

RESEARCH ARTICLE SUMMARY

NEUROSCIENCE

Secreted amyloid- β precursor protein functions as a GABA_BR1a ligand to modulate synaptic transmission

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INTRODUCTION: More than 30 years have passed since the amyloid- β precursor protein (APP) was identified. Although the role of APP in Alzheimer's disease has been studied widely, its normal physiological function in the brain has remained elusive. APP undergoes ectodomain shedding by α -, β -, or η -secretase to release secreted APP (sAPP α , sAPP β , or sAPP η , respectively). sAPP α affects synaptic transmission and plasticity and is sufficient to rescue synaptic defects in *App* knockout mice. This has led to speculation of a yet unidentified cell-surface receptor for sAPP α .

RATIONALE: To elucidate the physiological function of APP, we sought to identify the cell-

surface receptor mediating its effects on synaptic function. To identify candidate synaptic interactors for sAPP α , we performed affinity-purification experiments using recombinant sAPP α to pull down interacting proteins from synaptosome extracts, followed by mass spectrometric analysis of bound proteins. We identified the γ -aminobutyric acid type B receptor (GABA_BR), the metabotropic receptor for the inhibitory neurotransmitter γ -aminobutyric acid (GABA), as the leading candidate for a synaptic, cell-surface receptor for sAPP α . We then performed a combination of cell-surface binding assays and in vitro biophysical techniques to determine the interacting domains and structural consequences of binding. We inves-

tigated whether sAPP α can modulate GABA_BR function by assessing miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs, respectively) and synaptic vesicle recycling in mouse hippocampal neuron cultures, short-term plasticity in acute hippocampal slices from mice, and in vivo neuronal activity in the hippocampus of anesthetized mice.

RESULTS: Recombinant sAPP α selectively bound to GABA_BR subunit 1a (GABA_BR1a)-expressing cells. Binding was mediated by the flexible, partially structured extension domain in the linker region

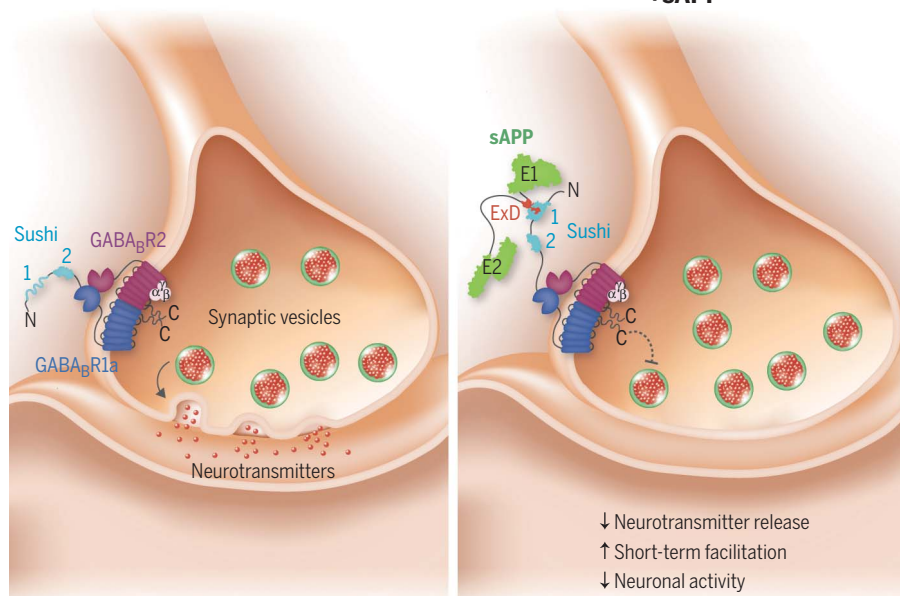
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sAPP β and sAPP η , which both contain the extension domain, also bound to GABA_BR1a-expressing cells. Conversely, APP family members APP-like proteins 1 and 2, which lack a conserved extension domain, failed to bind GABA_BR1a-expressing cells. Acute application of sAPP α reduced the frequency of mEPSCs and mIPSCs and decreased synaptic vesicle recycling in cultured mouse hippocampal neurons. In addition, sAPP α enhanced short-term facilitation in acute hippocampal slices from mice. Together, these findings demonstrate that sAPP reduces the release probability of synaptic vesicles. These effects were dependent on the presence of the extension domain in sAPP and were occluded by a GABA_BR antagonist. A short APP peptide corresponding to the GABA_BR1a binding region within APP stabilized the natively unstructured sushi 1 domain of GABA_BR1a, allowing determination of its solution structure using nuclear magnetic resonance spectroscopy and the generation of a structural model of the APP-sushi 1 complex. Application of a 17-amino acid APP peptide mimicked the effects of sAPP α on GABA_BR1a-dependent inhibition of synaptic vesicle release and reversibly suppressed spontaneous neuronal activity in vivo.

CONCLUSION: We identified GABA_BR1a as a synaptic receptor for sAPP and revealed a physiological role for sAPP in regulating GABA_BR1a function to modulate synaptic transmission and plasticity. Our findings provide a potential target for the development of GABA_BR1a isoform-specific therapeutics, which is relevant to a number of neurological disorders in which GABA_BR signaling is implicated. ■

+sAPP



sAPP is a functional GABA_BR1a-specific ligand. In the presence of sAPP (right), the extension domain (ExD) of sAPP binds the sushi 1 domain specific to GABA_BR1a. Binding induces a conformational change in the sushi 1 domain and leads to increased short-term facilitation and decreased neuronal activity via inhibition of neurotransmitter release. N, amino terminus; C, carboxyl terminus; α , β , and γ , G protein subunits coupled to GABA_BR subunit 2 (GABA_BR2); E1 and E2, sAPP domains.

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RESEARCH ARTICLE

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Secreted amyloid- β precursor protein functions as a GABA_BR1a ligand to modulate synaptic transmission

Heather C. Rice^{1,2}, Daniel de Malmazet^{3,4}, An Schreurs⁵, Samuel Frere⁶, Inge Van Molle⁷, Alexander N. Volkov^{7,8}, Eline Creemers^{1,2}, Irena Vertkin⁶, Julie Nys^{1,2}, Fanomezana M. Ranaivosoa^{9*}, Davide Comoletti^{9,10†}, Jeffrey N. Savas¹¹, Han Remaut⁷, Detlef Balschun⁵, Keimpe D. Wierda^{1,2}, Inna Slutsky⁶, Karl Farrow^{3,4,12,13}, Bart De Strooper^{1,2,14‡}, Joris de Wit^{1,2‡}

Amyloid- β precursor protein (APP) is central to the pathogenesis of Alzheimer's disease, yet its physiological function remains unresolved. Accumulating evidence suggests that APP has a synaptic function mediated by an unidentified receptor for secreted APP (sAPP). Here we show that the sAPP extension domain directly bound the sushi 1 domain specific to the γ -aminobutyric acid type B receptor subunit 1a (GABA_BR1a). sAPP-GABA_BR1a binding suppressed synaptic transmission and enhanced short-term facilitation in mouse hippocampal synapses via inhibition of synaptic vesicle release. A 17-amino acid peptide corresponding to the GABA_BR1a binding region within APP suppressed in vivo spontaneous neuronal activity in the hippocampus of anesthetized Thy1-GCaMP6s mice. Our findings identify GABA_BR1a as a synaptic receptor for sAPP and reveal a physiological role for sAPP in regulating GABA_BR1a function to modulate synaptic transmission.

Amyloid- β precursor protein (APP), a type 1 transmembrane protein, was first identified more than 30 years ago (1–4) as the precursor to the amyloid- β (A β) peptide, the primary constituent of amyloid plaques found in the brains of Alzheimer's disease (AD) patients. APP undergoes ectodomain shedding by α -, β -, or η -secretase to release secreted APP (sAPP α , sAPP β , or sAPP η , respectively) (5, 6). Evidence suggests that the synaptic function of APP (7–13) is carried out by sAPP (14, 15). sAPP α affects synaptic transmission and plasticity, including a reduction in synaptic activity and an enhancement of long-term potentiation (LTP) (16–19). More-

over, sAPP α is sufficient to rescue synaptic defects in *App* knockout (KO) mice, including defects in spine density (20), LTP (21, 22), and spatial learning (27). Together, this has led to speculation of a yet-unidentified cell-surface receptor for sAPP to mediate its synaptic function (15, 23, 24).

Proteomics screen for synaptic interactors of sAPP identifies GABA_BR

We first confirmed, using biochemical fractionation and structured illumination imaging, that APP was abundantly expressed at presynaptic terminals (25) of excitatory and inhibitory hippocampal synapses (fig. S1, A and B). Next, to identify candidate synaptic receptors for sAPP, we performed an extensive series of affinity-purification experiments using recombinant sAPP-Fc [C-terminal Fc tag; affinity purified from transfected-human embryonic kidney (HEK) 293T cell supernatants; fig. S2, A and B] to pull down interacting proteins from synaptosome extracts, followed by mass spectrometric analysis of bound proteins [affinity purification-mass spectrometry (AP-MS)] (Fig. 1A) (26). We consistently identified, among a few intracellular proteins (Fig. 1B; fig. S3, A and B; and table S1), the γ -aminobutyric acid type B receptor subunit 1 (GABA_BR1) as the most abundant and reproducible cell-surface protein, using sAPP α or sAPP β as bait, in wild-type (WT) and in *App/Aplp1* (APP-like protein 1) double KO synaptosome extracts (Fig. 1B; fig. S3, A and B; and table S1). Supporting our observations, APP has previously been identified in a GABA_BR interactome analysis (27). Together, the sAPP AP-MS experiments

identified GABA_BR as the leading candidate for a synaptic, cell-surface receptor for sAPP.

The extension domain of APP binds the sushi 1 domain of GABA_BR1a

GABA_BR, the metabotropic receptor for the inhibitory neurotransmitter γ -aminobutyric acid (GABA), regulates presynaptic neurotransmitter release and postsynaptic membrane excitability (28). It consists of two subunits: GABA_BR1, which binds GABA, and GABA_BR2, which couples to G proteins (29). Two major isoforms, GABA_BR1a and GABA_BR1b, differ by two N-terminal sushi repeats only present in the α -variant (29) (Fig. 1C). To validate the proteomics results, we performed cell-surface binding assays, applying recombinant sAPP α -Fc to HEK293T cells expressing the GABA_BR ectodomain on the plasma membrane using the pDisplay vector. sAPP α -Fc, but not Fc alone, bound strongly to GABA_BR1a-expressing cells, but not to GABA_BR1b- or GABA_BR2-expressing cells (Fig. 1D). Biolayer interferometry experiments using recombinant sAPP α (Fc tag enzymatically removed; fig. S2, C to F) and GABA_BR1a sushi domains showed that the sushi 1 peptide was sufficient for binding sAPP α (Fig. 1E). Accordingly, excess sushi 1 peptide blocked binding of sAPP α -Fc to GABA_BR1a-expressing cells (Fig. 1F). Isothermal titration calorimetry (ITC) determined the dissociation constant (K_D) for sAPP α -sushi 1 to be 431 nM (Fig. 1G). Thus, sAPP α binds directly and selectively to the sushi 1 domain of GABA_BR1a with submicromolar affinity.

The ectodomain of the APP695 isoform of APP contains several functional domains (Fig. 2A). Surprisingly, growth factor-like domain (GFLD)-Fc, copper binding domain (CuBD)-Fc, and E2-Fc each failed to bind GABA_BR1a-expressing cells (Fig. 2B). However, a peptide corresponding to the natively unstructured linker region between the APP695 E1 and E2 domains (Fig. 2A) strongly bound to GABA_BR1a-expressing cells (Fig. 2B). The linker region includes the acidic domain (AcD) and the recently defined extension domain (ExD), which is a flexible, partially structured region (30). The binding affinity of the purified ExD-AcD fragment (Fc tag enzymatically removed) to sushi 1 in ITC experiments (Fig. 2C) was in the same range as that of full-length sAPP α binding to sushi 1 (Fig. 1G). To further narrow down the minimal domain in the APP linker region required for sushi 1 binding, we generated ExD-Fc and AcD-Fc fragments. ExD-Fc, but not AcD-Fc, bound to GABA_BR1a-expressing cells (Fig. 2B), identifying the ExD as the minimal domain required for sushi 1 binding. Consequently, deletion of the ExD in sAPP α (sAPP α Δ ExD-Fc) abolished binding to GABA_BR1a-expressing cells (Fig. 2B). sAPP β -Fc and sAPP η -Fc, a product of the recently described η -secretase processing pathway (6), which both contain the ExD, also bound to GABA_BR1a-expressing cells (Fig. 2D). APP family members APP-like protein 1 and 2 (APLP1 and APLP2) (31), on the other hand, lack a conserved ExD and failed to bind GABA_BR1a-expressing cells (Fig. 2E). Thus, the sAPP ExD is necessary and sufficient to bind to the GABA_BR1a sushi 1 domain.

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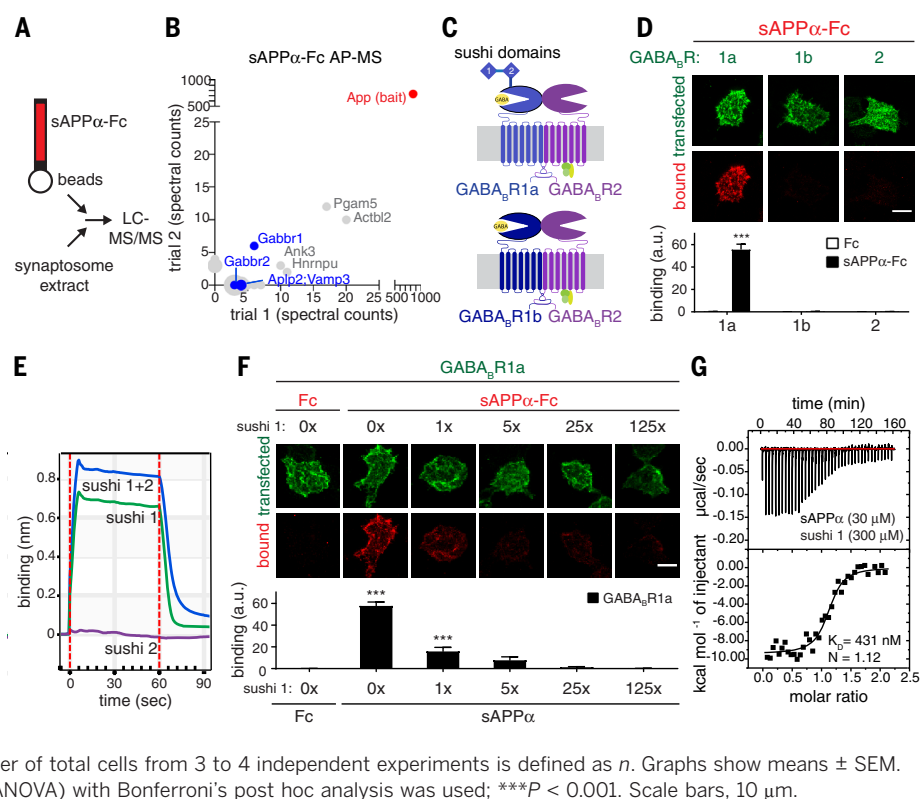
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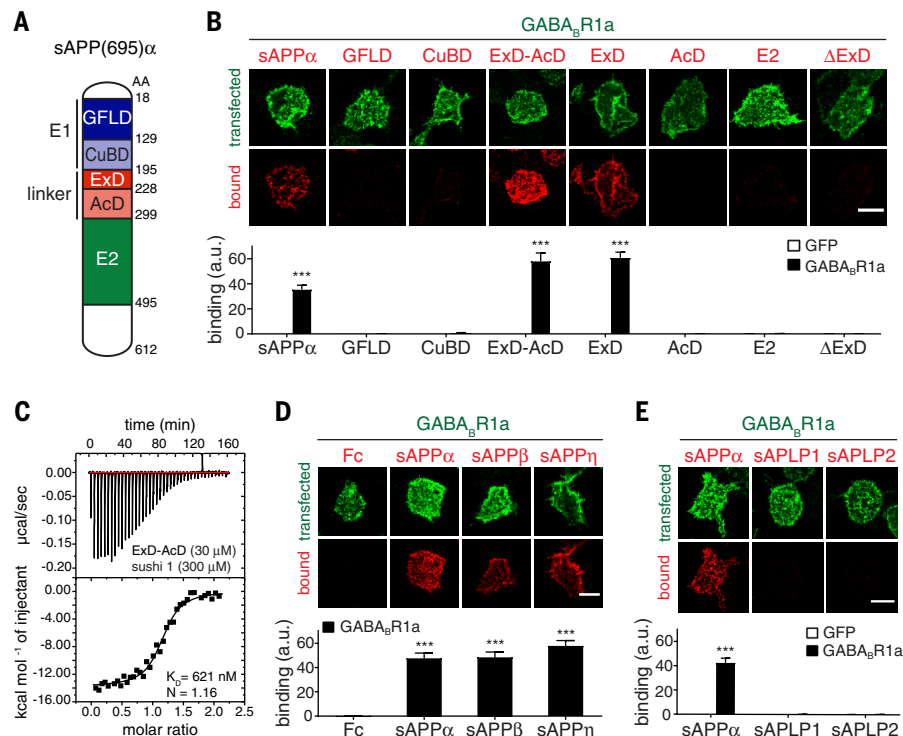
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Fig. 1. sAPP selectively binds the sushi 1 domain of GABA_BR1a.

