis
All results are shown as means±S.E.M. For electrophysiological comparison, data was analyzed using one way ANOVA and one-way repeated measure ANOVA and Tukey post-hoc test. In addition, a p value less than 0.05 was considered significant.

3. Results

3.1. Input/output (I/O) functions

Stimulus-response curves were obtained from dentate gyrus following stimulation of medial perforant pathway to assess the synaptic potency (Fig. 1). In this respect, one-way repeated measure ANOVA showed that PS amplitude and fEPSP slope prior to application of paired pulse and high frequency stimulation (HFS) protocols did not significantly differ between the groups (F(2,14)=1.94, p>0.05).
**Fig. 1:** Input–output curves shown as fEPSP slope and PS amplitude in dentate gyrus. Each point represents data obtained from 6 rats. Specimen recordings showed changes in baseline recording and LTP recording in 60 min after HFS. Each recording is the average of 10 consecutive recordings at 100 s with an interval of 10 s. There was no statistically significant difference amongst the groups.

### 3.2. Paired pulse responses

As shown in Fig. 2, the paired pulse protocols were applied to medial perforant pathway and recording was obtained from dentate gyrus at different inter-pulse intervals (IPIs) consisting of 10, 20, 30, and 50 ms. One-way repeated measure ANOVA showed no significant differences regarding EPSP slope index and paired-pulse index amongst the experimental groups.
Fig. 2: The effect of Aβ on paired pulse responses in hippocampal dentate gyrus at intervals of 10, 20, 30, and 50, as shown by fEPSP slope ratio (second response/first response ratio) and PS amplitude ratio (second response/first response ratio) (n=6 per group) and traces recorded at
dentate gyrus at an inter-stimulus interval of 50 ms. There was no statistically significant differences amongst the groups. recording is the average of 10 consecutive population spikes evoked by paired stimuli in 100 s.

3.3. Long-term potentiation (LTP)

Related data have been presented in Figure 3. As it is evident, LTP responses were recorded from dentate gyrus from different groups. Statistical analysis for different time points before application of HFS did not indicate a significant difference (baseline data for fEPSP slope and PS amplitude) amongst the groups. In other words, intrahippocampal bilateral microinjection of aggregated amyloid beta1-40 did not significantly affect baseline responses. In contrast, one-way repeated measure ANOVA indicated a significant difference amongst the groups for fEPSP slope (F(2,15)=1.15.3, p<0.01) and PS amplitude (F(2,15)=13.8, p<0.01) following application of HFS. In this respect, fEPSP slope was significantly lower in amyloid beta group relative to control group at all time points (F(2,15)=11.7,13.9, p<0.05-0.01). A similar significant difference was also found out for PS amplitude after application of HFS amongst the groups (F(2,15)=13.5, p<0.01). In this regard, PS amplitude was depressed and significantly lower in amyloid beta group as compared to control group at all time points (F(2,15)=14.7, p<0.01).
Fig 3: The effect of Aβ on LTP in hippocampal dentate gyrus using HFS as shown by fEPSP slope and PS amplitude and representative traces of evoked responses in the dentate gyrus of the rat hippocampus following stimulation of the medial perforant path. (n=6 per group). * p<0.05, ** p<0.01 (versus control)
4. Discussion

The main objective of this research study was to exactly determine differential effect of amyloid beta1-40 on dentate gyrus short-term and long-term plasticity in a rat model of AD through intracerebral microinjection of aggregated Aβ1-40. Our findings demonstrated that long-term synaptic plasticity as LTP is severely impaired following amyloid beta with no significant change of short-term plasticity as determined by application of a paired-pulse protocol. In this respect, we observed that intrahippocampal injection of Aβ1–40 severely dampens fEPSP slope and PS amplitude following execution of LTP protocol. Our finding regarding suppression of LTP were in agreement with earlier reports which have shown that exposure to soluble oligomers of Aβ could lower neuronal excitability and related synaptic plasticity and LTP phenomena in hippocampal regions including dentate gyrus (Wang et al., 2002). On interest, such oligomers do not significantly affect long-term depression (LTD) aspect related to memory processes (Wang et al., 2002). In this regard, amyloid beta exposure could contribute to the pathogenesis of AD both by impairing LTP and memory formation at the cellular level and by developing neuroplasticity imbalance in addition to impaired capacity for neurons to recover (Wang et al., 2002). In contrast, Li et al in 2009 showed that oligomers of amyloid beta could positively affect hippocampal LTD via disrupting neuronal uptake of the neurotransmitter glutamate (S. Li et al., 2009).

