Multiple effects of β-amyloid on single excitatory synaptic connections in the PFC

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Prefrontal cortex (PFC) is recognized as an AD-vulnerable region responsible for defects in cognitive functioning. Pyramidal cell (PC) connections are typically facilitating (F) or depressing (D) in PFC. Excitatory post-synaptic potentials (EPSPs) were recorded using patch-clamp from single connections in PFC slices of rats and ferrets in the presence of β-amyloid (Aβ). Synaptic transmission was significantly enhanced or reduced depending on their intrinsic type (facilitating or depressing), Aβ species (Aβ40 or Aβ42) and concentration (1–200 nM vs. 0.3–1 μM). Nanomolar Aβ40 and Aβ42 had opposite effects on F-connections, resulting in fewer or increased EPSP failure rates, strengthening or weakening EPSPs and enhancing or inhibiting short-term potentiation [STP: synaptic augmentation (SA) and post-tetanic potentiation (PTP)], respectively. High Aβ40 concentrations induced inhibition regardless of synaptic type. D-connections were inhibited regardless of Aβ species or concentration. The inhibition induced with bath application was hard to recover by washout, but a complete recovery was obtained with brief local application and prompt washout. Our data suggests that Aβ40 acts on the prefrontal neuronal network by modulating facilitating and depressing synapses. At higher levels, both Aβ40 and Aβ42 inhibit synaptic activity and cause irreversible toxicity once diffusely accumulated in the synaptic environment.

Keywords: β-amyloid (Aβ), synaptic connection, synaptic dynamics, excitatory post-synaptic potential (EPSP), short term potentiation (STP), synaptic augmentation (SA), post-tetanic potentiation (PTP)

INTRODUCTION

In patients and animal models of the early stages of Alzheimer’s Disease (AD), declines in episodic or spatial memory and cognition are correlated with an increase in brain levels of soluble β-amyloid (Aβ) (Lue et al., 1999; Walsh et al., 2002; Rowan et al., 2003). A causal link between the accumulation of Aβ in a soluble, toxic state and impairment of neuronal mechanisms that support memory was demonstrated in a mature βAPP transgenic mouse model wherein a single systemic injection of an antibody to Aβ eliminated the memory deficit (Dodart et al., 2002). There is a major focus on the synapse as the initial site of damage in AD (Selkoe, 2002; Nimmrich and Ebert, 2009). Synaptic dysfunction as a consequence of diffusible Aβ is also inferred from studies showing reduced basal transmission and altered plasticity (Klyubin et al., 2005; Shankar et al., 2008; Minano-Molina et al., 2011). In anatomic terms, synapse numbers are reduced early in some AD brain regions (Davies et al., 1987), especially in the prefrontal cortex (PFC) and medial temporal lobe (Morris and Baddeley, 1988). Meanwhile, additional studies in the recent decade indicate that low levels of Aβ peptides could be essential for the modulation of synaptic plasticity (Parihar and Brewer, 2010).

In AD, limbic and association cortices are selectively involved while primary cortical areas remain relatively preserved. These regions of neuronal vulnerability in fact correspond to the degree to which neuronal plasticity can be demonstrated in them (Arendt, 2001). The PFC is a critical association region associated with executive type cognitive function. PFC also orchestrates a unique form of short-term memory termed “working memory.” Working memory is a limited capacity system that supports non-routine types of daily activity. It is a temporary storage system for maintaining and rapidly manipulating information, and is closely connected with attention, strategic information flow and action (Goldman-Rakic, 1996). Experimentally, prefrontal cortical neurons are found to remain persistently active during the delay between sensory cue and an executed response task. The ongoing activity, an electrical correlate of working memory, is stable to the interference from distractors (Goldman-Rakic, 1995). Further, fMRI studies confirm the role of PFC in strategic encoding and goal directed control over the retrieval process in episodic type memory processes (Simons and Spiers, 2003). Les appreiated than episodic memory, working memory is also impaired in the early stages of AD, according to clinical and in vivo studies (Morris and Baddeley, 1988). Impairment of PFC function may precede the pathological changes of AD in other cortical association areas (Reid et al., 1996). Correspondingly, soluble Aβ accumulates in the PFC to one of the highest and earliest levels across several cortical regions in the pre-tangle stages of AD (Gouras et al., 2000) and in transgenic mice (Zhuo et al., 2008). Tangle formation in the PFC is also highly correlated with the transition to clearly recognizable dementia (Wang and Al, 2001).
In the PFC network, excitatory synaptic connections in layer V show both facilitated (F-connection) and depressed (D-connection) excitatory post-synaptic potentials (EPSPs) in response to short train stimuli (Wang et al., 2006). F-connections are formed predominantly by complex-type pyramidal cells (PCs) which feature dual apical dendrites, a high degree of interconnectivity and of reciprocity in chemical synaptic connections. In contrast, D-connections are typically formed by simple PCs that are common to primary cortices. A computer simulation study revealed that these facilitating synapses play a crucial role in the formation of persistent neuronal activity, consistent with the properties of working memory (Mongillo et al., 2008). The critical role of the PFC in working memory and early involvement in AD make it a suitable region to examine the electrophysiologic effects of Aβ.

Numerous studies of AD-promoting factors (e.g., Aβ) have examined their effects on field electrophysiological characteristics in the hippocampus and cortex. Because of competing synaptic inputs and influences from other local networks, the use of field recordings may account for seemingly contradictory early reports, where Aβ either increased excitability through membrane depolarization and enhancing long term potentiation (LTP) or depressed both synaptic transmission and LTP induction (Wu et al., 1995; Selkoe, 2002; Walsh et al., 2002; Esteban, 2004; Puzzo et al., 2008; Li et al., 2011). Up until now, a study focused on activity-dependent plasticity specific to the association cortex has not been reported. It is therefore timely to carry out an investigation of an in situ neural network, especially at the resolution of individual synaptic connections, within an association cortical area such as the PFC.

In this study, using multi-neuron patch clamp recording from PFC slices, we found that synaptic responses of single excitatory synaptic connections were significantly enhanced or reduced depending on their intrinsic type (facilitating or depressing), the tested Aβ species (40 or 42 amino acids) and concentration (low dose 1–200 nM vs. high dose 0.3–1 μM). Low-doses of Aβ40 enhanced F-connections and inhibited D-connections in the PFC. In contrast, high-doses of Aβ40 and low-doses of Aβ42 inhibited all types of excitatory synaptic connections. Further, the inhibition induced with bath application was commonly difficult to recover or even became worse by washout. However, direct local and brief application of the peptides by pipette at comparable concentrations produced similar inhibitions with a rapid and complete recovery upon washout. Based on the principles of synaptic dynamics that have been well-studied in our previous computer simulation of synaptic responses of single synaptic connections, the effects of Aβ were considered to be produced via both pre- and post-synaptic mechanisms.

MATERIALS AND METHODS

ELECTROPHYSIOLOGICAL RECORDINGS

Prefrontal cortical slices were prepared using a published protocol (Wang et al., 2006). Briefly, brain was dissected from normal adolescent Wistar rats (day 25–35) or young adult ferrets (7–9 weeks old). PFC slices (300 μM) were sectioned using a vibratome (DTK 1000 Zero 1 Microslicer) and then incubated in artificial cerebrospinal fluid (ACSF) before transfer to a recording chamber (at 34°C). Neurons in layer V of the medial PFC were visually identified using infrared differential contrast videomicroscopy (BX50WI, Olympus). An advanced technique consisting of quadruple patch clamp recording was used to record from candidate cell bodies (somata), and single synaptic connections formed between neuron pairs were determined electrophysiologically according to standard characteristics of chemical synaptic transmission (Wang et al., 2006). Somatic whole-cell signals (6–12 mΩ pipette resistance) were amplified using Axoclamp-200B amplifiers (Axon Instruments, USA). Recordings were sampled over real time and filtered using the program Igor (Igor WaveMetrics, Lake Oswego, OR, USA), digitized by an ITC-18 interface (Instrutech, Great Neck, NY, USA) and stored on hard drive (Macintosh G5 computer) for off-line analysis (Igor). Stimulating and voltage recording glass micropipettes were filled with (mM): 100 potassium gluconate, 20 KCl, 4 ATP-Mg, 10 phosphocreatine, 0.3 GTP, 10 Hepes (pH 7.3) and 0.4% biocytin (Sigma). Presynaptic action potentials (AP) were elicited using short (3 ms), suprathreshold, intracellular depolarizing current pulses. The extracellular recording solution consisted of ACSF, containing (mM): 125 NaCl, 2.5 KCl, 25 glucose, 25 NaHCO3, 1.25 NaH2PO4, 2 CaCl2, and 1 MgCl2. Only neurons with stable access resistance were included in the statistical analyses. Membrane potentials were routinely voltage-clamped at −70 ± 2 mV to maintain Vm against drift by using small current injections. Neurons were filled with 0.4% biocytin (Sigma) by diffusion at the end of the recordings for later identification of neuronal types.

Once synaptic connections were obtained, the EPSP failure rate, certain dynamic features of EPSPs and short term potentiation components including synaptic augmentation (SA) and post-tetanic potentiation (PTP), were recorded (Wang et al., 2006). For the EPSP failure rate, single APs were repeatedly evoked (0.5 Hz, 15–30 times) in a presynaptic cell and the number of corresponding EPSP failures in a postsynaptic cell were counted. For the synaptic dynamic features, an EPSP train was evoked by 6–8 presynaptic APs at 10–20 Hz followed by a recovery test response (RTR) after a 500 ms delay. SA and PTP were induced by giving a 15 pulse (tetanus) stimulus at 50 Hz. Single test responses (0.5 Hz) were recorded for 20 s before and up to 100 s after the train. This procedure was repeated four times, each preceded by a 2 min interval.

HISTOLOGICAL PROCEDURES AND 3D COMPUTER RECONSTRUCTION

After recording, the slices bearing biocytin-injected neurons were fixed for at least 24 h in cold 0.1 M phosphate buffer saline (PBS, pH 7.4) containing 2% paraformaldehyde, 1% glutaraldehyde, and 0.3% saturated picric acid. Thereafter, the slices were rinsed several times (10 min each) in PBS. To block endogenous peroxidases, slices were transferred into phosphate-buffered 3% H2O2 for 10–30 min. After five to six rinses in PBS (10 min each), slices were incubated overnight at 4°C in avidin-biotinylated horseradish peroxidase according to the manufacturer’s protocol (ABC-Elite, Vector Labs, Petersborough, UK) (2% A, 2% B, and 1% Triton-100). Following incubation and additional rinses, the reaction was developed with diaminobenzidine (DAB) under visual control using a bright-field microscope (Zeiss, NY,
USA) until all cell processes appeared clearly visible (usually after 2–4 min). The reaction was stopped upon transferring the sections into PBS. Slices were mounted in aqueous mounting medium.

3D neuron models were reconstructed from stained cells using the Neurolucida system (MicroBrightField Inc., USA) and a bright-field light microscope (Olympus, BX51, Japan). Reconstructed neurons subsequently underwent quantitative analysis using the NeuroExplorer (MicroBrightField Inc., USA; ×60 magnification, numerical aperture = 0.9; Z-axis resolution = 0.37 μm). Putative synapses were identified according to the criteria as published (Wang et al., 2002).

PREPARATION AND TREATMENT OF SOLUBLE SYNTHETIC Aβ
Soluble synthetic Aβ peptides (Aβ40, Aβ25–35, and Aβ42) were purchased from Biosource (Camarillo, CA) or the Harvard Protein Core laboratory and prepared as 0.1 mM stock following published methods and stocked at −80°C (Stine et al., 2003). The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels]. The quality of monomer stock solutions were examined periodically using western blots [NuPAGE Novex 4–12% Bis-Tris gels].

Different concentrations of Aβ were freshly prepared before use by defrosting and diluting the stock solution with ACSF. Only soluble Aβ was bath-applied either at low dosage (1–200 nM) or at high dosage (300 nM–1 μM) (see Chen et al., 2000). During recording, brain slices were continuously perfused with oxygenated ACSF at a flow rate of 0.75–1.0 ml/min. The ACSF volume in the tube leading to and including the recording chamber was 1.5–2.0 ml. This enabled quick replacement of the recording solutions (<3 min.) when switching between experimental procedures. Each recording procedure was repeated under three conditions: (1) pre-application, (2) application, and (3) washout of Aβ. The Aβ application recordings typically lasted for 30 min. whereas washout recordings lasted for 10–30 min.

MODELING ANALYSIS OF SYNAPTIC RESPONSES
The quantitative analysis of basal synaptic dynamic properties of excitatory connections has been carried out using a well-known computer model of a combination of EPSP train and a RTR evoked with a 500 ms delay (Markram et al., 1998; Tsodyks et al., 2000; Wang et al., 2006). The RTR is used to test the recovery of synaptic facilitation or depression, which characterizes the synapse type. The model extracts four key parameters of the connection: DFUA (D, the time constant of recovery from depression (ms); F, the time constant of recovery from facilitation (ms); U, utilization of synaptic resources, analogous to the neurotransmitter release probability (p); A, the absolute strength of a synaptic connection (nA), defined as its maximum response when p = 1). This modeling approach is based on fitting the mean output behavior of synaptic connections and therefore requires inputting only averaged responses (i.e., average EPSP traces). Generally speaking, reductions in A correspond to the situation when the amplitudes of all EPSPs in the train and RTR become smaller, keeping an unchanging EPSP pattern. In the case of reductions in U, the amplitude of the 1st EPSP is reduced whereas subsequent EPSPs are facilitated. When normalized to the first EPSP in such recordings, the subsequent EPSP amplitudes, but not the RTR, are magnified. In the case of a larger D value, both EPSPs (subsequent to the 1st) and the RTR show reductions. Oppositely, a high value for the parameter F correlates to the situation in which both EPSPs (subsequent to the 1st) and the RTR are increased.

STATISTICAL ANALYSIS
Paired student t-test was used to compare EPSP responses between different conditions: [(1) pre-application, (2) application, and (3) washout of Aβ]. Unpaired student t-test was used for the comparison of EPSP responses between different treatments of Aβ. The statistical analyses of EPSP train, SA and PTP were all based on an intrinsic comparison of individual single synaptic connections between the Aβ application or washout condition with the pre-application condition. In order to lessen the influence from the variance of synaptic strength between individual connections, the statistical comparisons were made using normalized values. In the analysis of EPSP train, all EPSP values of a connection were normalized to the mean of EPSPs recorded under the pre-application condition. In the analysis of SA and PTP, all EPSP values of a connection were normalized to the mean of pre-tetanus EPSPs obtained in the pre-Aβ application condition. Furthermore, an EPSP pattern defined by a certain “8-EPSP train + RTR” configuration for each type of synapse, allowed each EPSP value to be treated as an independent outcome value in the statistical comparison. Multiple outcome values per connection were therefore used in the EPSP train analysis. The same principle was also applied to the analyses of SA and PTP.

RESULTS
Quadruple patch clamp recording was performed to record synaptically coupled pairs (n = 100) in layer V of the PFC of rats (n = 92, age P25–P35) and ferrets (n = 8, age 7–9 weeks old). Since synaptic dynamics (i.e., depressing and facilitating types) of synapses are consistent across species (Wang et al., 2006), the data of the two species were pooled together in order to maximally utilize the obtained data. The studied connections comprise synapses formed between PCs (PC–PC, n = 86 pairs) and those formed by a PC onto an interneuron (PC–IN, n = 14 pairs) (Figure 1). The neuronal type was identified according to the morphology (PCs and interneurons—mainly basket cells and Marttinoti cells) combined with the firing pattern of APs evoked by depolarizing current steps injected into neuronal somata (Wang et al., 2002, 2006). EPSP responses of a postsynaptic cell were induced by APs evoked by brief depolarizing current injections delivered into the presynaptic neuronal soma. We previously characterized the excitatory neuronal network in layer V of the PFC (Wang et al., 2006). Facilitation-dominant synapses (F-connections) are abundant in the PFC while depression-dominant synapses (D-connections), typically common in primary cortical areas, form a minor population in
the PFC. In order to examine effects of beta-amyloid peptides (Aβ40, Aβ25–35, Aβ42) on single F- and D-connections, low- (1–200 nM) and high-dosages (0.3–1 μM) of Aβ were continuously bath-applied while recording of EPSPs. The failure rate, synaptic dynamics and STP were investigated. The experimental procedure of applying Aβ was successfully performed in 48% of recorded connections (48 out of 100 pairs: rats, PC–PC, n = 35 pairs and PC–IN, n = 7 pairs; ferrets, PC–PC, n = 6 pairs). Unfortunately, synaptic responses of the other 52 connections became unstable or even disappeared in the middle of Aβ application. These unstable connections were excluded from the data analysis.

**INFLUENCES OF Aβ ON FAILURE RATES OF F- AND D-CONNECTIONS**

Presynaptic APs can fail to induce the release of neurotransmitter resulting in failures of evoked EPSPs. F-connections generally display higher failure rates than do D-type connections because the initial release probability of F-connections is usually lower. Single APs in presynaptic cells were generated at 0.5 Hz and EPSP failure rates of F- and D-connections were observed, respectively, under conditions of pre-application (in ACSF only), Aβ application and washout. An example of widening transmission failure in an F-type synapse exposed to 1 μM Aβ40 and moderate recovery upon washout is given in Figure 2A. Surprisingly, we found that lower doses of Aβ40 tended to reduce the synaptic failure rate in F-connections. In contrast, the transmission failure rate became increased under all other studied conditions, including low-dose Aβ40-bathed D-connections, high-dose Aβ40 or low-dose Aβ42 applied to F- or D-connections (Figure 2B). The increase in failure rate was statistically significant for the cases of low and high doses of Aβ40 to D-connections (P = 0.01 and P = 0.024, respectively). A trend toward higher failure rate, although not statistically significant, was clearly visible in the cases of high-dose Aβ40 to F-connection (n = 3) and low-dose Aβ42 to F- (n = 3) and D- (n = 4) connections. The opposing directions in failure of synaptic responses was present in Figure 2C, wherein, the net failure reduction in the case of low-dose Aβ40 to F-connections was opposite in direction to the net failure increase in all other cases. After washout for 10–30 min, these contrasting net changes in failure rates virtually remained (Figure 2D). Note the failure rate was further increased in the case of low-dose Aβ42 to F- and D-connections following washout (Figure 2B, far right set: compared with pre-application, P = 0.04; compared with Aβ application, P = 0.05). This phenomenon indicates that Aβ42 is selectively more toxic to synaptic connections in the PFC.

**DIFFERENTIAL EFFECTS OF Aβ ON SYNAPTIC DYNAMICS OF F- AND D-CONNECTIONS**

EPSP trains generated by 5–8 APs and RTR 500 ms later were used in a phenomenological modeling strategy to estimate dynamic

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**FIGURE 1 | Excitatory synaptic connections in layer V of PFC.** (A) A facilitating (F-type) PC-PC connection in layer V of the PFC of an 8-weeks old ferret. Left panel: 3D computer reconstruction of the connection: Both pre- (PC1 in black) and post-synaptic (PC2 in green) cells are complex PCs featured by an apical dendrite with multiple early-bifurcated major branches. A total of 20 putative synapses are marked with red stars onto the basal, apical, oblique and tuft dendrites of PC2. Right panel: Physiological responses of pre- (upper, in black) and post-synaptic (middle, in green) cells were induced by injections of depolarization currents into their somata. Excitatory postsynaptic potentials (EPSPs, down, in red) were recorded from PC2 by giving brief current injections to induced action potentials (APs, bottom, in black) in PC1. The postsynaptic response train is composed of 8 EPSPs at a 20 Hz frequency followed by a recover test response (RTR) with 500 ms delay. (B) A depressing (D-type) PC-IN connection in layer V of the PFC of a P30 rat. The color coding for the reconstructed pre- and post-synaptic cells and their physiological and synaptic responses are the same as the PC-PC connection in (A). A total of 7 putative synapses are marked with red stars onto basal dendrites of the postsynaptic interneuron. Note: The postsynaptic interneuron appears to be a fast-spiking basket cell according to its axonal and dendritic morphologies and fast AP firing induced by depolarization current injection to its soma.
Synaptic failure rates vary according to synaptic type and upon Aβ species and concentration. (A) Superimposed 15 single EPSP traces recorded at 0.5 Hz from an F-type connection in pre-application, application and washout phases of 1 μM Aβ40. EPSPs were generally reduced and the number of failures increased during application of Aβ for 20 min, which tended to recover on washout for 10 min. (B) Average failure rates of F- and D-connections in pre-application, application and washout phases in the presence of Aβ were charted according to Aβ species and concentration, and connection type. The synaptic failure rate tended to decrease in F-connections after low-dose Aβ40 applied. In contrast, the failure rates appear to increase in all other cases. Low and high doses of Aβ40 applied to D-connections reached significance (P = 0.01 and P = 0.024, respectively). A trend to enhance failure rates is shown in the case of high-dose Aβ40 to F-connection (n = 4) and low-dose Aβ42 to F- (n = 3) and D- (n = 4) connections. After washout for 10–30 min., the failure rate are further exacerbated in applications of low-dose Aβ42 to F- and D-connections (compared with pre-application, P = 0.04; compared with Aβ application, P = 0.05). Note: * compared with pre-application, # compared with Aβ application; * or # P < 0.05; ** P < 0.01. (C) Net changes in average failure rates following exposure to Aβ (the failure rate in Aβ application - the failure rate in pre-application). The net rate change in low-dose Aβ40 to F-connections was opposite in direction to that of the other cases. The difference between the net rate changes corresponding to low-dose Aβ40 vs. high-dose Aβ40 to F-connections did not quite reach statistically significance possibly due to the low n (n = 3) in the latter case. (D) Net changes to average failure rates by washout of Aβ (the failure rate in washout of Aβ - the failure rate in pre-application). The differential change in failure rates remained virtually similar to that in (C). Notably, the net rate change became smaller (from 41 to 14%) in high-dose Aβ40 to F-connection, but became bigger (from 21 to 49%) in the case of low-dose Aβ42 to F- and D-connections.
enhancement, both subsequent EPSPs and RTR are reduced. In the case of F enhancement, both subsequent EPSPs and RTR are increased.

In accordance to this model (Tsodyks and Markram, 1997; Markram et al., 1998; Wang et al., 2006), EPSP trains evoked by 5–8 presynaptic APs and a RTR recorded after a 500 ms delay were analyzed for the estimation of synaptic dynamics based on their amplitudes and patterns of EPSPs, respectively, in pre-application, various Aβ application and washout conditions (Figure 3). For comparison between pre-application and Aβ...
application and washout, average EPSP amplitudes of individual synaptic connections were first normalized to the mean of EPSPs in pre-application conditions (Figure 3 middle row graphs) of either low dose Aβ40 (Figures 3A,B) or high dose Aβ40 (Figures 3C,D) or low dose Aβ42 (Figures 3E,F) applications, respectively. Next, in order to better present changes in EPSP patterns, the same responses were alternatively normalized to the 1st EPSP of their own trains (Figure 3 lower row graphs).

Compared with the pre-application, EPSP amplitudes were significantly increased in F-connections exposed to low-dose Aβ40 (Figure 3A, n = 12 pairs). The amplitude increase of the EPSP train was followed by a comparably larger increment in the Aβ40 (lower row graph), which indicated the enhanced facilitation, F. In all other conditions examined (low-dose Aβ40 to D-connections, high-dose Aβ40 to F- or D-connections and low-dose Aβ42 to F- or D-connections) (Figures 3B–F), the EPSP amplitudes were all significantly diminished compared to their own pre-applications, respectively, (Table 1). In both low-dose Aβ40 and low-dose Aβ42 to D-connections (Figure 3B, n = 7 pairs; Figure 3F, n = 5 pairs), the amplitudes of EPSPs were evenly reduced and the EPSP pattern virtually remained the same as in the pre-application. This change is represented as a typical reduction in the absolute synaptic strength, A. Interestingly, high-dose Aβ40 and low-dose Aβ42 in F-type connections (Figure 3C, n = 4 pairs; Figure 3E, n = 8 pairs) similarly resulted in an uneven reduction of EPSP trains and RTR, in which the decrements of the 1st EPSPs were greater. This result indicated a reduction in absolute synaptic strength, A (according to a decline in all EPSPs) accompanied by a reduced release probability, U (according to the greater decrements of the 1st EPSPs). In the high-dose Aβ40 to D-connections (Figure 3D, n = 7 pairs), the 1st EPSP was notably reduced while the amplitudes of steady state EPSPs (4th–8th EPSPs) remained unchanged. The notable decrement of the 1st EPSP represented a U reduction, which typically leads to an immediate facilitation of subsequent EPSPs of the train. However, such an immediate facilitation was not visible. Instead, the unchanged steady state EPSPs was followed by a reduced RTR. This phenomenon could be attributed to an interplay of the reduction in both U and A. The immediate facilitation of subsequent EPSPs due to the notable U reduction, would counterbalance the reduction of these EPSPs due to the reduction of parameter A, keeping them unchanged.

High-dose Aβ25–35 was used in a few test recordings considering the fact that this short peptide has neurotoxic action and aggregating property (Chen et al., 2000). The high-dose Aβ25–35 showed inhibiting effects on EPSP trains of F- (n = 2 pairs) and D-connections (n = 3 pairs). The changes in EPSP train induced by Aβ25–35 were similar to those induced by the high-dose Aβ40.

**Effects of Aβ Are Fully Reversible When Applied Briefly and Locally**

It is noteworthy that upon washout of the various Aβ-containing mediums, only the enhancement of F-connections by low-dose Aβ40 recovered (P < 0.05 in Table 1, Figure 3). The reductions in EPSP train and RTR in all the other cases did not (P < 0.05 in Table 1). Rather than reflecting inefficient washout, we suspect a damaging effect on synapses was induced by prolonged Aβ conditions such as in the presence of high concentrations of Aβ40 or low-dose Aβ42. To test this, we briefly applied 1nM Aβ42 (n = 2) or 1 μM Aβ40 (n = 1) locally to a synaptic connection via a “puff” using a 3rd pipette (a representative recording is shown in Figure 4, estimating that the local concentration of Aβ remained close to the concentration in the pipette). Recordings were obtained prior to and at the end of peptide application (only 2 min) and bath washout phases (10 min). The synaptic

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**Table 1 | Comparison results of EPSP trains recorded in pre-application, application and washout of Aβ.**

|                | low-dose Aβ40 | high-dose Aβ40 and Aβ25–35 | low-dose Aβ42 |
|----------------|---------------|-----------------------------|---------------|
|                | F-connection  | D-connection                | F-connection  | D-connection |
| ctrl vs. Aβ    | P = 0.02      | P < 0.001                   | P < 0.001     | P < 0.001    |
| ctrl vs. washout | P = 0.96    | P < 0.001                   | P < 0.001     | P < 0.001    |

Paired student t-test was used with multiple outcome values per connection.

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responses almost completely disappeared at the end of application of Aβ. The recovery of EPSPs, however, began quickly at ~1 min after the application phase of Aβ had terminated and was largely recovering from inhibition within 2 min into the washout (Figure 4, right panel). A full recovery was observed 10 min into the washout (Figure 4, the bottom trace in left panel). These results suggest that brief, highly local exposures of synapses to Aβ (even at a high level of concentration) produce reversible inhibition. With this evidence, the aforementioned irreversible inhibitory effects of bath-applied peptides becomes understandable if either modest diffuse accumulations of Aβ40 or abnormally high levels of Aβ40, may be enough to damage synapse function.

OPPOSITE EFFECTS OF LOW NANOMOLAR Aβ40 AND Aβ42 ON SHORT TERM POTENTIATION OF F-CONNECTIONS

In the excitatory neuronal network of the PFC, the F-type connections prominently exhibit forms of short term potentiation (STP) termed SA and PTP (Wang et al., 2006). We next examined effects of low-dose Aβ40 and Aβ42 on the SA and PTP in F-connections. Compared with pre-application, the low-dose Aβ40 to F-connections significantly enhanced synaptic responses during all recording phases, i.e., pre-tetanus baseline, SA induction and PTP induction (Figure 5A1, paired t-test with multiple outcome values per connection: all P < 0.01, n = 4 connected pairs). After washout for 10–30 min, the pre-tetanus EPSPs recovered (P = 0.557), however, the enhanced EPSPs still remained at a significantly higher level during the SA and PTP phases (Figure 5A1 inset table, both P < 0.01). Thus, on average, the low-dose Aβ40 enhanced pre-tetanus EPSP by 23 ± 6% (Figure 5A2, P = 0.01), which recovered after washout for 10–30 min (P = 0.556). Meanwhile, the induction of SA was enhanced nearly 2-fold by the low-dose Aβ40 (30 ± 13% vs. 16 ± 9% in pre-application, P = 0.141) and the enhancement to nearly 4-fold persisted after washout for 10–30 min (58 ± 16% vs. 16 ± 9%, P = 0.05). Similarly, the induction of PTP was enhanced

**FIGURE 5 | Differential effects of low nanomolar Aβ40 and Aβ42 on the SA and PTP.** (A1) Low-dose Aβ40 enhanced synaptic responses (i.e., EPSPs) under all the recording phases (pre-tetanus baseline, SA induction and PTP induction) in F-connections (all P < 0.01, n = 4 pairs). After washout for 10–30 min, the EPSPs during the pre-tetanus phase recovered to the pre-application level (P = 0.557), but still remained significantly higher during the SA and PTP induction phases (both P < 0.01, inset table). EPSP amplitudes were normalized to the mean of pre-tetanus EPSPs in pre-application. Paired t-test with multiple outcome values per connection was performed between pre-application and Aβ40 application, and between pre-application and washout phases. (A2) Comparison of increments during pre-tetanus, SA and PTP induction in the case of low-dose Aβ40 application. Compared with the baseline level (0 ± 3%) of increment during pre-tetanus phase of pre-application condition, low-dose Aβ40 enhanced the average baseline EPSP by 23 ± 6% (P = 0.01), recovering after a 10–30 min washout (4 ± 4%, P = 0.556). Compared with 16 ± 9% in pre-application, the SA appeared to be enhanced by low-dose Aβ40 to 30% ± 13% (P = 0.141), and remained enhanced at an average level of 58 ± 16% after a 10–30 min washout (P = 0.05). Similarly, compared with 2 ± 2% in pre-application condition, the PTP appeared to be enhanced by low-dose Aβ40 to 5 ± 3% (P = 0.284), and remained enhanced to a statistically significant level after a 10–30 min. washout (10 ± 2%, P = 0.01). (B1) Low-dose Aβ42 depressed synaptic responses at the pre-tetanus baseline, and significantly at the SA and PTP inductions (both P < 0.01, n = 6 pairs). After a 10–30 min. washout, the EPSPs under all the recording phases (pre-tetanus, SA induction and PTP induction) became significantly depressed (P < 0.01, inset table). (B2) Comparison of increments during pre-tetanus, SA and PTP inductions in low-dose Aβ42 applications. Compared with the baseline level (0 ± 2%) of increment during the pre-tetanus phase of pre-application condition, low-dose Aβ42 depressed the average baseline EPSP by ~6 ± 5% (P = 0.08). This became statistically significant after washout (~28 ± 4%, P < 0.01). Compared with 33 ± 5% in pre-application, the SA was significantly depressed by low-dose Aβ42 to 22 ± 5% (P = 0.01), recovering after washout (27 ± 7%, P = 0.530). Compared with 6 ± 1% of the increment in pre-application, the PTP was significantly depressed by low-dose Aβ42 to ~4 ± 1% (P = 0.01), again recovering after washout (6 ± 2%, P = 0.154). *P < 0.05; **P < 0.01.
more than 2-fold by the low-dose Aβ40 (5 ± 3% vs. 2 ± 2% in pre-application, \( P = 0.284 \)) and the enhancement to 5-fold persisted after washout (10 ± 2% vs. 2 ± 2%, \( P < 0.01 \)).

In contrast, low-dose Aβ42 significantly depressed synaptic responses corresponding to the SA and PTP inductions (Figure 5B1, both \( P < 0.01, n = 6 \) connected pairs). After washout for 10–30 min, the EPSPs comprising the SA and PTP phases of STP were further depressed (both \( P < 0.01 \)).

Meanwhile, the EPSPs of the pre-tetanus baseline were also significantly depressed (Figure 5B1 inset table, \( P < 0.01 \)).

In Figure 5B2, the opposing actions of low-dose Aβ42 highly contrast the actions of low-dose Aβ40 in Figure 5A2.

The experiments in the current study have explored the effects of soluble monomer predominant extracellular Aβ peptides on synaptic failure rates, synaptic dynamic properties and STP (including SA and PTP) of single excitatory connections in normal PFC. The PFC is highly vulnerable to the effects of aging and neurodegeneration but is relatively understudied in AD. To our knowledge, these are the first whole-cell patch clamp recordings from pairs of individual connections formed by pyramidal neurons in PFC that examine Aβ modulation and toxicity on chemical synaptic transmissions. The advantage of this technique over more conventional field studies is that influences from other afferents and reverberant circuits and influences by exciting neuromodulatory fibers are virtually avoided. In addition, the results are highly repeatable based on single synaptic connections that are classified according to their unitary synaptic dynamics.

We found that the transmission involving individual synaptic connections was significantly enhanced or reduced depending on their intrinsic type (facilitating or depressing), the tested Aβ species (40 or 42 amino acids) and concentration (low dose 1–200 nM vs. high dose 0.3–1 \( \mu \)M). Our main findings are that bath applications of low nanomolar Aβ40 have opposite actions on basal and STP properties of F-connections compared with high nanomolar Aβ40 or low nanomolar Aβ42. Specifically, when applied to F-connections, low nanomolar Aβ40 reduces failure rate and enhances EPSP trains and SA and PTP, whereas higher nanomolar Aβ40 and low nanomolar Aβ42 alike inhibit them. Interestingly, low nanomolar Aβ40 inhibits D-connections, acting similarly thereon as high nanomolar Aβ40 or low nanomolar Aβ42.

In addition, the inhibitory effects of these bath-applied peptides often appeared irreversible despite long-time washout. Nevertheless, reversibility could be demonstrated when Aβ was applied very locally, briefly and followed with a thorough washout.

Normal concentrations of Aβ in CSF and plasma are in the picomolar range (Bohrmann et al., 1999; Teunissen et al., 2002; Lewczuk et al., 2004), but likely higher in the synaptic cleft. Our differential results in the nanomolar range may add new insight into the modulatory role of Aβ by balancing facilitation and depression of synaptic connections to influence activity of synaptic networks in the PFC. Specifically, Aβ40 at physiological levels moderately reduced EPSP failure rate and significantly enhanced EPSP trains and STP of F-connections while damping D-connections. The net functional result would be to enhance network activity relevant to working memory while limiting incoming distracting signals, respectively. The first study to show that Aβ40 actually increased LTP was Wu et al. (1995), moreover the effect was noticed at 200 nM, same as our “low dose” upper limit. In addition, our results add to the notion from other work that Aβ40 could actually have a beneficial role to moderate Aβ42 effects (Kim et al., 2007). They are also in line with the differential effects of Aβ42 and Aβ25–35 peptides on hippocampal network activation, specifically on \( \theta \), \( \beta \), and \( \gamma \) oscillations (Adaya-Villanueva et al., 2010). However, the possibility is not excluded that Aβ42 at much lower levels such as in the picomolar range also plays a similar physiological role as does Aβ40 in synaptic modulation. In a former study of recordings from hippocampal slices, low picomolar concentrations of Aβ42 caused a marked increase of long-term potentiation in excitatory cells, whereas high nanomolar concentrations lead to the reduction of the potentiation (Puzzo et al., 2008). It may be necessary to study the effects of picomolar Aβ42 on single synaptic connections in future experiments.

In recent years, it has been found that Aβ is physiologically released from synaptic terminals depending on the levels of synaptic activity (Kamenetz et al., 2003; Cirrito et al., 2005). In turn, Aβ may play an inhibitory feedback role to balance the homeostasis of neuronal networks (Kamenetz et al., 2003; Hsieh et al., 2006; Venkitaramani et al., 2007). A feature of this feedback loop is that Aβ42 peptides are eventually cleared by endocytosis and diffusion (Venkitaramani et al., 2007). Our results imply that Aβ40 at high nanomolar concentrations or Aβ42 at concentrations as studied here induced an inhibition that might serve as feedback to limit synaptic activity and Aβ production. This is supported by our observation that inhibition of synaptic responses fully recovers when Aβ (40 or 42) is applied briefly and locally followed by a prompt washout (which may be closer to the physiological processes.
of endocytosis and diffusion). Conversely, inhibition becomes more difficult to recover from after longer-time bath applications of these peptides. These considerations make it likely that the toxic effects of these peptides on synaptic functions become irreversible once they accumulate near synapses to concentrations that overload endocytic and enzymatic removal mechanisms.

The potential toxicity to synaptic function as evidenced by the resistance to recovery following washout could result from the formation of aggregated Aβ oligomers around synapses aided by long bath application times and high concentrations. The Aβ aggregation is dependent on protein concentration and time (Harper and Lansbury, 1997). Aggregated Aβ oligomers may suppress synaptic responses by disrupting synaptic vesicle endocytosis (Kelly and Ferreira, 2007), inhibiting NMDA receptors (Chen et al., 2002) and P/Q-type calcium currents (Nimmrich et al., 2008) and/or via forming artificial ion pores on neuronal membranes (Small et al., 2009). Aβ40 on the other hand may enhance synaptic facilitation by acting on P-type calcium channels, but once forming oligomers appears to lose the facilitating effect, turning to suppressing responses (Ramsden et al., 2002). This could explain the opposite effects of Aβ40 at low vs. high concentrations as observed in the current study. Since Aβ42 aggregates more readily than the other Aβ species (Snyder et al., 1994), it is not surprising that Aβ42 might only enhance synaptic activity at picomolar levels (Puzzo et al., 2008). Otherwise, Aβ42 induces synaptic depression at concentrations at or above the low nanomolar range. In recent years, several studies reported that extrasynaptic NMDA receptors are activated by Aβ oligomers, leading to synaptic dysfunction. Soluble Aβ oligomers increase activation of extrasynaptic NR2B receptors inhibiting NMDAR-dependent LTP (Li et al., 2011). Aβ oligomers also reduce baseline synaptic transmission and spontaneous neuronal network activity and induce retraction of synaptic contacts (Ronicke et al., 2011), some of which may be dependent on extrasynaptic sites of action. Prolonged activation of extrasynaptic NMDAR by Aβ oligomers may also play a key role in pathogenic mechanisms of glutamate excitotoxicity (Stanika et al., 2009), and cell death (Hardingham et al., 2002; Papadia and Hardingham, 2007). While our results pertain to synaptic dysfunction at the resolution of single synaptic connections, future studies are foreseeable to address any extrasynaptic contributions.

An important factor to consider is whether such effects of Aβ peptides on synaptic transmission and plasticity occur at the pre- or post-synaptic element. With our research scheme, this can be speculated upon according to changes in the synaptic dynamic parameters, D-F-U-A. The enhancement to F-connections by low-dose Aβ40 occurs via an increase in the parameter F, a presynaptic mechanism. This is further supported by the enhancement to the SA and PTP by the low-dose Aβ40. It is well-known that synaptic facilitation is mediated by presynaptic residual calcium (Kamiya and Zucker, 1994; Mongillo et al., 2008) and the induction of SA and PTP relies on pre-synaptic mechanisms (Hempel et al., 2000; Zucker and Regehr, 2002). It has also been reported that Aβ acts via presynaptic mechanisms as a positive endogenous modulator for hippocampal synapses in rodent hippocampal cultures and slices (Abramov et al., 2009). The enhancement of F-connections we observe could therefore be related to an effect of Aβ40 acting on P-type calcium channels, a pre-synaptic calcium channel that mediates synaptic facilitation (Ramsden et al., 2002; Tamse et al., 2003; Ilegorova et al., 2010). Both low-dose Aβ40 and Aβ42 inhibit D-connections via reducing synaptic strength, reflected in parameter A. The A parameter represents the synaptic response when the probability of synaptic transmitter release equals 1 at the maximal level. Therefore, changes in synaptic strength, A, basically represents alterations in the postsynaptic elements. High-dose Aβ40 inhibits both F- and D-connections via reducing the release probability, U, and the synaptic strength, A, which involves both pre- and post-synaptic mechanisms. With respect to the same mechanisms, low-dose Aβ42 inhibits F-connections. Experimentally, an inhibition on presynaptic transmission is reported after Aβ42 injection through a block of vesicle fusion in the terminal (Moreno et al., 2009), and the inhibition by Aβ on postsynaptic sites has previously been verified to occur at multiple molecular structures such as AMPA receptors and metabotropic glutamate receptors (Puchti and Sweat, 1962; Wang et al., 2004; Hsieh et al., 2006; Shemer et al., 2006; Minano-Molina et al., 2011).