(A) Cartoon illustrating the AP-MS workflow. LC-MS/MS, liquid chromatography–tandem mass spectrometry. (B) Spectral counts of proteins identified by MS from two independent sAPP α -Fc pull-downs on rat synaptosome extracts. Only proteins which were absent in the Fc controls and present with >2 spectral counts in a single trial are included. Cell-surface proteins are highlighted in blue. (C) Cartoon of GABA_BR subunits and isoforms. (D) Confocal images (top) and quantifications (bottom) of immunostaining for sAPP α -Fc or Fc binding to GABA_BR1a-, 1b-, or 2-expressing HEK293T cells ($n = 24$). a.u., arbitrary units. (E) Binding of sAPP α purified protein to immobilized Fc-tagged sushi 1, sushi 2, or sushi 1 and 2 peptides by biolayer interferometry. (F) Confocal images (top) and quantifications (bottom) of immunostaining for Fc control or sAPP α -Fc binding to GABA_BR1a-expressing HEK293T cells in the presence of increasing concentrations of untagged sushi 1 peptide ($n = 24$ to 31). X notation indicates fold increase in sushi 1 peptide concentration. (G) Binding of purified sAPP α and sushi 1 proteins (Fc-tag enzymatically removed from both constructs) by ITC. The red line indicates baseline. N, stoichiometry. The number of total cells from 3 to 4 independent experiments is defined as n . Graphs show means \pm SEM. Two-way (D) or one-way (F) analysis of variance (ANOVA) with Bonferroni's post hoc analysis was used; *** $P < 0.001$. Scale bars, 10 μ m.

**Fig. 2. The ExD of sAPP binds GABA_BR1a.**

(A) Cartoon of sAPP α domains. AA, amino acid. (B) Confocal images (top) and quantifications (bottom) of immunostaining for sAPP α -Fc, GFLD-Fc, CuBD-Fc, ExD-AcD-Fc, ExD-Fc, AcD-Fc, E2-Fc, or sAPP α ΔExD-Fc binding to green fluorescent protein (GFP)– or GABA_BR1a-expressing HEK293T cells ($n = 24$ to 32). (C) Binding of purified ExD-AcD and sushi 1 proteins (Fc-tag enzymatically removed from both constructs) by ITC. (D) Confocal images (top) and quantifications (bottom) of immunostaining for Fc control, sAPP α -Fc, sAPP β -Fc, and sAPP η -Fc binding to GABA_BR1a-expressing HEK293T cells ($n = 24$ to 30). (E) Confocal images (top) and quantifications (bottom) of immunostaining for sAPP α -Fc, sAPLP1-Fc, or sAPLP2-Fc binding to GFP- or GABA_BR1a-expressing HEK293T cells ($n = 24$). The number of total cells from three to five independent experiments is defined as n . Graphs show means \pm SEM. Two-way (B) and (E) or one-way (D) ANOVA with Bonferroni's post hoc analysis was used; *** $P < 0.001$. Scale bars, 10 μ m.

**sAPP suppresses probability of presynaptic vesicle release via GABA_BR1a**

Sushi domain-containing GABA_BR1a is the predominant isoform localized to presynaptic compartments at excitatory synapses (32–34), where it functions to inhibit neurotransmitter release (28). To test whether sAPP α can modulate GABA_BR

function, we simultaneously measured miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs, respectively), which were separated on the basis of their distinct decay kinetics as described (35), in cultured mouse hippocampal neurons (12 to 17 days in vitro) (Fig. 3A). Consistent with previous observations (36, 37),

acute exposure of hippocampal neurons to 30 μ M baclofen, a GABA_BR agonist, reduced the frequency of mEPSCs by 63 \pm 5% ($n = 14$ cells; $P < 0.001$) (fig. S4, A and B). Likewise, 250 nM sAPP α (Fc tag removed) reduced the frequency of mEPSCs by 39 \pm 5% ($n = 13$ cells; $P < 0.001$) (Fig. 3, B and C), an effect that was already apparent at 25 nM

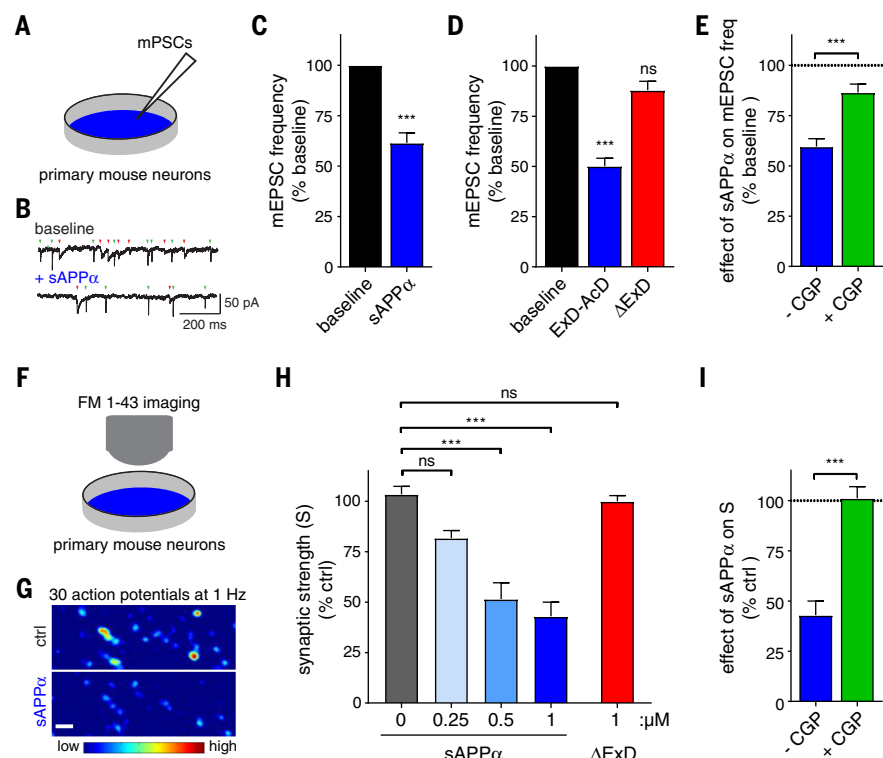


Fig. 3. sAPP α reduces the release probability of synaptic vesicles via presynaptic GABA $_B$ 1a.

(A) Cartoon of mPSC (mEPSC and mIPSC) measurements in cultured hippocampal mouse neurons reported in (B) to (E). (B and C) Example traces of mEPSCs (green arrowheads) and mIPSCs (red arrowheads) (B) and average mEPSC frequency (C) normalized to baseline recorded from primary neurons before (baseline) and after treatment with sAPP α (250 nM, Fc tag enzymatically removed; $n = 13$, $N = 3$, paired Student's t test). (D) Same as (C) but with either ExD-AcD or sAPP α ΔExD (Fc tag enzymatically removed; $n = 17$ to 20, $N = 3$, one-way ANOVA with Dunnett's post hoc analysis). (E) Same as (C) but with sAPP and either without (blue) or with (green) preincubation with CGP55845 (CGP, 5 μ M), a GABA $_B$ antagonist. Dotted line indicates baseline ($n = 14$ to 17, $N = 3$, unpaired Student's t test). (F) Cartoon of FM1-43 measurements in cultured hippocampal mouse neurons reported in (G) to (I). (G) High-magnification ΔF images before and after application of sAPP α (1 μ M, Fc tag enzymatically removed) to primary neurons. ctrl, control. (H) Summary of the dose-dependent inhibitory effect of sAPP α on presynaptic strength (S) ($N = 5$ to 8, one-way ANOVA analysis with post hoc Tukey's analysis). (I) Summary of sAPP α effect on presynaptic vesicle recycling in hippocampal neurons with or without CGP54626 (normalized to control) ($N = 8$). The number of neurons is defined as n , and the number of independent experiments or mice is defined as N . Graphs show means \pm SEM. *** $P < 0.001$; ns, not significant.

(fig. S4, D and E), without affecting mEPSC amplitude (fig. S4C). sAPP β similarly reduced mEPSC frequency (fig. S4, D and E). Acute application of the APP695 ExD-AcD fragment reduced mEPSC frequency to a similar degree as sAPP α (Fig. 3D and fig. S4F), whereas application of sAPP α ΔExD had no effect (Fig. 3D and fig. S4F), indicating that the ExD of sAPP is necessary and sufficient for the suppression of spontaneous glutamatergic synaptic transmission by sAPP α . Accordingly, acute application of sAPLP1, which lacks a conserved ExD, did not reduce mEPSC frequency (fig. S4G), although we observed a minor ($17 \pm 9\%$; $n = 17$ cells; $P < 0.05$) reduction in mIPSC frequency (fig. S4H). Pretreatment with the GABA $_B$ R antagonist CGP55845 (5 μ M) attenuated the sAPP α -mediated reduction of mEPSC frequency (Fig. 3E and fig. S4I), showing that the effect is mediated by GABA $_B$ R.

