Supplementary Information

Spike bursts increase amyloid-β 40/42 ratio by inducing a presenilin-1 conformational change

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Supplementary Figures

Supplementary Figure 1 Pharmacological manipulations perturbing spontaneous neuronal activity uniformly regulate Aβ40 and Aβ42 isoforms in rat hippocampal cultures.

(a) Blocking spikes by 1 μM tetrodotoxin reduced [Aβ40]₀ by 34% and [Aβ42]₀ by 31%

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(TTX, n = 3, *P < 0.05); blocking GABA<sub>A</sub> receptors by 20 μM gabazine increased [Aβ<sub>40</sub>]<sub>o</sub> by 93.5% and [Aβ<sub>42</sub>]<sub>o</sub> by 103% (GBZ, n = 3, **P < 0.01); ↑Ca/Mg ratio resulted in increase of [Aβ<sub>40</sub>]<sub>o</sub> by 45% and [Aβ<sub>42</sub>]<sub>o</sub> by 51% (↑Ca/Mg, n = 3). All the treatments have been applied for 1h at 37 °C. (b) The Aβ40/42 ratio associated with single and burst patterns in acute hippocampal slices for bursts consisting of 5 AP @ 100 Hz with increased inter-burst interval (30 sec, n = 3). (c) [Aβ<sub>40</sub>]<sub>o</sub> measurements during single spikes and spike bursts in hippocampal slices of WT mice, App<sup>−/−</sup> mice (n.d. - non detected, n = 3), and WT slices pretreated for 3h by β-secretase inhibitor (BSI, BACE1 inhibitor IV, 5 μM, n = 3) or by γ-secretase inhibitor (GSI, L-685,458, 2 μM, n = 3). (d) [Aβ<sub>42</sub>]<sub>o</sub> measurements during single spikes and spike bursts in the same experiments as described in (c). Data represent the mean ± s.e.m. ANOVA analysis with post hoc Dunnett’s multiple comparison tests indicated significance. *P < 0.05, **P < 0.01, ***P < 0.001.
Supplementary Figure 2 Differential regulation of Aβ40 and Aβ42 isoforms by temporal pattern of afferent input in rat hippocampal cultures. (a) [Aβ40]o and [Aβ42]o measured during different stimulation patterns (at constant mean rate of 1 Hz). [Aβ40]o was 47% higher during spike bursts (n = 4) and 44% higher during “natural” discharges (n = 4) comparing to single spikes. Conversely [Aβ42]o was not significantly altered by bursts (n = 4, P > 0.05). (b) Aβ40/42 ratio was higher during regular spike bursts (n = 4) and “natural” bursts (n = 4) comparing to single spikes. (c) Comparison between Aβ40/42 ratio measured under spontaneous neuronal activity and stimulation by single pattern at 1 Hz (n = 6, P > 0.3). (d) Analysis of APP processing by western blot in rat hippocampal slices. 3600 stimuli were delivered for 1h by single spikes (s) or spike burst (b) as described in Fig. 1c. The homogenates of hippocampal slices were assessed for the levels of full length APP (FL-APP), soluble APP (sAPPα), APP C-terminal fragments (CTFα) and for actin as a house keeping gene. Left: Representative western blots of FL-
APP, sAPP\(\alpha\), CTF\(\alpha\) and actin in rat hippocampal slices. \textit{Right:} There was no significant differences between FL-APP, sAPP and \(\alpha\)CTF following single and burst stimulation patterns (\(n = 4\) per each group, \(P > 0.5\)). *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\).
**Supplementary Figure 3** Measurements of the intracellular Aβ40 and Aβ42 concentrations in acute hippocampal slices during periods of single and burst inputs. (a) Effects of TeTx (33 nM, n = 4) and BAPTA-AM (100 μM, n = 4) on [Aβ40]i during single spikes and spike bursts. (b) Measurements of [Aβ42]i during single spikes and spike bursts in the same experiments as in (a). (c) Effects of TeTx and BAPTA-AM on Aβ40/42 ratio associated with single and burst patterns (n = 4). (d) TeTx and BAPTA-AM abolished the Aβ40/42 pattern dependency (n = 4). Data represent the mean ± s.e.m. ANOVA analysis with post hoc Bonferroni’s multiple comparison tests. ***P < 0.001.
Supplementary Figure 4 Effects of dynasore on synaptic vesicle endocytosis and exocytosis. (a) Cer-PS1-Cit FRET ratio (FR, defined as F_{Cer}/F_{Cer}) was not affected by changing the temporal stimulation pattern from single spikes to spike bursts (n = 25, P > 0.5, paired t-test) or by increasing stimulation frequency of from 1 to 5 Hz (n = 20, P > 0.9, paired t-test) in a fast timescale of seconds. (b) Dynasore (80 µM) completely inhibits synaptic vesicle recycling detected by FM1-43 dye as described previously\(^1\). Dynasore was added 30 min before and during the second FM1-43 staining. Scale bar: 2 µm. (c) Left: Representative fEPSP recordings in CA3-CA1 connections before (Cnt, DMSO) and after application of dynasore for 30 min during stimulation by bursts (5 spikes at 100 Hz, inter-spike-interval 10 ms, inter-burst-interval, 5 sec). Right: Dynasore, applied for 30 min during burst stimulation, does not affect short-term synaptic plasticity (n = 3, P > 0.6). (d) Increase in the mean stimulation rate through decreasing inter-burst-
interval (IBI) from 5 to 0.5 sec abolished the effect of burst on Cer-PS1-Cit FRET efficiency in the presence of dynasore \((n = 38, P > 0.8)\).
**Supplementary Figure 5** Dependency of Aβ40 and Aβ42 isoforms on synaptic vesicle exocytosis from the presynaptic active zone. (a) [Aβ40]₀ decreased by 95% during single (1 Hz) or burst (5 pulses with inter-spike-interval of 10 ms and inter-burst-interval of 5 s) stimulation patterns in Munc13−/− comparing to [Aβ40]₀ in WT hippocampal cultures (n = 4). (b) Spike-evoked [Aβ42]₀ decreased by 90% in Munc13−/− comparing to [Aβ42]₀ in WT hippocampal cultures (n = 4). ANOVA analysis with post hoc Bonferroni’s multiple comparison tests. ***P < 0.001.
Supplementary Figure 6 Effect of EGTA-AM on short-term synaptic facilitation. (a) Representative recordings of AMPAR-mediated EPSCs (holding potential -70 mV) evoked by single spikes and bursts in CA3-CA1 connections in acute hippocampal slices under control conditions and following application of EGTA-AM (50 μM). Scale bars: 25 pA, 20 ms. (b) The magnitude of short-term plasticity determined as the burst / single ratio of EPSC charge transfer per spike (Q_{EPSC}) was lower following EGTA-AM application (n = 11). (c) Average relative slopes of EPSCs within the burst, normalized to the first peak. EGTA-AM reduced short-term synaptic facilitation (n = 11). Paired t-test, *P < 0.05, **P < 0.01, ***P < 0.001.
Supplementary Figure 7  Effects of presynaptic modulators on synaptic function (a)