Acting on both pre- and post-synaptic sites, Aβ peptides are likely to have multiple actions on multi-synaptic activity in neuronal networks. Low nanomolar concentrations of Aβ40 significantly enhance synaptic facilitation and both SA and PTP forms of STP of F-connections, meanwhile, inhibiting the synaptic strength (A) of D-connections. This finding suggests that Aβ40 differentially enhances F-connections via presynaptic sites and inhibits D-connections via postsynaptic sites. High nanomolar Aβ40, on the other hand, inhibits both F- and D-connections through reductions in both U and A parameters. Aβ42, even at low nanomolar concentrations, inhibits not only D-connections by reducing A, but also to F-connections by reducing both U and A. These peptides at relatively higher concentrations are therefore expected to play a physiological negative feedback role and/or to produce toxic effects on synaptic functions via both pre- and post-synaptic mechanisms. In addition to pre- and post-synaptic regulation of synaptic activity by physiological levels of Aβ and the depression of excitatory transmission by pathological levels, Aβ peptides are also shown to trigger aberrant synchronous circuit activity, even epileptic discharges, at the network level (Minkeviene et al., 2009; Palop and Mucke, 2010). Our previous work shows that high levels of soluble Aβ may be involved in aberrant synchronous circuit activity via enhancing neuronal excitability and acting on electrical networks. Here again, physiological levels of Aβ act oppositely playing a negative feedback role to dampen electrical network activity by reducing neuronal excitability (Wang et al., 2009). Future patch clamp recording of inhibitory synaptic connections formed between interneuron and PC pairs can further address this issue from another point of view.
The PFC network has the capacity to support persistent activities during recurrent weak inputs, without resorting to the metabolic expenditure of AP generation, precisely because of some special built-in functions such as synaptic facilitation and predominant STP (Hempel et al., 2000; Wang et al., 2006; Mongillo et al., 2008). Facilitation lastings hundreds of milliseconds (and outlasting depression), SA lasting up to 10 s, and PTP lasting up to minutes are each likely to be important mechanisms to sustain network activity during short-term storage and manipulation processes such as working memory tasks (Magleby, 1987; Fisher et al., 1997; Mongillo et al., 2008). STP is an especially important correlate in the PFC to organizing sequential behavior, mental flexibility and planning (Grafman, 1995; Hempel et al., 2000; Mongillo et al., 2008). Understanding endogenous modulators of the working memory network and its processes is increasingly important to the cognition and neurodegeneration fields. At the resolution of single excitatory synaptic connections, our results show that Ab may be a homeostatic modulator and play multiple roles depending on intrinsic synapse types, soluble Ab species and their levels in the synaptic environment. Thus, we predict that Ab influences persistent neuronal activity during working memory tasks in the PFC. High concentrations and mild accumulation of Ab around synapses likely lead to declines in memory and cognition such as in the early stages of AD.

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APPENDIX

FIGURE A1 | Western blot monitoring of Aβ stock solutions. Monomer predominant preps as shown in lane 1 were used for the recording of single synaptic connections. Synthetic Aβ peptides were solubilized in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), aliquoted, dried and stored at −80°C. A working stock solution (0.1 mM) was then prepared and stored at −80°C for dilution immediately before use. Aged or oligomerized preps as appear in lane 5 were not used in the current work.

FIGURE A2 | Computer simulation of synaptic responses. The 10 Hz EPSP train followed by a RTR with 500 ms delay was recorded from a PC-IN connection (A). Using a model of dynamic synaptic transmission, the synaptic dynamics were assessed by fitting the EPSPs (B). The modeling extracts four key parameters of the connection: DFUA (D, the time constant of recovery from depression; F, the time constant of recovery from facilitation; U, utilization of synaptic resources used analogously to release probability, p; A, the absolute synaptic strength). If samplings were big enough, these parameters could be quantitatively analyzed enabling a quantitative comparison of basal synaptic dynamic changes induced by Aβ. Trends of changes induced by Aβ were revealed despite low samplings in most data sets (see Table A1).

Table A1 | Changes of synaptic dynamic properties in the modeling analysis.

|                      | A     | U     | D     | F     | F/D   |
|----------------------|-------|-------|-------|-------|-------|
| Low-dose Aβ40        | Figure 3A | F-connection | control (n = 11) | 3.68 | 0.24 | 300 | 589 | 1.97 |
|                      |       |       | Aβ (n = 11) | 3.17 | 0.29 | 248 | 926 | 3.73 |
|                      |       |       | washout (n = 9) | 3.86 | 0.16 | 291 | 1071 | 3.69 |
| High-dose Aβ40&Aβ25–35 | Figure 3B | D-connection | control (n = 6) | 2.61 | 0.50 | 359 | 165 | 0.46 |
|                      |       |       | Aβ (n = 6) | 2.21 | 0.39 | 623 | 457 | 0.73 |
|                      |       |       | washout (n = 3) | 1.50 | 0.42 | 594 | 382 | 0.64 |
|                      | Figure 3C | F-connection | control (n = 3) | 4.68 | 0.32 | 235 | 807 | 3.43 |
|                      |       |       | Aβ (n = 3) | 3.92 | 0.26 | 243 | 905 | 3.73 |
|                      |       |       | washout (n = 3) | 9.75 | 0.28 | 200 | 896 | 4.49 |
|                      | Figure 3D | D-connection | control (n = 6) | 1.83 | 0.48 | 679 | 149 | 0.22 |
|                      |       |       | Aβ (n = 5) | 1.36 | 0.42 | 430 | 465 | 1.08 |
|                      |       |       | washout (n = 5) | 1.72 | 0.34 | 902 | 223 | 0.25 |
| Low-dose Ab42        | Figure 3E | F-connection | control (n = 7) | 5.94 | 0.39 | 382 | 588 | 1.54 |
|                      |       |       | Aβ (n = 7) | 5.84 | 0.26 | 428 | 1093 | 2.55 |
|                      |       |       | washout (n = 3) | 4.97 | 0.31 | 451 | 964 | 2.14 |
|                      | Figure 3F | D-connection | control (n = 5) | 3.71 | 0.55 | 646 | 119 | 0.25 |
|                      |       |       | Aβ (n = 5) | 3.71 | 0.55 | 646 | 119 | 0.25 |
|                      |       |       | washout (n = 3) | 2.90 | 0.45 | 826 | 114 | 0.14 |
|                      |       |       | Aβ (n = 5) | 2.90 | 0.45 | 826 | 114 | 0.14 |
|                      |       |       | washout (n = 3) | 2.54 | 0.47 | 825 | 142 | 0.17 |