GABA $_B$ R1a also localizes to GABAergic boutons (34). Consistent with previous observations (37, 38), acute exposure of hippocampal neurons to 30 μ M baclofen reduced the frequency of mIPSCs by $62 \pm 5\%$ ($n = 14$ cells; $P < 0.001$) (fig. S5A). Acute application of 250 nM sAPP α to hippocampal neurons reduced the frequency of mIPSCs by $44 \pm 5\%$ ($n = 13$ cells; $P < 0.001$) (Fig. 3B and fig. S5B). Application of sAPP α caused a minor (14%) reduction in mIPSC amplitude (fig. S5C), possibly owing to a small postsynaptic effect of sAPP α on GABA $_B$ R1a at postsynaptic GABAergic sites (39). The APP695 ExD-AcD fragment, but not sAPP α ΔExD, reduced mIPSC frequency to a similar extent as sAPP α (figs. S4F and S5D). The effect of sAPP α on mIPSC frequency was blocked by pretreatment with the GABA $_B$ R antagonist CGP55845 (5 μ M) (figs. S4I and S5E). Thus, sAPP α acutely reduces both glutamatergic and

GABAergic quantal synaptic transmission through a GABA $_B$ R1a isoform-dependent mechanism.

sAPP α might exert its effect on synaptic transmission by interfering with a complex of full-length APP and GABA $_B$ R1a. In neurons lacking APP, however, sAPP α still reduced mEPSC and mIPSC frequency (fig. S6, A and B), excluding this possibility. Application of 30 μ M baclofen similarly reduced mEPSC and mIPSC frequency in *App/Aplp1* double KO cultures (fig. S6, C and D) as in WT cultures (Fig. 3C and fig. S5B), suggesting that the absence of full-length APP does not cause major alterations in GABA $_B$ R localization to presynaptic terminals. However, the possibility that full-length APP also interacts with and affects GABA $_B$ R signaling separate from the effects of sAPP α reported here cannot be excluded.

The decrease in mEPSC frequency, but not amplitude, following acute sAPP α application suggests a change in presynaptic release properties. We therefore assessed the effect of sAPP α on presynaptic vesicle recycling using the fluorescent membrane dye FM1-43. We measured presynaptic strength by measuring the density (D) of FM1-43-positive boutons per image area and the change in fluorescence intensity (ΔF) of FM1-43 signals at individual boutons of cultured hippocampal neurons using a combined FM1-43 loading-unloading stimulation paradigm (Fig. 3F). Application of sAPP α decreased the total presynaptic strength ($S = \Delta F \times D$) across synaptic populations (Fig. 3G and fig. S7A) in a dose-dependent manner (Fig. 3H), reaching $57 \pm 7\%$ ($n = 8$ experiments; $P < 0.001$) reduction at 1 μ M sAPP α . This decrease was not observed with deletion of the ExD (sAPP α ΔExD, 1 μ M) (Fig. 3H and fig. S7B) and was occluded by the GABA $_B$ R antagonist CGP54626 (10 μ M) (Fig. 3I and fig. S7C), indicating that GABA $_B$ R1a mediates the presynaptic inhibition induced by sAPP α .

sAPP enhances short-term plasticity at Schaffer collateral synapses in a GABA $_B$ R1a-dependent manner

We next assessed the effect of sAPP α on synaptic transmission in an intact circuit at CA3-CA1 Schaffer collateral (SC) synapses, which exclusively contain GABA $_B$ R1a receptors (32). We measured field excitatory postsynaptic potentials (fEPSPs) evoked by low-frequency stimulation (0.1 Hz) at varying intensities (30 to 150 μ A) in CA1 stratum radiatum after 90 min preincubation of acute hippocampal slices with or without 1 μ M sAPP α (Fig. 4A). Treatment with sAPP α reduced fEPSP amplitude and decreased the slope of the input-output (i-o) curve by 23% (fig. S8A), indicating that sAPP α suppresses basal synaptic transmission at SC synapses. To specifically assess if sAPP α affects presynaptic properties, we applied a burst of five stimuli at three different frequencies (20, 50, and 100 Hz) to induce short-term facilitation, which inversely correlates with the probability of neurotransmitter release. Facilitation was higher for each frequency tested in sAPP α -incubated slices compared with control slices (Fig. 4B and fig. S8, B and C). Analysis of the paired-pulse ratio (PPR) for the first two

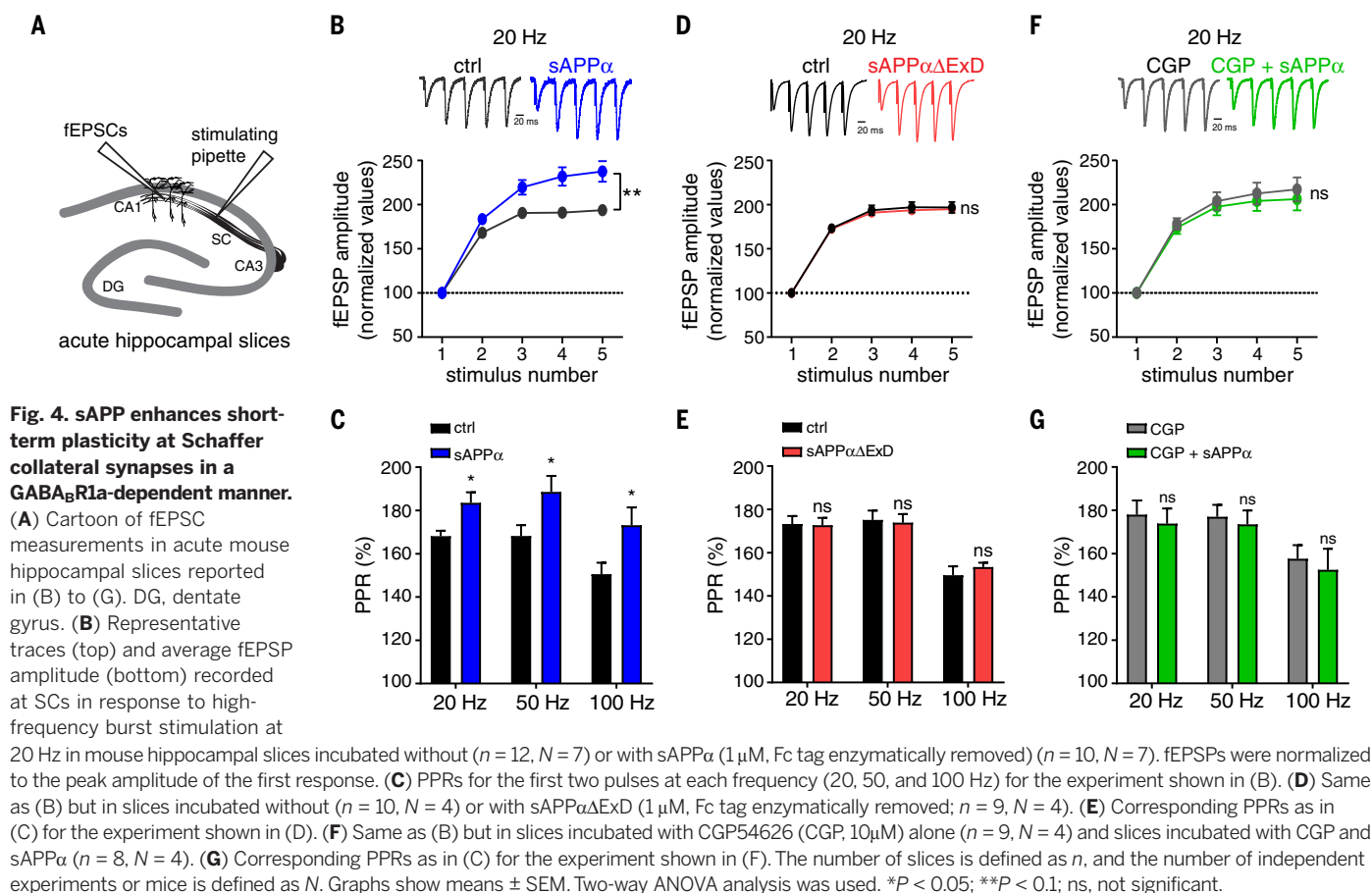
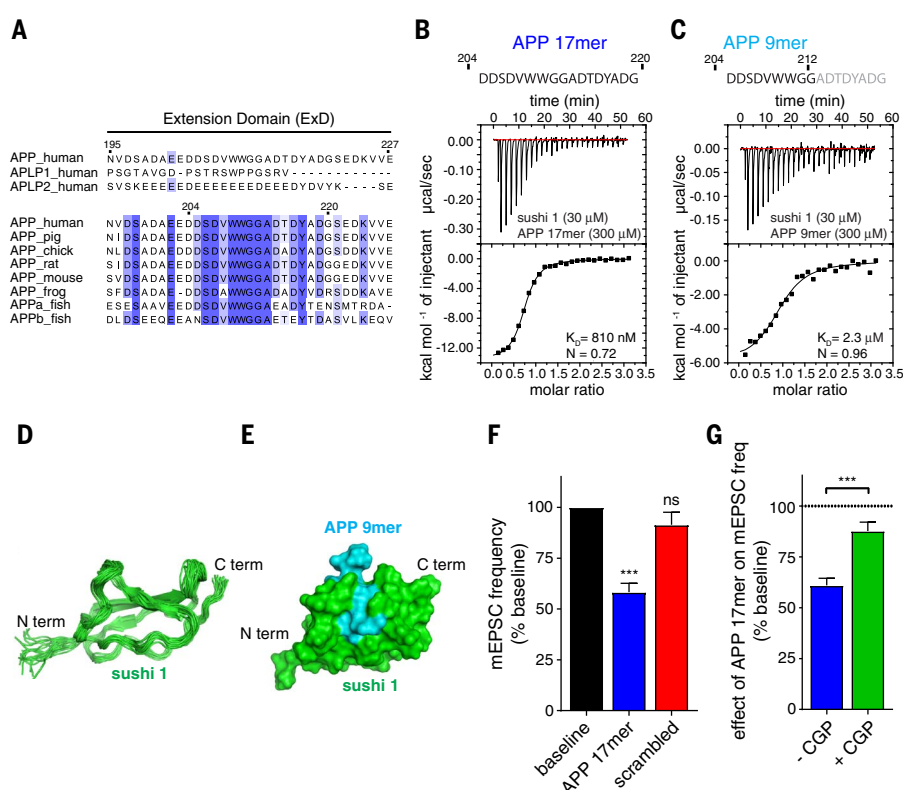