Input-output relationship between stimulation amplitude (delivered at 0.1 Hz) and the slope of fEPSP in control slices ($n = 8$, slope of linear fit is $0.78 \pm 0.04$) and in slices following 1h stimulation in the presence of the following drugs: modified ACSF solution containing 1.6 mM Ca$^{2+}$ and 0.8 mM Mg$^{2+}$ (↑Ca/Mg, $n = 6$, slope of linear fit is $1.13 \pm 0.03$), ↑Ca/Mg + 2 μM ω-CgTx ($n = 8$, slope of linear fit is $0.82 \pm 0.03$), ↑Ca/Mg + 1 μM baclofen ($n = 10$, slope of linear fit is $0.77 \pm 0.02$), and ↑Ca/Mg + 2 nM CCPA ($n = 6$, slope of linear fit is $0.49 \pm 0.03$). (b-d) Whole-cell, voltage-clamp recordings of EPSC-AMPA in pyramidal CA1 neurons. Stimulation of SC pathway. (b) Representative recordings of EPSCs evoked by burst before and after application of 1 μM baclofen. (c) Average relative slopes of EPSCs within the burst, normalized to the first peak. Baclofen increased short-term synaptic facilitation ($n = 8$). (d) The magnitude of short-term plasticity determined as the burst / single ratio of EPSC charge transfer per spike ($Q_{EPSC}$) was higher following baclofen application ($n = 8$). Paired t-test, **$P < 0.01$, ***$P < 0.001$. 

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Supplementary Figure 8  Deficits in synaptic and Aβ40 dynamics in the FAD mouse model can be reversed by a GABA<sub>B</sub>R agonist. (a) Representative fEPSP recordings evoked by single and bursts in CA3-CA1 connections in WT, APP/PS1 and baclofen-treated (1 μM, 3h) APP/PS1 slices. Following pre-incubation, recordings were made in normal ACSF. Scale bars: 0.1 mV, 20 ms. (b) Baclofen reversed reduction in synaptic facilitation in APP/PS1 mice (n = 6). (c) The Q<sub>fEPSP</sub> burst / single ratio was lower in APP/PS1 than in WT slices (n = 4 - 6) and reversed by baclofen in APP/PS1 slices (n = 5). (d) Higher [Aβ40]<sub>o</sub> was measured during single spikes in APP/PS1 comparing to WT.
slices (n = 5 - 7), while [Aβ40]₀ did not differ between WT and APP/PS1 during bursts (n = 5 – 7, P > 0.4). Baclofen did not affect [Aβ40]₀ in APP/PS1 slices during single spikes (n = 5 , P > 0.5), while increased it during bursts. (e) [Aβ42]₀ was higher in APP/PS1 comparing to WT slices during either stimulation pattern (n = 5 - 7). Baclofen did not affect [Aβ42]₀ during either pattern (n = 5, P > 0.5). (f) Baclofen increased Aβ40/42 associated with burst patterns in APP/PS1 slices (n = 6). (g) Baclofen rescued Aβ40/42 pattern dependency in APP/PS1 slices (n = 6 - 7). Data represent the mean ± s.e.m. ANOVA analysis with post hoc Bonferroni’s multiple comparison tests. *P < 0.05, **P < 0.01, ***P < 0.001.
Supplementary Figure 9 Verification of Aβ40 and Aβ42 measurements (a - d) by WAKO ELISA kit for human Aβ (n = 3) and (e – h) by Millipore ELISA kit for human Aβ (n = 3). Unpaired t-tests, *P < 0.05, ***P < 0.001.
Supplementary Figure 10 Scheme of the experience-dependent regulation of the Aβ40/42 ratio and its implication to AD etiology. Experience-dependent reduction in release probability (Pr) results in enhanced synaptic and Aβ40 facilitation, inducing shift of PS1 towards an ‘open’ conformation, thus boosting Aβ40/42. In contrast, experience-dependent increase in release probability causes a decrease in synaptic and Aβ40 facilitation during bursts, inducing shift of PS1 towards a ‘closed’ conformation, thus leading to Aβ40/42 decline and subsequent AD pathogenesis.

Reference: