

Research report

# Soluble oligomers of $\beta$ amyloid (1-42) inhibit long-term potentiation but not long-term depression in rat dentate gyrus

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## Abstract

The dementia in Alzheimer disease (AD) is usually attributed to widespread neuronal loss in conjunction with the pathologic hallmarks of intracellular neurofibrillary tangles and extracellular plaques containing amyloid (A $\beta$ ) in fibrillar form. Recently it has been demonstrated that non-fibrillar assemblies of A $\beta$  possess electrophysiologic activity, with the corollary that they may produce dementia by disrupting neuronal signaling prior to cell death. We therefore examined the effects of soluble oligomers of A $\beta_{1-42}$  on long-term potentiation (LTP) and long-term depression (LTD), two cellular models of memory, in the dentate gyrus of rat hippocampal slices. Compared with vehicle controls, slices pre-incubated 60 min in the presence of A $\beta$ -derived diffusible ligands (ADDLs) showed no differences in threshold intensity to evoke a synaptic response, slope of field excitatory post-synaptic potentials (EPSPs), or the input/output function. Tetanus-induced LTP and reversal of LTD were strongly inhibited in ADDLs-treated slices whereas LTD was unaffected. These data suggest that soluble non-fibrillar amyloid may contribute to the pathogenesis of AD both by impairing LTP/memory formation at the cellular level and by creating 'neuroplasticity imbalance' manifested by unopposed LTD in the setting of impaired capacity for neural repair via reversal of LTD or LTP. © 2002 Elsevier Science B.V. All rights reserved.

*Theme:* Disorders of the nervous system

*Topic:* Degenerative disease: Alzheimer's - beta amyloid

*Keywords:* Neuroplasticity; Long-term potentiation; Long-term depression; Dentate gyrus; A $\beta$ -derived diffusible ligand

## 1. Introduction

Requisite features in the neuropathologic diagnosis of Alzheimer disease (AD) include neuritic plaques composed of amyloid beta protein (A $\beta$ ) in fibrillar form, neuronal dystrophy with intracytoplasmic neurofibrillary tangles, and widespread neuronal loss [1]. The proximity of A $\beta$  fibrils to dystrophic neurites and reactive glia in mature plaques in post mortem tissue, as well as the correlation of in vitro toxicity of A $\beta$  with its aggregation state, have led

to the assumption that fibril accumulation per se underlies neuronal degeneration in AD. Dementia, in turn, has been attributed to neuronal loss and cerebral atrophy.

Recently these assumptions have been challenged in two important ways. One is the recognition that other A $\beta$  derivatives, such as protofibrils (intermediates in the process of fibrillogenesis [19,20,43]) or oligomers formed independent of or in the absence of fibril formation [28], have neurotoxic potential. The second is the hypothesis that memory loss in AD may result from synaptic dysfunction or neuronal signaling abnormalities that precede massive neuronal degeneration [8,9,13,21,28,40]. Long-term potentiation (LTP) and long-term depression (LTD) are complementary cellular models of learning and memory that constitute an attractive means of detecting per-

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turbations of synaptic functioning in the absence of overt neuronal death. We have recently demonstrated that small diffusible oligomers of  $A\beta_{1-42}$  (referred to as ADDLs for  $A\beta$ -derived diffusible ligands) cause death of hippocampal pyramidal and granule cells at low concentrations in organotypic central nervous system cultures after 24-h incubation [28]. In the present study we report the effects of ADDLs on basic neurotransmission, short-term plasticity, LTP, and LTD. We found that ADDLs differentially affect the latter opposing forms of long-term plasticity by strongly inhibiting tetanically-induced potentiation at both naive and previously depressed synapses but completely sparing low-frequency induced long-term depression.

## 2. Materials and methods

### 2.1. Electrophysiology

#### 2.1.1. Animal age and slice preparation

Synaptic LTP (measured in field excitatory post synaptic potentials, EPSPs) is inconsistently elicited at the medial perforant path-granule cell synapse in dentate gyrus of rats  $\leq 19$  days old, but consistently demonstrated in animals over 20 days old [41,42]. In contrast, LTD is considerably more robust in younger animals. To maximize our ability to detect amyloid  $\beta$  effects on synaptic plasticity, we therefore used 20–30-day-old rats in experiments involving LTP but substituted 14–19-day-old rats when LTD was studied. Measures of basic synaptic function and short-term plasticity were made in 20–30-day-old rats only.

Hippocampal slices (350  $\mu\text{m}$ ) from Sprague–Dawley rats of both genders were prepared as previously described [42]. Artificial cerebrospinal fluid (ACSF) was of the following composition (mM): NaCl 124, KCl 3,  $\text{CaCl}_2$  2.4,  $\text{MgSO}_4$  1.3,  $\text{NaH}_2\text{PO}_4$  1.25,  $\text{NaHCO}_3$  26, and glucose 10 (gassed with 95%  $\text{O}_2$ /5%  $\text{CO}_2$ , pH 7.4). Slices were incubated at room temperature for at least 60 min in ACSF containing either F12 vehicle (5 ml/l) or soluble  $\beta$ -amyloid oligomers ( $A\beta$ ) in F12 (final  $A\beta$  oligomer concentration 500 nM, see below), transferred as needed to a small volume perfusion chamber maintained at 32°C, and allowed to equilibrate  $\geq 15$  min before electrode placement.

#### 2.1.2. Recording

The perfusion solutions during recording were identical to the incubation solutions except for the addition of 100  $\mu\text{M}$  picrotoxin (added directly to the ACSF without solvent, stirred at room temperature for 1 h) to isolate the excitatory responses. Stimulating electrodes were placed in the middle third of the suprapyramidal limb of the dentate gyrus in the middle third of the molecular layer to activate the medial perforant path. Recording electrodes (tip impedance 1–5 M $\Omega$ , filling solution 2 M NaCl) were also placed in the middle molecular layer to record field EPSPs.

Confirmation of medial perforant path activation was obtained by demonstrating paired pulse depression (PPD) at an interstimulus interval (ISI) of 50 ms. Constant current square wave pulses were generated by stimulus isolation units (World Precision Instruments, Model A 360) driven by Grass S88 or S8800 stimulators, driven in turn by custom written software or by the Clampex module of pClamp software (Axon Instruments, v. 6.04), both of which were also used for data acquisition. Stimulation parameters were standardized across slices by identifying the current intensity required to produce a threshold response at a pulse width of 50  $\mu\text{s}$  (usual range 70–150  $\mu\text{A}$ ). Current was then fixed and a stimulus–response curve was constructed over range of pulse widths (50–200  $\mu\text{s}$ ). The pulse width that produced a 70% maximal response (usually 90–100  $\mu\text{s}$ ) was used throughout a given experiment for both monitoring test pulses (0.033–0.05 Hz) and plasticity induction. High frequency stimulation (HFS) to induce LTP consisted of four trains at 100 Hz for 1-s duration with 20-s intertrain interval. Low frequency stimulation (LFS) to induce LTD was delivered at 1 Hz for 15 min. Slope and amplitude of evoked EPSPs were measured using the Clampfit subroutines of pClamp software. Responses were binned in 2-min epochs and were normalized to a 10-min baseline recording period within each experiment for comparison across experiments, statistical analysis and graphing. All values are given as mean  $\pm$  S.E. and comparisons were by Student's *t*-test and ANOVA.

### 2.2. Amyloid $\beta$ ( $A\beta$ )

#### 2.2.1. Preparation of soluble oligomers (ADDLs)

$A\beta_{1-42}$  (American Peptide) was dissolved in hexafluoro-2-propanol (HFIP) and aliquoted to microcentrifuge tubes. HFIP was allowed to evaporate overnight in a fume hood after which residual traces of HFIP were removed by drying in a SpeedVac (Savant Instruments) at 6 mTorr. The tubes were stored desiccated at  $-20^\circ\text{C}$ . To prepare the soluble oligomer solution, an aliquot of  $A\beta_{1-42}$  was dissolved in neat, cold dimethylsulfoxide (DMSO; freshly opened vial) to make a 5-mM solution. The DMSO stock was immediately diluted into cold phenol-free F12 medium (Life Technologies) to make a 100- $\mu\text{M}$   $A\beta$  solution. This solution was verified to be fibril-free by atomic force microscopy (AFM). It was then incubated at 4°C for 24–30 h and centrifuged at 14,000 $\times g$  for 10 min. The supernatant was used at a 1:10 or 1:20 dilution in F12 medium (for MTT cytotoxicity assays, see below) or 1:200 dilution in ACSF (for electrophysiology experiments). The samples diluted in F12 were fibril-free by SDS–PAGE as previously described [10,28].

#### 2.2.2. Cellular toxicity of amyloid $\beta$ (ADDLs)

To control for the possibility that absence of electrophysiologic effects of ADDLs might simply reflect lack