LTP is itself considered a major synaptic mechanism that is valuable for assessment of long-term synaptic plasticity in rats and mice. Post-tetanic LTP is postulated a physiological form of synaptic plasticity and its appearance in cortical and subcortical regions is considered as a valuable tool for assessment of learning and memory at cellular and/or molecular levels (Bliss & Collingridge, 1993). According to previous reports, LTP induction and maintenance is significantly disturbed after application of tetanic stimulation in animal models of AD (Lambert et al., 1998; Walsh et al., 2002) that is also consistent with our finding. Mechanistically, LTP is greatly dependent on NMDA receptors and is a widely-accepted mechanism for occurrence of synaptic plasticity via presynaptic release of the neurotransmitter glutamate and consequent depolarization of pos-synaptic target due to activation of NMDA receptors and ensuing inward
calcium currents. The neurotoxic peptide Aβ could target glutamate receptors and in this way exert its synaptotoxic effects. In this regard, earlier researches have shown that amyloid beta could decrease expression of NMDA receptors and this could decrease NMDA and AMPA receptor-mediated synaptic transmission through enhancing receptors endocytosis (Hsieh et al., 2006).

In addition, brain nicotinic acetylcholine receptors are strongly engaged in learning and memory process through inducing LTP (Drever, Riedel, & Platt, 2011; Maurer & Williams, 2017). Experimental evidences indicate that amyloid beta fragments could lead to cholinergic dysfunction and consequent cognitive decline in individuals with AD (Nordberg, 2001). Amyloid beta peptides could bind to some kinds of nicotinic receptors which leads to accumulation of intracellular Aβ and subsequent deficits in synaptic function (D'Andrea & Nagele, 2006), finally leading to lower release of acetylcholine as an excitatory neurotransmitter and this could negatively affect LTP. Furthermore, Aβ fragments could inhibit NMDA receptor-dependent synaptic neurotransmission partly through lowering inward calcium current via NMDA receptors, in this way reducing phosphorylation of calcium and calmodulin-dependent protein kinase II (CaMKII) and ensuing attenuation of LTP process (Zhao, Watson, & Xie, 2004). In this regard, Lisman et al in 2002 showed that CaMKII molecules are strongly involved in occurrence of LTP in hippocampal dentate gyrus (Lisman, Schulman, & Cline, 2002).

In this research investigation, we also assessed short-term plasticity in dentate gyrus through application of paired-pulse protocol. Paired-pulse ratio is correlated with presynaptic release of neurotransmitters and is regarded as short-term plasticity event (Colino, Munoz, & Vara, 2002; Fortune & Rose, 2002; Zucker & Regehr, 2002). Short-term plasticity is itself dependent on residual calcium in presynaptic terminals. Enhanced level of calcium following application of the first stimulus could elevate the probability of neurotransmitter release as a result of the application of the second stimulus (Debanne, Guerineau, Gahwiler, & Thompson, 1996). In addition, GABAergic inhibitory interneurons could affect excitability of hippocampal granular and pyramidal cells through feed forward and feed back circuits, in this way modulating short-term plasticity related to presynaptic regions (Jiang, Sun, Nedergaard, & Kang, 2000). In our research, amyloid beta1–40 exposure did not significantly affect PS amplitude and fEPSP slope
due to application of paired-pulse stimulation protocols at IPIs of 10, 30, 40, and 50 ms. Thus, it is reasonable to tell that reduction of PS amplitude and EPSP slope following HFS application is possibly unrelated to presynaptic function/activity and post-synaptic regions are affected to a greater degree following amyloid beta.

To conclude, findings of this study clearly demonstrated that long-term synaptic plasticity as LTP is severely impaired following amyloid beta with no significant change of short-term plasticity as determined by application of a paired-pulse protocol.

Acknowledgment
This study was part of a Ph.D. thesis project that was approved and financially supported by Physiology Research Center affiliated to Iran University of Medical Sciences (grant # 93-03-130-24998).

Conflict of interest: The authors declare no conflict of interests.
References