Fig. 5. A short peptide within the APP ExD suppresses synaptic vesicle release via GABA_BR1a. (A) Sequence alignment for the ExD of human APP with APLP1 and 2 and with seven vertebrate APP sequences. Numbers indicate residue positions. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (B and C) ITC binding experiments of purified sushi 1 and synthetic peptides within the ExD corresponding to residues 204 to 220 (B) or residues 204 to 212 (C) of APP695. (D) An ensemble of 20 lowest-energy NMR structures of the sushi 1 domain of GABA_BR1a when bound to the APP 9-mer peptide. (E) A structural model of the complex between the sushi 1 domain of GABA_BR1a (green) and the APP 9-mer peptide (cyan) shown as a molecular surface. Protein termini are indicated by the labels N term and C term. (F) Average mEPSC frequency normalized to baseline recorded from mouse primary neurons before (baseline) and after treatment with 17-mer APP peptide (250 nM, APP695 residues 204 to 220) ($n = 20$, $N = 3$) or scrambled 17-mer control peptide (250 nM; $n = 18$, $N = 4$) (one-way ANOVA with Dunnett's post hoc analysis). (G) Quantification of the effect of 250 nM 17-mer APP peptide (APP695 residues 204 to 220) on mEPSC frequency normalized to baseline either without ($n = 14$, $N = 3$) or with preincubation with CGP55845 (CGP, 5 μ M; $n = 16$, $N = 3$) (unpaired Student's t test). Dotted line indicates baseline. The number of neurons is defined as n , and the number of independent experiments is defined as N . Graphs show means \pm SEM. *** $P < 0.001$; ns, not significant.



stimuli showed an increased PPR for each frequency after sAPP α treatment (Fig. 4C), indicating a decreased release probability. Deletion of the ExD (sAPP α ΔExD, 1 μ M) abolished the sAPP α -mediated effect on the i-o curve (fig. S9D), short-term facilitation (Fig. 4D and fig. S8, E and F), and PPR (Fig. 4E). In addition, preincubation of slices with the GABA $_B$ R antagonist CGP54626 (10 μ M) abolished the sAPP α -mediated decrease in the slope of the i-o curve (fig. S8G) and occluded the sAPP α -induced increase in short-term facilitation and PPR at each frequency (Fig. 4, F and G, and fig. S8, H and I), demonstrating the GABA $_B$ R-dependence of these effects. Thus, sAPP α controls vesicle release at SC synapses by acting on presynaptic GABA $_B$ R1a.

A short peptide within the APP ExD suppresses synaptic vesicle release via GABA $_B$ R1a

A GABA $_B$ R1a isoform-specific modulator has potential therapeutic implications for a number of neurological disorders involving GABA $_B$ R signaling (29). Because we observed that purified protein corresponding to the linker region of

APP (Fig. 2A) was sufficient to mimic the effects of sAPP α on mEPSC frequency (Fig. 3D), we set out to identify the minimally active region within the ExD. Alignment of the sAPP ExD [amino acid residues 195 to 227 of APP695] from seven vertebrate species revealed the strongest conservation within a 17-amino acid stretch (residues 204 to 220; Fig. 5A). The corresponding synthetic APP 17-mer peptide bound sushi 1 of GABA $_B$ R1a with a K_D of 810 nM (Fig. 5B), in the same range as the binding affinity of the entire linker region (Fig. 2C). Shortening the peptide to a synthetic 9-mer consisting of APP695 residues 204 to 212 (APP 9-mer) lowered the K_D to 2.3 μ M (Fig. 5C), whereas residues 211 to 220 failed to bind sushi 1 (fig. S9A). Thus, a conserved, minimal 9-amino acid sequence within the sAPP ExD is sufficient for direct binding to the sushi 1 domain of GABA $_B$ R1a.

To gain further insight into the binding of the APP 9-mer to the GABA $_B$ R1a sushi 1 domain, we used nuclear magnetic resonance (NMR) spectroscopy. As previously reported (40), we observed that the sushi 1 domain of GABA $_B$ R1a is natively unstructured (fig. S9B). Notably, APP 9-mer bind-

ing stabilized the sushi 1 domain of GABA $_B$ R1a, allowing determination of its solution structure (Fig. 5D and fig. S9C) and generation of a structural model of the complex (Fig. 5E). In our model, valine and tryptophan at residues 208 and 209, respectively, of APP695 bind within a pocket of sushi 1, formed by the loops and the short β strand in the N-terminal part of the protein (residues 32 to 53 of full-length GABA $_B$ R1a) (fig. S9D). Thus, APP binding induces a conformational change in the natively unstructured sushi 1 domain of GABA $_B$ R1a. This structure-function relationship strongly supports the physiological relevance of the interaction.

Because the affinity for sushi 1 was better retained in the APP 17-mer compared with the 9-mer (Fig. 5, B and C), we next tested whether the APP 17-mer could functionally mimic sAPP α . Acute application of the APP 17-mer peptide, but not of a scrambled 17-mer control peptide, reduced mEPSC frequency in hippocampal neurons to a similar degree as sAPP α (Fig. 5F and fig. S9E) and was already apparent at 25 nM (fig. S9F). Pretreatment with the GABA $_B$ R antagonist CGP55845 (5 μ M) blocks this effect (Fig. 5G and fig. S9G).

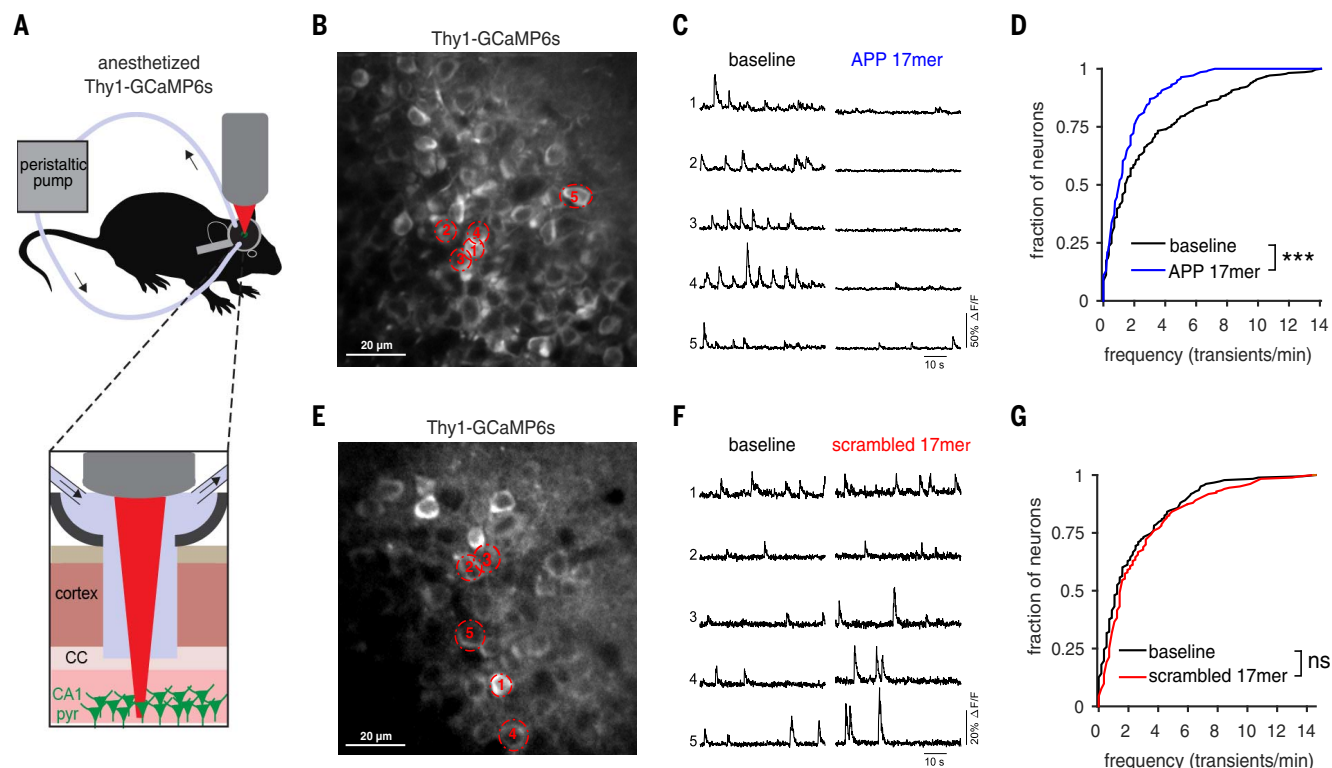


Fig. 6. A 17-amino acid peptide corresponding to the GABA $_B$ R1a binding region within APP suppresses neuronal activity in vivo.