of bioactivity in the sample, each batch used in the present experiments was tested for toxicity in the neuron-like PC12 cell line by the colorimetric MTT assay (Roche Diagnostics). This assay detects toxin-induced dysfunction in vesicle trafficking and cellular oxidative activity [30,39]. To ensure linearity, PC-12 cells were plated at a concentration of 30,000–50,000 cells/well on collagen coated 96-well plates and grown overnight. Plates were inverted to remove media and cultures then given fresh F12K media with 1% serum and either ADDLs or vehicle control. After 4–24-h incubation, cultures were given MTT and the conversion to formazan measured according to the manufacturer's instruction. ADDL tests were run using dosages of 5 and 10  $\mu\text{M}$  (with respect to total A $\beta$  concentration) to monitor maximal response. We routinely observed that 10  $\mu\text{M}$  had little or no increased impact relative to 5  $\mu\text{M}$ , consistent with dose–response curves (not shown). When compared over all experiments, cultures at 30,000 cells/well averaged 52.8% (S.E.M.  $\pm$  2.1%) and 58.4% (S.E.M.  $\pm$  2.3%) decreases in MTT reduction after 4-h exposure to 5 and 10  $\mu\text{M}$  ADDLs, respectively. At this cell density, no greater impact was observed at 24 h. In all cases, ADDLs used for electrophysiology showed robust activity in the MTT assay.

### 3. Results

#### 3.1. Basic synaptic function and short-term plasticity

To distinguish ADDLs effects on long-term plasticity from effects on neurotransmission we examined several aspects of basic synaptic function and short-term plasticity.

We examined the effects of ADDLs on overall slice excitability by comparing threshold current intensities and the slope and amplitude distributions of EPSPs evoked in slices pre-incubated in ADDLs versus vehicle-pre-incubated slices. At a fixed pulse width of 50  $\mu\text{s}$ , the threshold current required to evoke an EPSP did not differ between ADDLs and vehicle-treated control slices ( $111 \pm 10$  vs.  $109 \pm 10$   $\mu\text{A}$ ,  $n = 10$  pairs,  $P = 0.17$ ). Using the pulse width that produced a 70% maximal response at the threshold current in these slices, EPSP slopes ( $2.51 \pm 0.13$  mV/ms in ADDLs vs.  $2.56 \pm 0.16$  in vehicle,  $P = 0.125$ ) and amplitudes ( $2.89 \pm 0.11$  vs.  $2.87 \pm 0.08$ ,  $P = 0.38$ ) were comparable in the two groups. In a separate set of experiments we plotted the ratio of EPSP slope/presynaptic fiber volley amplitude over a comparable range of stimulus current (180–200  $\mu\text{A}$ ) and pulse widths (50–120  $\mu\text{s}$  in increments of 10  $\mu\text{s}$ ) for ADDLs-treated ( $n = 5$ ) and control ( $n = 7$ ) slices (Fig. 1A). This ratio serves as a measure of the synaptic response size produced by stimulation of a given number of presynaptic axons. ADDLs had no significant effect on the distribution or mean values of this ratio ( $7.44 \pm 0.37$  in ADDLs vs.  $8.19 \pm 1.61$  in vehicle,  $P = 0.09$ ).

At the medial perforant path-granule cell synapse the

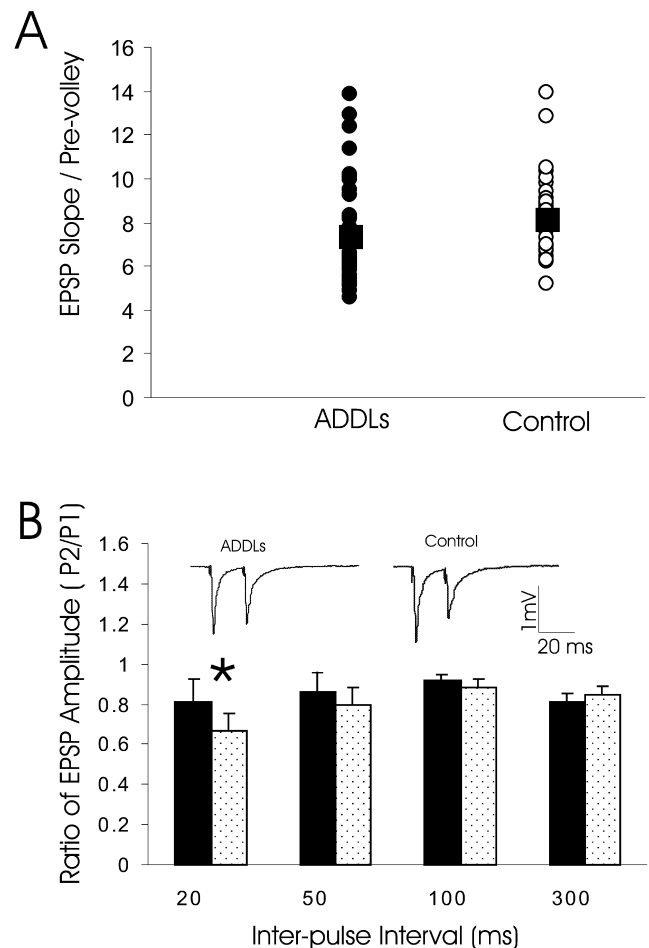


Fig. 1. ADDLs do not affect the input–output function, but do decrease paired pulse depression (PPD) at short interstimulus intervals. (A) The ratio of EPSP slope to pre-synaptic volley for ADDLs-treated ( $n = 5$ ) and control ( $n = 7$ ) slices over a range of pulse widths (50–120  $\mu\text{s}$  in increments of 10  $\mu\text{s}$ ) at comparable stimulus current (180–200  $\mu\text{A}$ ). The distribution (circles) and mean ratios (squares) did not differ between groups ( $7.44 \pm 0.37$  in ADDLs vs.  $8.19 \pm 0.24$  in vehicle,  $P = 0.09$ ). (B) The ratios of the amplitude of pulse 2/pulse 1 for ADDLs pre-incubated slices (solid black bars) and control slices (stippled black bars). ADDLs decreased PPD at the 20-ms interstimulus interval only (paired pulse ratios  $0.81 \pm 0.1$  vs.  $0.67 \pm 0.1$ ,  $P < 0.002$ ). Inset shows representative responses to paired stimuli in ADDLs-treated slices and control at 20-ms interstimulus interval.

response to the second of a pair of stimuli at brief interstimulus intervals (ISI) is smaller than the first (paired pulse depression, PPD; [3,11]). We examined the ratios (pulse 2/pulse 1) of EPSP slope and amplitude of the paired responses evoked over a range of interstimulus intervals (20, 50, 100, 300 ms) in ADDLs- and vehicle-treated groups ( $n = 4$  pairs, Fig. 1B). ADDLs did not affect PPD at 50-, 100-, and 300-ms interstimulus intervals but caused a decrease in PPD (increased paired pulse ratio) at the 20-ms interval that was significant only for the amplitude measure ( $0.81 \pm 0.1$  mV in ADDLs vs.  $0.67 \pm 0.1$  mV in control vehicle,  $P < 0.002$ ). Since the first pulse was comparable in ADDLs-treated and control groups

( $1.96 \pm 0.47$  mV vs.  $1.90 \pm 0.49$  mV,  $P=0.89$ ), the decrease in PPD resulted from an increase in the second pulse.

### 3.2. ADDLs prevent LTP induced by HFS

In five pairs of slices (Fig. 2A), robust LTP was seen in vehicle control ( $204.5 \pm 24.8\%$  baseline at 30 min following tetanus, and  $193.1 \pm 20.7\%$  at 60 min) whereas ADDLs-treated slices showed minimal early potentiation ( $120.2 \pm 5.4\%$  at 30 min) that decayed completely to  $95.7 \pm 8.5\%$  baseline by 60 min.

To test the hypothesis that ADDLs might induce LTP, and thus block it by occlusion, we performed a set of wash-in experiments ( $n=6$  pairs) in which naive slices (pre-incubated only in ACSF) were exposed for the first time in the recording chamber to either ADDLs or vehicle control (Fig. 2B). Duration of ADDLs exposure in the recording chamber was 60 min, the same duration that blocked LTP via pre-incubation. A repeated measures ANOVA was performed with one between subjects measure (ADDLs vs. vehicle wash-in) and one within subjects measure (time). Mean EPSP slope for each of three epochs was calculated within each experiment as follows: the 10-min baseline period prior to the start of wash-in, the 10 min spanning 25–35 min after wash-in began, and the 10 min spanning 50–60 min after wash-in began. The ANOVA showed no main effect for ADDLs versus vehicle ( $P=0.582$ ), or for time ( $P=0.487$ ) and no interaction ( $P=0.877$ ). These results demonstrate that ADDLs had no effect on EPSP slope during 60 min of wash-in. The control EPSP slope was  $101.1 \pm 2.0$  and  $97.5 \pm 2.6\%$  baseline (mean  $\pm$  S.E.) at 30 and 60 min, respectively. The EPSP slope in ADDLs-treated slices was  $102.6 \pm 4.2$  and  $100.2 \pm 4.3\%$  baseline at 30 and 60 min, respectively.

### 3.3. ADDLs permit LTD

In five additional pairs of slices pre-incubated in ADDLs or vehicle for  $\geq 1$  h, LFS was delivered to induce LTD. As is shown in Fig. 3A, ADDLs had no effect on LTD: the EPSP slope was  $72.3 \pm 5.6\%$  baseline 30 min after LFS in the control group and  $70.0 \pm 3.9\%$  baseline in the ADDLs-treated group ( $P=0.43$ ).

### 3.4. ADDLs prevent reversal of LTD

We have previously demonstrated that LTD in dentate gyrus (DG) is reversible by the administration of tetanic stimulation [42]. In the final set of experiments (Fig. 3B,  $n=4$  pairs) we induced LTD in ADDLs pre-incubated ( $70.2 \pm 13.7\%$  baseline) and control ( $73.8 \pm 1.5\%$  baseline,  $P=0.62$  at 20 min after LFS) slices and then delivered tetanic stimulation in an attempt to reverse it. Despite the fact that younger (14–19 days) animals were used in these experiments we found that tetanic stimulation identical to that used for LTP induction readily restored the response

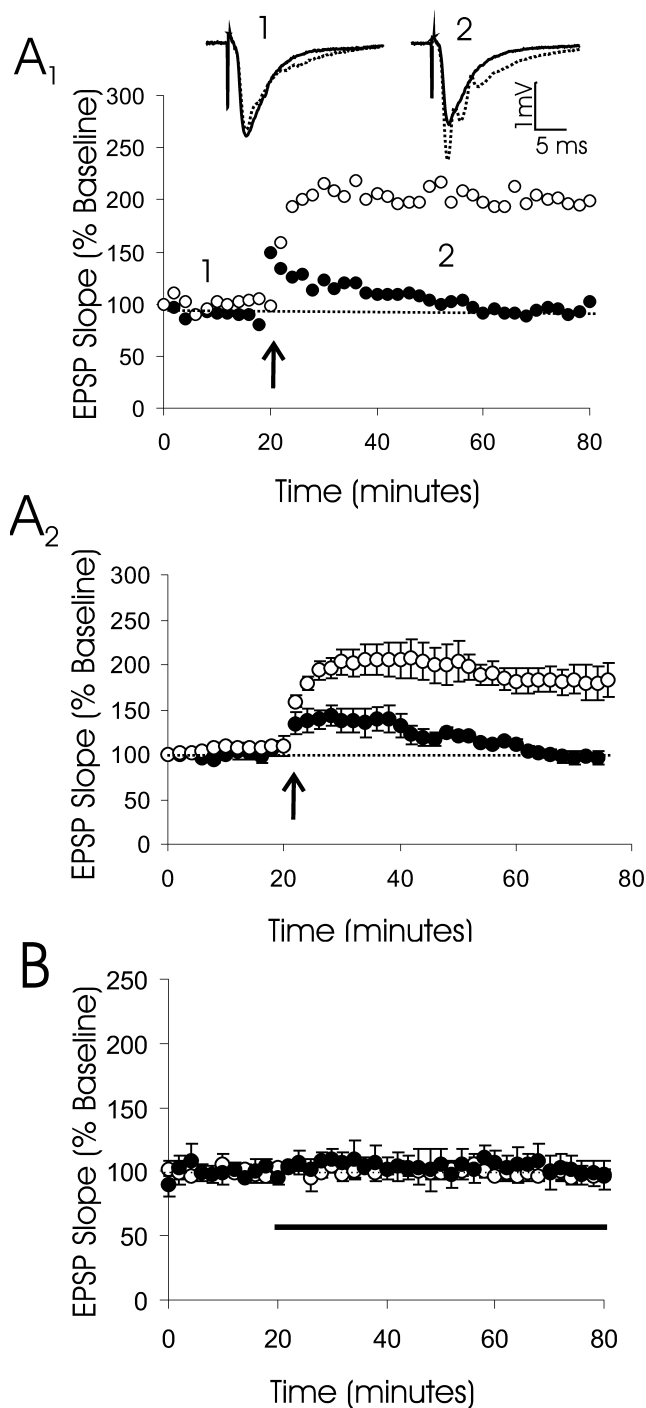


Fig. 2. ADDLs block but do not occlude LTP. (A) Control slices (open circles) show robust LTP in response to tetanic stimulation (vertical arrow) whereas ADDLs-treated slices show limited transient potentiation with decay to baseline by 60 min.  $A_1$  shows representative ADDLs and vehicle control experiments. Insets are sweeps (average of four traces) showing superimposed ADDLs-treated (heavy solid line) and control (dotted line) responses from these experiments obtained at the indicated times.  $A_2$  is a composite of five pairs of experiments. (B) Neither control responses (open circles) nor ADDLs-treated slices (filled circles) show a change from baseline during wash-in. Graph represents composite of six pairs of slices. Baseline recording was obtained in ACSF and solid bar represents infusion of ADDLs or vehicle.

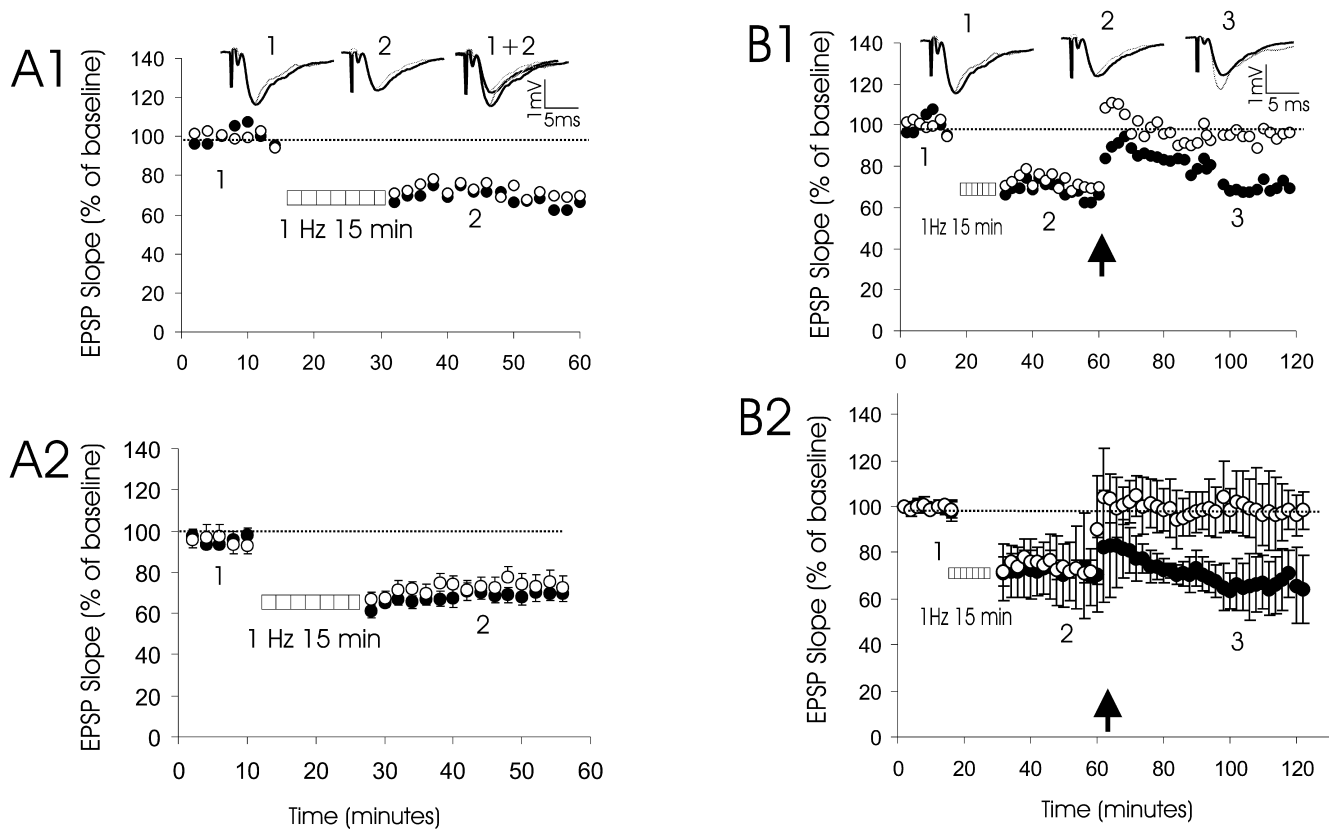


Fig. 3. ADDLs do not affect LTD but prevent the reversal of LTD by tetanic stimulation. (A) Low frequency stimulation induced LTD in both control (open circles) and ADDLs pre-incubated (closed circles) slices.  $A_1$  shows representative ADDLs and control experiments. Insets represent superimposed sweeps (average of four traces) from ADDLs-treated (heavy line) and control (thin line) slices from these experiments at indicated times.  $A_2$  is a composite of five pairs of slices. (B) ADDLs-treated (filled circles) and control (open circles) slices both demonstrated LTD ( $n=4$  pairs), but tetanic stimulation (arrow) reversed LTD only in control slices.  $B_1$  shows representative ADDLs and control experiments. Insets represent superimposed sweeps (average of four traces) from ADDLs-treated (heavy line) and control (thin line) slices from these experiments at indicated times.  $B_2$  is a composite of five pairs of slices.

back to baseline in stable fashion in control slices ( $98.8 \pm 9.1\%$  baseline at 20 min,  $98.2 \pm 17.6\%$  baseline at 60 min), but had no effect on the depressed response in ADDLs-treated slices ( $72.4 \pm 5.3\%$  baseline at 20 min and  $68.4 \pm 13.6\%$  baseline at 60 min).

#### 4. Discussion

In this study we examined the effects of soluble oligomers of  $A\beta_{1-42}$  (ADDLs) on neurotransmission and plasticity at the medial perforant path-granule cell synapse in hippocampal dentate gyrus in vitro. Our major finding is that brief (60 min) pre-incubation in nanomolar concentrations of ADDLs strongly inhibits both synaptic LTP and the reversal of LTD when these are induced by tetanic stimulation, but does not affect the induction of LTD by low frequency stimulation.

##### 4.1. ADDLs do not impair basic synaptic function

Several lines of evidence confirm that the observed

effects of ADDLs on long-term plasticity are specific, and not attributable to disruption of normal synaptic processes. This is important because it establishes that the impairment of LTP of the population spike (PS) that we previously identified after ADDLs pre-incubation was not the result of a decrease in neuronal excitability [28]. Using EPSPs as a more faithful representation of synaptic events than is the PS, we now demonstrate that ADDLs do not affect threshold stimulus intensity, evoked response size, or the input–output function. Further, despite the fact that ADDLs are potentially cytotoxic ([28], and MTT assay), the concentration (500 nM in total  $A\beta$ ) used in these experiments did not appear to cause acute cytotoxicity since there was no decay of previously established evoked responses when naive slices were exposed to ADDLs during recording (Fig. 2B).

##### 4.2. ADDLs affect short-term plasticity

ADDLs pre-incubated slices showed decreased PPD of EPSP amplitude at short (20 ms) ISI that resulted from an increase in the second pulse in each pair; ADDLs did not

affect intermediate (50–200 ms) or longer (300 ms) ISI. Several prior investigations have specifically examined PPD of the dendritically recorded field EPSP in response to medial perforant path stimulation [3,6,23,34]. Of these, only two [3,34] included the very short ISI (10 and 25 ms, respectively) comparable to those affected by ADDLs in the present study. In particular Asztely et al. [3] demonstrate that PPD is diminished at ISI as short as 25 ms by both targeted disruption of the brain derived neurotrophic factor (BDNF) gene and by sequestration of endogenous BDNF with TrkB-IgG fusion protein. Thus ADDLs may diminish PPD by acting as an antagonist at the TrkB receptor or by interfering with the action of BDNF, the endogenous ligand for this receptor. The BDNF effect is believed to be pre-synaptic, in part because it persisted in companion whole cell recordings despite the use of calcium chelation and voltage clamp of the membrane potential in the postsynaptic neuron [3].

Class II/III metabotropic glutamate receptors (mGluRs) are the other major candidate mediators of PPD at the medial perforant path-granule cell synapse. Agonists at these receptors diminished PPD at ISI of 10–200 ms [34], 40 ms [6], and 40–100 ms [23]. Thus ADDLs might mimic mGluR agonist activity to decrease PPD. These effects are usually attributed to a pre-synaptic mechanism such as activation of autoreceptors that diminish transmitter release. However, the exclusive involvement of a very short ISI (20 ms) in the present study raises the additional possibility that ADDLs act to diminish desensitization of postsynaptic AMPA receptors (i.e. make more receptors available in response to the second glutamate pulse). Conceivably this would involve diminished affinity of the receptor for glutamate, or an increase in the transition rate out of a desensitized state, and might implicate a post-synaptic mGluR.

#### 4.3. ADDLs disrupt LTP but spare LTD

We found that ADDLs differentially affected the two forms of long-term plasticity, antagonizing LTP but not altering LTD. The animal ages selected for these experiments (20–30 days old for LTP, and 14–19 days old for LTD) corresponded to developmental stages at which the form of plasticity under study was most robust. In particular, since we have previously found that LTP of the dentate field EPSP cannot be consistently evoked in 14–19-day-old rats [42], and LTD of the field EPSP is similarly inconsistent (and of smaller magnitude when present) in 20–30-day-old rats [42], the use of only one age group for both types of experiment would risk falsely obscuring or exaggerating the effects of ADDLs on plasticity. In consideration of potential age-related ADDLs effects, however, the experiments in 14–19-day-old animals contained an internal control. Thus after the induction of LTD in the 14–19-day-old ADDLs-treated slices, tetanic stimulation identical to that used for LTP induction

was delivered. ADDLs pre-incubation blocked this tetanus-induced LTD reversal, whereas in vehicle-treated control slices the response was restored to baseline following the tetanus. Although the mechanism of LTD reversal by tetanus may not be identical to the mechanism of LTP induction, these experiments clearly demonstrate that ADDLs differentially affect tetanus-induced synaptic strengthening but not low frequency induced synaptic weakening within a single age group.

The mechanisms of the ADDLs effects on LTP are unknown. One possibility is an alteration of the granule cells' responsiveness to stimulation pattern. For example, whereas high frequency stimuli (100 Hz) are usually optimal for LTP induction, and low frequency stimuli (1–3 Hz) are optimal for LTD induction, work in CA1 has shown that intermediate frequencies (10 Hz) constitute a null point (no plastic change) that may be altered by previous stimulation or by the pharmacologic milieu [5]. Thus ADDLs may act to raise the null point such that a high frequency stimulus of 100 Hz no longer produces a plastic change.

Another possibility, suggested by our findings related to PPD, is that ADDLs impair LTP via a direct effect on mGluRs or the TrkB-BDNF complex. TrkB receptor immunoreactivity has been demonstrated both pre- and post-synaptically in dentate granule cells [15]. The relationship with LTP is supported by the observations that both BDNF and TrkB mRNA expression are enhanced in dentate following tetanic stimulation [14], and BDNF promotes LTP in CA1 [16,24,31].

ADDLs may also induce NMDA receptor dysfunction. The disparity between LTP and LTD effects in this setting would be consistent with prior demonstrations that LTD is NMDA-independent in DG [41,42,44], whereas LTP is NMDA-dependent [7,11,41] throughout the age spectrum. If the NMDA receptor is affected, this in turn may relate to a role for the non-receptor protein tyrosine kinase Fyn. Fyn is involved in LTP [18], is anchored in the post-synaptic density where it phosphorylates and modifies the activity of the NR2A subunit of the NMDA receptor [36,40], and in our prior studies was shown to be required for hippocampal neuron death caused by chronic ADDLs incubation [28].

Finally, a limited number of studies have addressed the mechanism of impairment of LTP by other A $\beta$  species (i.e. truncated fragments of A $\beta$ <sub>31-35</sub> and A $\beta$ <sub>25-35</sub>) in both dentate [37] and CA1 [45] and have demonstrated their dependence on p38 mitogen-activated protein kinase and hippocampal cholinergic terminals, respectively.

#### 4.4. Alzheimer disease as neuroplasticity imbalance

Our demonstration of LTP blockade by ADDLs is consistent with emerging evidence that soluble A $\beta$  structures, whether introduced by genetic engineering or by exogenous application, have biologic activity in the ab-

sence of fibril or plaque formation. For example, they may alter neuronal and synaptic properties such as membrane potential and firing frequency [20], synaptic transmission [8], and synaptic plasticity [8,9,13,28]. ADDLs, amyloid protofibrils [20], and similar A $\beta$  products presumably exert their effects in mobile circulating form rather than in proximity to plaques. This mechanism may help reconcile the lack of correlation, both in quantity and in location, between amyloid plaque deposition and clinical dementia [32].

Importantly, our findings support the hypothesis of ‘neuroplasticity failure’ that has been proposed as a unifying theory of AD pathogenesis [32]. In this context, neuroplasticity (e.g. synaptic remodeling, axonal sprouting, neurite extension, synaptogenesis, neurogenesis, alterations in dendritic ramification, and LTP) is understood both as a substrate of learning and as a lifelong mediator of neuronal responses to attrition and injury. AD risk factors represent barriers to this ‘structural upkeep’ that prompt a compensatory upregulation in plasticity. AD results when plasticity becomes excessive and maladaptive; the selective vulnerability of limbic and paralimbic structures (e.g. hippocampus, entorhinal cortex) to neurofibrillary tangle formation is attributed to their retention of the potential for plasticity in adulthood.

Our results suggest an additional related mechanism of AD pathogenesis best described as ‘neuroplasticity imbalance’ (unopposed LTD). Although LTD is occasionally proposed as a model of forgetting [2], it is far more likely that LTD complements LTP to refine neuronal circuitry and maintain normal memory storage during adulthood [29], possibly via competition for synaptic stabilization as occurs during brain development [12]. Best studied at the developing myoneuronal junction [4] and visual cortex [26,35], the process of synaptic refinement entails a transition from early diffuse patterns of innervation by multiple axons to mature patterns in which inputs are precisely segregated. The formation and elimination of specific synapses during this process are influenced by the timing and balance of both spontaneous and sensory-driven electrical activity [35]. Further, at the cerebellar climbing fiber-Purkinje cell synapse both synapse elimination and LTD are impaired in mGluR1 null mutant mice and both are rescued by introduction of the mGluR1 transgene [22], suggesting that LTD or like processes may specifically contribute to physiologic synapse elimination. We hypothesize that by selectively preserving LTD, ADDLs may lead to accelerated and pathologic synapse elimination in granule cells, thus increasing the demand for restorative plasticity while simultaneously rendering that demand more difficult to meet.

Whether ADDLs-induced plasticity imbalance is unique to the dentate (i.e. hinges on the differential NMDA-dependence of LTP and LTD that we find in this region) or can be generalized to brain regions that demonstrate NMDA-dependent LTD (e.g. CA1, visual cortex) remains

to be investigated. The phenomenon is important nonetheless because dentate is a specific target of AD-related neuropathologic change including A $\beta$  deposition and synaptic loss [38]. Further, with its lifelong neurogenesis [17,27,33], that can be augmented by environmental enrichment and even physical exercise [25], the dentate is an attractive focus for interventions that might stem disease progression or aim for reversibility.

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