(A) Cartoon of in vivo two-photon calcium imaging of CA1 hippocampus of anesthetized Thy1-GCaMP6s mice with superfusion of APP 17-mer or scrambled control 17-mer. CC, corpus callosum; CA1 pyr, CA1 pyramidal cells. (B) In vivo two-photon image of CA1 hippocampal neurons of Thy1-GCaMP6s mice analyzed in (C) and (D). Representative neurons are indicated with a dotted outline. (C) Calcium traces of five representative neurons, labeled in (A), before (baseline) and during bath application of 5 μ M APP 17-mer peptide corresponding to the GABA $_B$ R1a binding region within APP (APP 17-mer). $\Delta F/F$, change in fluorescence intensity/resting fluorescence

intensity. (D) Cumulative distribution of the frequency of calcium transients at baseline (black line) and during APP 17-mer bath application (blue line) ($n = 277$, $N = 3$). (E) In vivo two-photon image of CA1 hippocampal neurons of Thy1-GCaMP6s mice analyzed in (F) and (G). Representative neurons are indicated with a dotted outline. (F) Calcium traces of five representative neurons, labeled in (D), before (baseline) and during bath application of 5 μ M scrambled 17-mer control peptide (scrambled 17-mer). (G) Cumulative distribution of the frequency of calcium transients at baseline (black line) and during scrambled 17-mer bath application (red line) ($n = 183$, $N = 3$). The number of neurons is defined as n , and the number of mice is defined as N . Wilcoxon rank sum test was used. *** $P < 0.001$; ns, $P > 0.05$ (not significant).

Together, these findings show that the APP 17-mer peptide mimics the effects of sAPP α on GABA $_B$ R1 α -dependent inhibition of synaptic vesicle release.

APP 17-mer peptide suppresses neuronal activity of CA1 pyramidal cells in vivo

In the final series of experiments, we used the APP 17-mer peptide as a tool to examine the effects of sAPP-GABA $_B$ R signaling on neuronal activity in vivo. Using two-photon calcium imaging, we measured calcium transients of CA1 hippocampal neurons in anesthetized transgenic mice expressing the genetically-encoded calcium indicator GCaMP6s under the Thy-1 promoter (Thy1-GCaMP6s) before (baseline) and after a 60 to 90 min superfusion of the exposed hippocampus with either baclofen (30 μ M), APP 17-mer (5 μ M), or scrambled 17-mer control peptide (5 μ M) (Fig. 6A). Application of 30 μ M baclofen caused a dramatic decrease in the frequency of calcium transients compared to baseline (fig. S10, A to C), indicating that activation of GABA $_B$ Rs strongly suppresses neuronal activity in CA1 pyramidal neurons in vivo. Consistent with our results in cultured hippocampal neurons, application of the APP 17-mer significantly reduced the frequency of calcium transients compared to baseline (Fig. 6, B to D, and movie S1). The frequency of calcium transients was restored back to baseline after a 2-hour wash-out of the peptide (fig. S10, D to F), indicating that the suppression of CA1 neuron activity by the APP 17-mer peptide is reversible. Furthermore, the scrambled 17-mer control peptide did not affect the frequency of calcium transients (Fig. 6, E to G; fig. S10, G to I; and movie S2). Thus, APP inhibits neuronal activity in vivo, and the GABA $_B$ R1 α binding domain is sufficient for such inhibition.

Discussion

Here, we found that sAPP acts as a GABA $_B$ R1 α -specific ligand to suppress synaptic vesicle release. Consequently, sAPP modulates hippocampal synaptic plasticity and neurotransmission in vivo. APP is among the most abundant proteins in synaptic boutons (25), and deletion of *App* in mice leads to synaptic deficits (7–9, 21, 22). Synaptic activity enhances proteolytic processing of APP (41, 42), and GABA $_B$ R is a key regulator of homeostatic synaptic plasticity (43). Our observations raise the possibility that the sAPP-GABA $_B$ R1 α interaction acts as an activity-dependent negative-feedback mechanism to suppress synaptic release and maintain proper homeostatic control of neural circuits. Although AD-causing mutations in APP all affect A β generation, it is not entirely clear whether other aspects of APP function contribute to AD. Network abnormalities such as hyperexcitability and hypersynchronization precede clinical onset of AD in human patients (44). Some studies indicate that sAPP levels may be altered in AD (14). Interestingly, a GABA $_B$ R antagonist can improve memory in animal models and patients with mild cognitive impairment (45–47). Moreover, because most transgenic AD mouse models overexpress sAPP, the role of sAPP in synaptic phenotypes of trans-

genic APP mice should be considered, particularly given evidence that network hyperexcitability in these mice is independent of A β production (48).

GABA $_B$ R signaling has been implicated in a number of neurological and psychiatric disorders, including epilepsy, depression, addiction, and schizophrenia (49). Selective binding partners of the GABA $_B$ R1 α sushi domains are of potential therapeutic interest owing to localization and functional differences of GABA $_B$ R1 isoforms (32, 50) as well as the adverse effects of current nonspecific agonists (29). The identification of sAPP as a functional GABA $_B$ R1 α -specific binding partner provides a target for the development of therapeutic strategies for modulating GABA $_B$ R1 α -specific signaling in neurological and psychiatric disorders. The identification of short APP peptides that confer structure in the GABA $_B$ R1 α sushi 1 domain and modulate neurotransmission in vivo are major steps toward development of a GABA $_B$ R1 α isoform-specific therapeutic.

Methods summary

To identify candidate synaptic interactors for sAPP, affinity-purification experiments were performed using recombinant sAPP-Fc to pull down interacting proteins from synaptosome extracts, followed by MS analysis of bound proteins. Cell-surface binding assays, biolayer interferometry, and ITC were used to determine domains of interaction and apparent binding affinities between sAPP and GABA $_B$ R. NMR spectroscopy was used to generate a structural model of the APP-GABA $_B$ R complex. The function of the sAPP-GABA $_B$ R interaction was investigated by accessing spontaneous postsynaptic currents and FM1-43 dye labeling in mouse hippocampal cultures, short-term facilitation in acute hippocampal slices, and two-photon in vivo calcium imaging in CA1 hippocampus of anesthetized Thy1-GCaMP6 mice. The details of each of these methods are described in the supplementary materials.

REFERENCES AND NOTES

- D. Goldgaber, M. I. Lerman, O. W. McBride, U. Saffiotti, D. C. Gajdusek, Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. *Science* **235**, 877–880 (1987). doi: [10.1126/science.3810169](https://doi.org/10.1126/science.3810169); pmid: [3810169](https://pubmed.ncbi.nlm.nih.gov/3810169/)
- N. K. Robakis, N. Ramakrishna, G. Wolfe, H. M. Wisniewski, Molecular cloning and characterization of a cDNA encoding the cerebrovascular and the neuritic plaque amyloid peptides. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4190–4194 (1987). doi: [10.1073/pnas.84.12.4190](https://doi.org/10.1073/pnas.84.12.4190); pmid: [3035574](https://pubmed.ncbi.nlm.nih.gov/3035574/)
- R. E. Tanzi et al., Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science* **235**, 880–884 (1987). doi: [10.1126/science.2949367](https://doi.org/10.1126/science.2949367); pmid: [2949367](https://pubmed.ncbi.nlm.nih.gov/2949367/)
- J. Kang et al., The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* **325**, 733–736 (1987). doi: [10.1038/325733a0](https://doi.org/10.1038/325733a0); pmid: [2881207](https://pubmed.ncbi.nlm.nih.gov/2881207/)
- C. Haass, C. Kaether, G. Thinakaran, S. Sisodia, Trafficking and proteolytic processing of APP. *Cold Spring Harb. Perspect. Med.* **2**, a006270 (2012). doi: [10.1101/cshperspect.a006270](https://doi.org/10.1101/cshperspect.a006270); pmid: [22553493](https://pubmed.ncbi.nlm.nih.gov/22553493/)
- M. Willem et al., η -Secretase processing of APP inhibits neuronal activity in the hippocampus. *Nature* **526**, 443–447 (2015). doi: [10.1038/nature14864](https://doi.org/10.1038/nature14864); pmid: [26322584](https://pubmed.ncbi.nlm.nih.gov/26322584/)
- G. R. Dawson et al., Age-related cognitive deficits, impaired long-term potentiation and reduction in synaptic marker density in mice lacking the beta-amyloid precursor protein.

- Neuroscience* **90**, 1–13 (1999). doi: [10.1016/S0306-4522\(98\)00410-2](https://doi.org/10.1016/S0306-4522(98)00410-2); pmid: [10188929](https://pubmed.ncbi.nlm.nih.gov/10188929/)
- G. R. Seabrook et al., Mechanisms contributing to the deficits in hippocampal synaptic plasticity in mice lacking amyloid precursor protein. *Neuropharmacology* **38**, 349–359 (1999). doi: [10.1016/S0028-3908\(98\)00204-4](https://doi.org/10.1016/S0028-3908(98)00204-4); pmid: [10219973](https://pubmed.ncbi.nlm.nih.gov/10219973/)
- S.-H. Tyan et al., Amyloid precursor protein (APP) regulates synaptic structure and function. *Mol. Cell. Neurosci.* **51**, 43–52 (2012). doi: [10.1016/j.mcn.2012.07.009](https://doi.org/10.1016/j.mcn.2012.07.009); pmid: [22884903](https://pubmed.ncbi.nlm.nih.gov/22884903/)
- S. M. Fitzjohn et al., Similar levels of long-term potentiation in amyloid precursor protein-null and wild-type mice in the CA1 region of picrotoxin treated slices. *Neurosci. Lett.* **288**, 9–12 (2000). doi: [10.1016/S0304-3940\(00\)01204-0](https://doi.org/10.1016/S0304-3940(00)01204-0); pmid: [10869803](https://pubmed.ncbi.nlm.nih.gov/10869803/)
- L. Yang, Z. Wang, B. Wang, N. J. Justice, H. Zheng, Amyloid precursor protein regulates Ca $_v$ 1.2 L-type calcium channel levels and function to influence GABAergic short-term plasticity. *Neuroscience* **29**, 15660–15668 (2009). doi: [10.1523/JNEUROSCI.4104-09.2009](https://doi.org/10.1523/JNEUROSCI.4104-09.2009); pmid: [20016080](https://pubmed.ncbi.nlm.nih.gov/20016080/)
- B. Wang et al., The amyloid precursor protein controls adult hippocampal neurogenesis through GABAergic interneurons. *J. Neurosci.* **34**, 13314–13325 (2014). doi: [10.1523/JNEUROSCI.2848-14.2014](https://doi.org/10.1523/JNEUROSCI.2848-14.2014); pmid: [25274811](https://pubmed.ncbi.nlm.nih.gov/25274811/)
- M. Chen et al., APP modulates KCC2 expression and function in hippocampal GABAergic inhibition. *eLife* **6**, e20142 (2017). pmid: [28054918](https://pubmed.ncbi.nlm.nih.gov/28054918/)
- B. G. Mockett, M. Richter, W. C. Abraham, U. C. Müller, Therapeutic potential of secreted amyloid precursor protein APPs. *Front. Mol. Neurosci.* **10**, 30 (2017). doi: [10.3389/fnmol.2017.00030](https://doi.org/10.3389/fnmol.2017.00030); pmid: [28223920](https://pubmed.ncbi.nlm.nih.gov/28223920/)
- U. C. Müller, T. Deller, M. Korte, Not just amyloid: Physiological functions of the amyloid precursor protein family. *Nat. Rev. Neurosci.* **18**, 281–298 (2017). doi: [10.1038/nrn.2017.29](https://doi.org/10.1038/nrn.2017.29); pmid: [28360418](https://pubmed.ncbi.nlm.nih.gov/28360418/)
- K. Furukawa, S. W. Barger, E. M. Blalock, M. P. Mattson, Activation of K $^+$ channels and suppression of neuronal activity by secreted beta-amyloid-precursor protein. *Nature* **379**, 74–78 (1996). doi: [10.1038/379074a0](https://doi.org/10.1038/379074a0); pmid: [8538744](https://pubmed.ncbi.nlm.nih.gov/8538744/)
- A. Ishida, K. Furukawa, J. N. Keller, M. P. Mattson, Secreted form of β -amyloid precursor protein shifts the frequency dependency for induction of LTD, and enhances LTP in hippocampal slices. *Neuroreport* **8**, 2133–2137 (1997). doi: [10.1097/00001756-199707070-00009](https://doi.org/10.1097/00001756-199707070-00009); pmid: [9243598](https://pubmed.ncbi.nlm.nih.gov/9243598/)
- C. J. Taylor et al., Endogenous secreted amyloid precursor protein- α regulates hippocampal NMDA receptor function, long-term potentiation and spatial memory. *Neurobiol. Dis.* **31**, 250–260 (2008). doi: [10.1016/j.nbd.2008.04.011](https://doi.org/10.1016/j.nbd.2008.04.011); pmid: [18585048](https://pubmed.ncbi.nlm.nih.gov/18585048/)
- M. Xiong et al., Secreted amyloid precursor protein-alpha can restore novel object location memory and hippocampal LTP in aged rats. *Neurobiol. Learn. Mem.* **138**, 291–299 (2017). doi: [10.1016/j.nlm.2016.08.002](https://doi.org/10.1016/j.nlm.2016.08.002); pmid: [27521248](https://pubmed.ncbi.nlm.nih.gov/27521248/)
- S. W. Weyer et al., Comparative analysis of single and combined APP/APLP knockouts reveals reduced spine density in APP-KO mice that is prevented by APPs α expression. *Acta Neuropathol. Commun.* **2**, 36 (2014). doi: [10.1186/2051-5960-2-36](https://doi.org/10.1186/2051-5960-2-36); pmid: [24684730](https://pubmed.ncbi.nlm.nih.gov/24684730/)
- S. Ring et al., The secreted β -amyloid precursor protein ectodomain APPs α is sufficient to rescue the anatomical, behavioral, and electrophysiological abnormalities of APP-deficient mice. *J. Neurosci.* **27**, 7817–7826 (2007). doi: [10.1523/JNEUROSCI.1026-07.2007](https://doi.org/10.1523/JNEUROSCI.1026-07.2007); pmid: [17634375](https://pubmed.ncbi.nlm.nih.gov/17634375/)
- M. Hick et al., Acute function of secreted amyloid precursor protein fragment APPs α in synaptic plasticity. *Acta Neuropathol.* **129**, 21–37 (2015). doi: [10.1007/s00401-014-1368-x](https://doi.org/10.1007/s00401-014-1368-x); pmid: [25432317](https://pubmed.ncbi.nlm.nih.gov/25432317/)
- T. Saitoh et al., Secreted form of amyloid β protein precursor is involved in the growth regulation of fibroblasts. *Cell* **58**, 615–622 (1989). doi: [10.1016/0092-8674\(89\)90096-2](https://doi.org/10.1016/0092-8674(89)90096-2); pmid: [2475254](https://pubmed.ncbi.nlm.nih.gov/2475254/)
- C. Reinhard, M. Borgers, G. David, B. De Strooper, Soluble amyloid- β precursor protein binds its cell surface receptor in a cooperative fashion with glypican and syndecan proteoglycans. *J. Cell Sci.* **126**, 4856–4861 (2013). doi: [10.1242/jcs.137919](https://doi.org/10.1242/jcs.137919); pmid: [23986479](https://pubmed.ncbi.nlm.nih.gov/23986479/)
- B. G. Wilhelm et al., Composition of isolated synaptic boutons reveals the amounts of vesicle trafficking proteins. *Science* **344**, 1023–1028 (2014). doi: [10.1126/science.1252884](https://doi.org/10.1126/science.1252884); pmid: [24876496](https://pubmed.ncbi.nlm.nih.gov/24876496/)
- J. N. Savas et al., Ecto-Fc MS identifies ligand-receptor interactions through extracellular domain Fc fusion protein

- bait and shotgun proteomic analysis. *Nat. Protoc.* **9**, 2061–2074 (2014). doi: [10.1038/nprot.2014.140](https://doi.org/10.1038/nprot.2014.140); pmid: [25101821](https://pubmed.ncbi.nlm.nih.gov/25101821/)
27. J. Schwenk *et al.*, Modular composition and dynamics of native GABA_B receptors identified by high-resolution proteomics. *Nat. Neurosci.* **19**, 233–242 (2016). doi: [10.1038/nn.4198](https://doi.org/10.1038/nn.4198); pmid: [26691831](https://pubmed.ncbi.nlm.nih.gov/26691831/)
 28. M. Gassmann, B. Bettler, Regulation of neuronal GABA_B receptor functions by subunit composition. *Nat. Rev. Neurosci.* **13**, 380–394 (2012). doi: [10.1038/nrn3249](https://doi.org/10.1038/nrn3249); pmid: [22595784](https://pubmed.ncbi.nlm.nih.gov/22595784/)
 29. J.-P. Pin, B. Bettler, Organization and functions of mGlu and GABA_B receptor complexes. *Nature* **540**, 60–68 (2016). doi: [10.1038/nature20566](https://doi.org/10.1038/nature20566); pmid: [27905440](https://pubmed.ncbi.nlm.nih.gov/27905440/)
 30. I. Coburger *et al.*, Analysis of the overall structure of the multi-domain amyloid precursor protein (APP). *PLOS ONE* **8**, e81926 (2013). doi: [10.1371/journal.pone.0081926](https://doi.org/10.1371/journal.pone.0081926); pmid: [24324731](https://pubmed.ncbi.nlm.nih.gov/24324731/)
 31. S. A. M. Shariati, B. De Strooper, Redundancy and divergence in the amyloid precursor protein family. *FEBS Lett.* **587**, 2036–2045 (2013). doi: [10.1016/j.febslet.2013.05.026](https://doi.org/10.1016/j.febslet.2013.05.026); pmid: [23707420](https://pubmed.ncbi.nlm.nih.gov/23707420/)
 32. R. Vigot *et al.*, Differential compartmentalization and distinct functions of GABA_B receptor variants. *Neuron* **50**, 589–601 (2006). doi: [10.1016/j.neuron.2006.04.014](https://doi.org/10.1016/j.neuron.2006.04.014); pmid: [16701209](https://pubmed.ncbi.nlm.nih.gov/16701209/)
 33. B. Biermann *et al.*, The Sushi domains of GABA_B receptors function as axonal targeting signals. *J. Neurosci.* **30**, 1385–1394 (2010). doi: [10.1523/JNEUROSCI.3172-09.2010](https://doi.org/10.1523/JNEUROSCI.3172-09.2010); pmid: [20107064](https://pubmed.ncbi.nlm.nih.gov/20107064/)
 34. P. C. Waldmeier, K. Kaupmann, S. Urwyler, Roles of GABA_B receptor subtypes in presynaptic auto- and heteroreceptor function regulating GABA and glutamate release. *J. Neural Transm. (Vienna)* **115**, 1401–1411 (2008). doi: [10.1007/s00702-008-0095-7](https://doi.org/10.1007/s00702-008-0095-7); pmid: [18665320](https://pubmed.ncbi.nlm.nih.gov/18665320/)
 35. K. D. B. Wierda, J. B. Sørensen, Innervation by a GABAergic neuron depresses spontaneous release in glutamatergic neurons and unveils the clamping phenotype of synaptotagmin-1. *J. Neurosci.* **34**, 2100–2110 (2014). doi: [10.1523/JNEUROSCI.3934-13.2014](https://doi.org/10.1523/JNEUROSCI.3934-13.2014); pmid: [24501351](https://pubmed.ncbi.nlm.nih.gov/24501351/)
 36. M. Scanziani, M. Capogna, B. H. Gähwiler, S. M. Thompson, Presynaptic inhibition of miniature excitatory synaptic currents by baclofen and adenosine in the hippocampus. *Neuron* **9**, 919–927 (1992). doi: [10.1016/0896-6273\(92\)90244-8](https://doi.org/10.1016/0896-6273(92)90244-8); pmid: [1358131](https://pubmed.ncbi.nlm.nih.gov/1358131/)
 37. M. Iyadomi, I. Iyadomi, E. Kumamoto, K. Tomokuni, M. Yoshimura, Presynaptic inhibition by baclofen of miniature EPSCs and IPSCs in substantia gelatinosa neurons of the adult rat spinal dorsal horn. *Pain* **85**, 385–393 (2000). doi: [10.1016/S0304-3959\(99\)00285-7](https://doi.org/10.1016/S0304-3959(99)00285-7); pmid: [10781911](https://pubmed.ncbi.nlm.nih.gov/10781911/)
 38. J. Rohrbacher, W. Jarolimek, A. Lewen, U. Misgeld, GABA_B receptor-mediated inhibition of spontaneous inhibitory synaptic currents in rat midbrain culture. *J. Physiol.* **500**, 739–749 (1997). doi: [10.1113/jphysiol.1997.sp022055](https://doi.org/10.1113/jphysiol.1997.sp022055); pmid: [9161988](https://pubmed.ncbi.nlm.nih.gov/9161988/)
 39. D. Ulrich, B. Bettler, GABA_B receptors: Synaptic functions and mechanisms of diversity. *Curr. Opin. Neurobiol.* **17**, 298–303 (2007). doi: [10.1016/j.conb.2007.04.001](https://doi.org/10.1016/j.conb.2007.04.001); pmid: [17433877](https://pubmed.ncbi.nlm.nih.gov/17433877/)
 40. S. Blein *et al.*, Structural analysis of the complement control protein (CCP) modules of GABA_B receptor 1a: Only one of the two CCP modules is compactly folded. *J. Biol. Chem.* **279**, 48292–48306 (2004). doi: [10.1074/jbc.M406540200](https://doi.org/10.1074/jbc.M406540200); pmid: [15304491](https://pubmed.ncbi.nlm.nih.gov/15304491/)
 41. R. M. Nitsch, S. A. Farber, J. H. Growdon, R. J. Wurtman, Release of amyloid beta-protein precursor derivatives by electrical depolarization of rat hippocampal slices. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 5191–5193 (1993). doi: [10.1073/pnas.90.11.5191](https://doi.org/10.1073/pnas.90.11.5191); pmid: [8506366](https://pubmed.ncbi.nlm.nih.gov/8506366/)
 42. F. Kamenetz *et al.*, APP processing and synaptic function. *Neuron* **37**, 925–937 (2003). doi: [10.1016/S0896-6273\(03\)00124-7](https://doi.org/10.1016/S0896-6273(03)00124-7); pmid: [12670422](https://pubmed.ncbi.nlm.nih.gov/12670422/)
 43. I. Vertkin *et al.*, GABA_B receptor deficiency causes failure of neuronal homeostasis in hippocampal networks. *Proc. Natl. Acad. Sci. U.S.A.* **112**, E3291–E3299 (2015). doi: [10.1073/pnas.1424810112](https://doi.org/10.1073/pnas.1424810112); pmid: [26056260](https://pubmed.ncbi.nlm.nih.gov/26056260/)
 44. J. J. Palop, L. Mucke, Network abnormalities and interneuron dysfunction in Alzheimer disease. *Nat. Rev. Neurosci.* **17**, 777–792 (2016). doi: [10.1038/nrn.2016.141](https://doi.org/10.1038/nrn.2016.141); pmid: [27829687](https://pubmed.ncbi.nlm.nih.gov/27829687/)
 45. Y. Li *et al.*, Implications of GABAergic neurotransmission in Alzheimer's disease. *Front. Aging Neurosci.* **8**, 31 (2016). doi: [10.3389/fnagi.2016.00031](https://doi.org/10.3389/fnagi.2016.00031); pmid: [26941642](https://pubmed.ncbi.nlm.nih.gov/26941642/)
 46. W. Froestl *et al.*, SGS742: The first GABA_B receptor antagonist in clinical trials. *Biochem. Pharmacol.* **68**, 1479–1487 (2004). doi: [10.1016/j.bcp.2004.07.030](https://doi.org/10.1016/j.bcp.2004.07.030); pmid: [15451390](https://pubmed.ncbi.nlm.nih.gov/15451390/)
 47. K. A. Helm *et al.*, GABA_B receptor antagonist SGS742 improves spatial memory and reduces protein binding to the cAMP response element (CRE) in the hippocampus. *Neuropharmacology* **48**, 956–964 (2005). doi: [10.1016/j.neuropharm.2005.01.019](https://doi.org/10.1016/j.neuropharm.2005.01.019); pmid: [15857622](https://pubmed.ncbi.nlm.nih.gov/15857622/)
 48. H. A. Born *et al.*, Genetic suppression of transgenic APP rescues hypersynchronous network activity in a mouse model of Alzheimer's disease. *J. Neurosci.* **34**, 3826–3840 (2014). doi: [10.1523/JNEUROSCI.5171-13.2014](https://doi.org/10.1523/JNEUROSCI.5171-13.2014); pmid: [24623762](https://pubmed.ncbi.nlm.nih.gov/24623762/)
 49. B. Bettler, K. Kaupmann, J. Mosbacher, M. Gassmann, Molecular structure and physiological functions of GABA_B receptors. *Physiol. Rev.* **84**, 835–867 (2004). pmid: [15269338](https://pubmed.ncbi.nlm.nih.gov/15269338/)
 50. J. D. Foster, I. Kitchen, B. Bettler, Y. Chen, GABA_B receptor subtypes differentially modulate synaptic inhibition in the dentate gyrus to enhance granule cell output. *Br. J. Pharmacol.* **168**, 1808–1819 (2013). doi: [10.1111/bph.12073](https://doi.org/10.1111/bph.12073); pmid: [23186302](https://pubmed.ncbi.nlm.nih.gov/23186302/)
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- SUPPLEMENTARY MATERIALS**
www.sciencemag.org/content/363/6423/eaao4827/suppl/DC1
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 Movies S1 and S2
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Secreted amyloid- β precursor protein functions as a GABA B R1a ligand to modulate synaptic transmission

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A physiological function for sAPP?

Although the pathological role of the amyloid- β precursor protein (APP) in Alzheimer's disease is well studied, the physiological role of this protein has remained elusive. Rice *et al.* found that the secreted ectodomain of APP (sAPP) binds to GABA B R1a, the metabotropic receptor for the inhibitory neurotransmitter γ -aminobutyric acid (GABA) (see the Perspective by Korte). Binding suppressed synaptic vesicle release and modulated synaptic transmission and plasticity in mice. A short, 17 –amino acid peptide in APP bound to GABA B R1a's sushi 1 domain, conferring structure to this unstructured domain. Therapeutics targeting this interaction could potentially benefit a range of neurological disorders in which GABA signaling is implicated.

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