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Amyloid β causes excitation/inhibition imbalance through dopamine receptor 1-dependent disruption of fast-spiking GABAergic input in anterior cingulate cortex

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Alzheimer's disease (AD) is the most common cause of dementia in the elderly. At the early stages of AD development, the soluble β -amyloid ($A\beta$) induces synaptic dysfunction, perturbs the excitation/inhibition balance of neural circuitries, and in turn alters the normal neural network activity leading to cognitive decline, but the underlying mechanisms are not well established. Here by using whole-cell recordings in acute mouse brain slices, we found that 50 nM $A\beta$ induces hyperexcitability of excitatory pyramidal cells in the cingulate cortex, one of the most vulnerable areas in AD, via depressing inhibitory synaptic transmission. Furthermore, by simultaneously recording multiple cells, we discovered that the inhibitory innervation of pyramidal cells from fast-spiking (FS) interneurons instead of non-FS interneurons is dramatically disrupted by $A\beta$, and perturbation of the presynaptic inhibitory neurotransmitter gamma-aminobutyric acid (GABA) release underlies this inhibitory input disruption. Finally, we identified the increased dopamine action on dopamine D1 receptor of FS interneurons as a key pathological factor that contributes to GABAergic input perturbation and excitation/inhibition imbalance caused by $A\beta$. Thus, we conclude that the dopamine receptor 1-dependent disruption of FS GABAergic inhibitory input plays a critical role in $A\beta$ -induced excitation/inhibition imbalance in anterior cingulate cortex.

Alzheimer's disease (AD), the leading cause of dementia in the elderly, is characterized by pathological hallmark of extracellular Amyloid β deposits¹. However, it is becoming increasingly clear that at early preclinical stages even before amyloid is deposited, the accumulated soluble $A\beta$ disrupts synaptic transmission, perturbs excitation/inhibition (E/I) balance and alters neuronal networks resulting in the cognitive decline in AD²⁻⁴. AD patients with early-onset dementia have an increased risk of epileptic seizures^{5,6}. Consistently, AD model mice that overexpress $A\beta$ also show hyperexcitation in individual neurons and higher epileptiform activity in cortical and hippocampal networks⁷⁻¹¹. Similarly, $A\beta$ at pathological relevant concentrations cause neuronal hyperexcitation in culture neurons^{9,11-14}. Hence, $A\beta$ -induced neuronal hyperexcitation and epilepsy are believed to represent the excitotoxic effect which leads to neuronal silencing and cognitive deficits^{8,15,16}. However, it is still not well established how $A\beta$ induces neuronal hyperexcitation.

Anterior cingulate cortex (ACC) is a part of the medial prefrontal cortex, which plays a pivotal role in memory, attention and emotion¹⁷⁻¹⁹. Dysfunction of ACC metabolism and functional connectivity are involved in

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aging-related cognitive decline^{20–22}. ACC is one of the earliest affected areas and “epicenters” in AD^{23–26}. ACC is also one of the most selective areas where A β accumulates at the very early stage in AD patients²³. However, how A β influences the local circuits in ACC is elusive.

In ACC, the proper GABAergic inhibitory innervation of excitatory pyramidal cells is important for spatial and temporal dynamics in cognitive processes. Disruption of excitation/inhibition balance is related to many psychiatric diseases such as schizophrenia, epilepsy and autism^{27–30}. Inhibitory interneurons can be classified as fast-spiking (FS) and non-FS cells based on their firing patterns³¹. FS interneuron is the predominant subtype in mammalian neocortex, and it primarily innervates the soma and the axonal initial segment of excitatory pyramidal cells to control action potential (AP) firing and synchronization, whereas non-FS interneurons preferentially target dendrites to control efficacy and plasticity of excitatory inputs^{32–34}. Interestingly in the frontal cortex, the inhibitory innervation of pyramidal cells from FS and non-FS interneurons can be regulated differently by the enriched dopaminergic input from areas such as the ventral tegmental area^{35,36}. Abnormal dopaminergic innervation of FS parvalbumin interneurons has been suggested to exaggerate schizophrenia symptom by disrupting E/I balance³⁷. In AD, A β promotes excessive dopamine release in the frontal cortex³⁸, and dopamine receptor 1 (D1 receptor) is involved in A β -induced epileptic activity³⁹. Nevertheless, whether the dopamine-related signaling pathway is directly involved in A β -induced neuronal hyperexcitation has not yet been studied.

Here by using whole-cell recordings in acute mouse brain slices, we found that 50 nM A β leads to hyperexcitability of excitatory pyramidal cells in ACC through specifically depressing inhibitory synaptic innervation from FS but not non-FS interneurons. We also discovered that perturbation of presynaptic GABA release is the main cause of this inhibitory input disruption. In addition, we identified that the excessive activation of dopamine D1 receptor of FS interneurons leads to A β -induced disruption of inhibitory innervation. More importantly, D1 receptor antagonist SCH23390 can reverse A β -induced hyperexcitability of pyramidal cells. This suggests that the increased dopamine action on D1 receptor of FS interneurons is the key mechanism in this pathological process.

Results

50 nM A β promotes pyramidal cell excitability in ACC. Whole cell recordings, as previously reported^{40,41}, were performed on ACC excitatory pyramidal cells in acute brain slices (Fig. 1A,B). Confocal images and AP firing patterns confirmed the identity of excitatory pyramidal cells (Fig. 1B,C,G). To evaluate the effects of A β on neuronal excitability, a series of depolarizing currents were injected to elicit APs before (Ctrl) and 5–10 mins after 50 nM A β administration (A β) into perfusing artificial cerebrospinal fluid (ACSF). The frequency of APs was dramatically increased after A β administration ($p = 0.0251$, compared with Ctrl, repeated-measures two-way ANOVA, $n = 10$ cells; Fig. 1C,D). Moreover, the input resistance was also significantly increased after A β administration (A β : 164.08 ± 16.50 M Ω , $n = 10$ cells, $p = 0.0372$ compared with Ctrl: 145.83 ± 12.30 M Ω , paired t test; Fig. 1E,F). At the same time, A β also increased the frequency but not the amplitude of spontaneous postsynaptic excitatory currents (sp EPSCs) (frequency: A β : 4.51 ± 0.90 Hz, $n = 8$ cells, $p = 0.0042$ compared with Ctrl: 3.86 ± 0.87 Hz; amplitude: A β : 6.56 ± 0.65 pA, $n = 8$ cells, $p = 0.3830$ compared with Ctrl: 6.06 ± 0.60 pA; paired t test; Supplemental Fig. 1A,B). As a control, the scrambled A β (Scr-A β) had no any effect on the AP firing frequency ($p = 0.6532$, compared with Ctrl, repeated-measures two-way ANOVA, $n = 14$ cells; Fig. 1G,H), input resistance (Scr-A β : 155.35 ± 12.08 M Ω , $n = 11$ cells, $p = 0.8724$ compared with Ctrl: 156.63 ± 10.60 M Ω , paired t test; Fig. 1I,J) and sp EPSCs (frequency: Scr-A β : 3.33 ± 0.80 Hz, $n = 8$ cells, $p = 0.4592$ compared with Ctrl: 3.42 ± 0.73 Hz; amplitude: Scr-A β : 6.72 ± 0.93 pA, $n = 8$ cells, $p =$ compared with Ctrl: 6.73 ± 0.82 pA; paired t test; Supplemental Fig. 1C,D). These results strongly indicate that A β promotes pyramidal cell excitation in ACC. Interestingly, this increased neuronal excitation caused by A β exposure was reversed 30 mins after A β washout (Washout: $p = 0.0287$ compared with A β , $n = 5$ cells; $p = 0.1709$ compared with Ctrl, $n = 5$ cells; $p = 0.0105$, A β compared with Ctrl; repeated-measures two-way ANOVA; Supplemental Fig. 2A,B), implying A β -induced neuronal hyperexcitability in ACC pyramidal cells is an acute effect. Taken together, all the results demonstrate A β exposure can promote neuronal excitation of excitatory pyramidal cells in ACC.

A β promotes pyramidal cell excitability by disrupting presynaptic inhibitory input. To assess if the inhibitory input disruption is related to the enhanced excitation of pyramidal cells caused by A β in ACC, miniature inhibitory postsynaptic currents (mini IPSCs) were recorded from pyramidal cells using high-chloride internal solution with TTX and NBQX incubated in the perfusing ACSF (Supplemental Fig. 3). A β caused a dramatic decrease of both frequency (A β : 2.07 ± 0.77 Hz, $n = 12$ cells, $p = 0.0005$ compared with ctrl: 2.95 ± 1.06 Hz, Wilcoxon signed-rank test; Fig. 2A,B) and amplitude (A β : 7.13 ± 1.27 pA, $n = 12$ cells, $p = 0.0034$ compared with ctrl: 12.12 ± 2.03 pA; Wilcoxon signed-rank test; Fig. 2A,C) of mini IPSCs. This indicates that the enhanced excitation of pyramidal cells during A β exposure is possibly attributed to inhibitory input disruption.

The decreased frequency of mini IPSCs is exclusively due to the perturbed presynaptic GABA release, but the reduced amplitude could be resulted from a decrease in either postsynaptic GABA receptor number or presynaptic multi-vesicular events⁴². We assumed that the reduced postsynaptic GABA receptor number decreases the amplitudes of all mini IPSCs uniformly, whereas the reduced presynaptic multi-vesicular events decreases the number of large-amplitude mini IPSCs selectively because the large-amplitude mini IPSCs are mostly resulted from multi-vesicular events⁴². In order to figure out which mechanism is the main cause of the overall decreased mini IPSCs amplitude, we simulated the amplitude distribution of mini IPSCs (Ctrl: 7595 events; A β : 5939 events) based on Fig. 2B,C, the frequency decreased 30% (from 2.97 Hz to 2.07 Hz) and the amplitude decreased 42% (from 12.12 pA to 7.13 pA) uniformly for all the mini IPSCs. Surprisingly, the number of large-amplitude mini IPSCs after A β administration was dramatically fewer compared to the simulated number (Fig. 2D), hinting the overall decreased mini IPSCs amplitude in Fig. 2A,C is probably due to the reduced presynaptic multi-vesicular events.

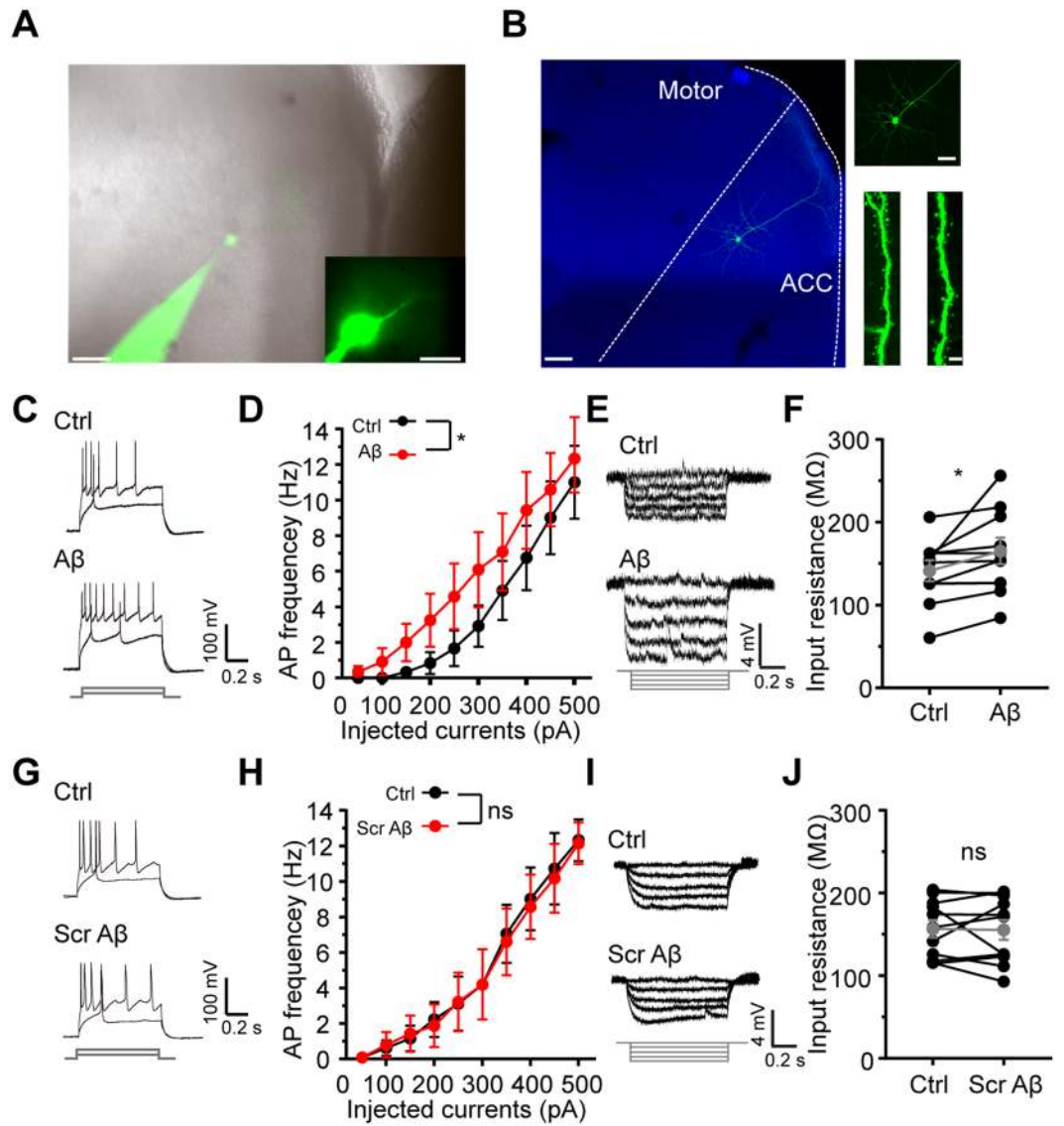


Figure 1. A β induces hyperexcitability of excitatory pyramidal cells in ACC. (A) DIC epifluorescent image of a pyramidal cell in whole-cell configuration in ACC. Alexa 488-conjugated biocytin (green, left) was included in the recording pipette to reveal cell morphology and confirm the cells identity. Scale bars: 100 μ m and 20 μ m; (B) high-resolution confocal scanning image of the pyramidal cell in (A) (left); zoom-in images showed typical pyramidal shape soma (right, top) and apical and basal dendrites and spines (right, down). Scale bars: 100 μ m, 50 μ m and 5 μ m; (C) examples of AP traces induced by 150 pA and 400 pA current injections before and after A β application; (D) quantification of the AP frequencies at different current injections before and after A β application; (E) examples of membrane potential responses induced by serial currents injection from -20 pA to 0 pA with 5 pA interval before and after A β application; (F) quantification of cellular input resistance before and after A β application; (G) examples of AP traces induced by 150 pA and 400 pA current injections before and after Scr-A β application; (H) quantification of the AP frequencies at different current injections before and after Scr-A β application; (I) examples of membrane potential responses induced by serial currents injection from -20 pA to 0 pA before and after Scr-A β application; (J) quantification of cellular input resistance before and after Scr-A β application. (* $p < 0.05$).

To directly prove that the presynaptic GABA release disruption is the main mechanism underlying A β -induced inhibitory input disruption, we recorded mini IPSCs in calcium-free perfusing ACSF as a lowered external calcium concentration can eliminate multi-vesicular events⁴². In calcium-free perfusing fluid, A β caused a significant decrease of the frequency (A β : 0.16 ± 0.03 Hz, $n = 10$ cells, $p = 0.0322$ compared with ctrl: 0.22 ± 0.03 Hz; Wilcoxon signed-rank test; Fig. 2E,F) but not amplitude (A β : 7.14 ± 0.59 pA, $n = 10$ cells, $p = 0.4131$ compared with ctrl: 7.94 ± 0.95 pA; Wilcoxon signed-rank test; Fig. 2E,G) of mini IPSCs. This suggests the impairment of presynaptic GABA release is the main mechanism underlying A β -induced inhibitory input disruption.

To determine whether disruption of GABAergic inhibitory input plays a causal role in the increased excitation of pyramidal cells induced by A β , GABA_A receptor antagonist bicuculline methiodide (BMI) was applied

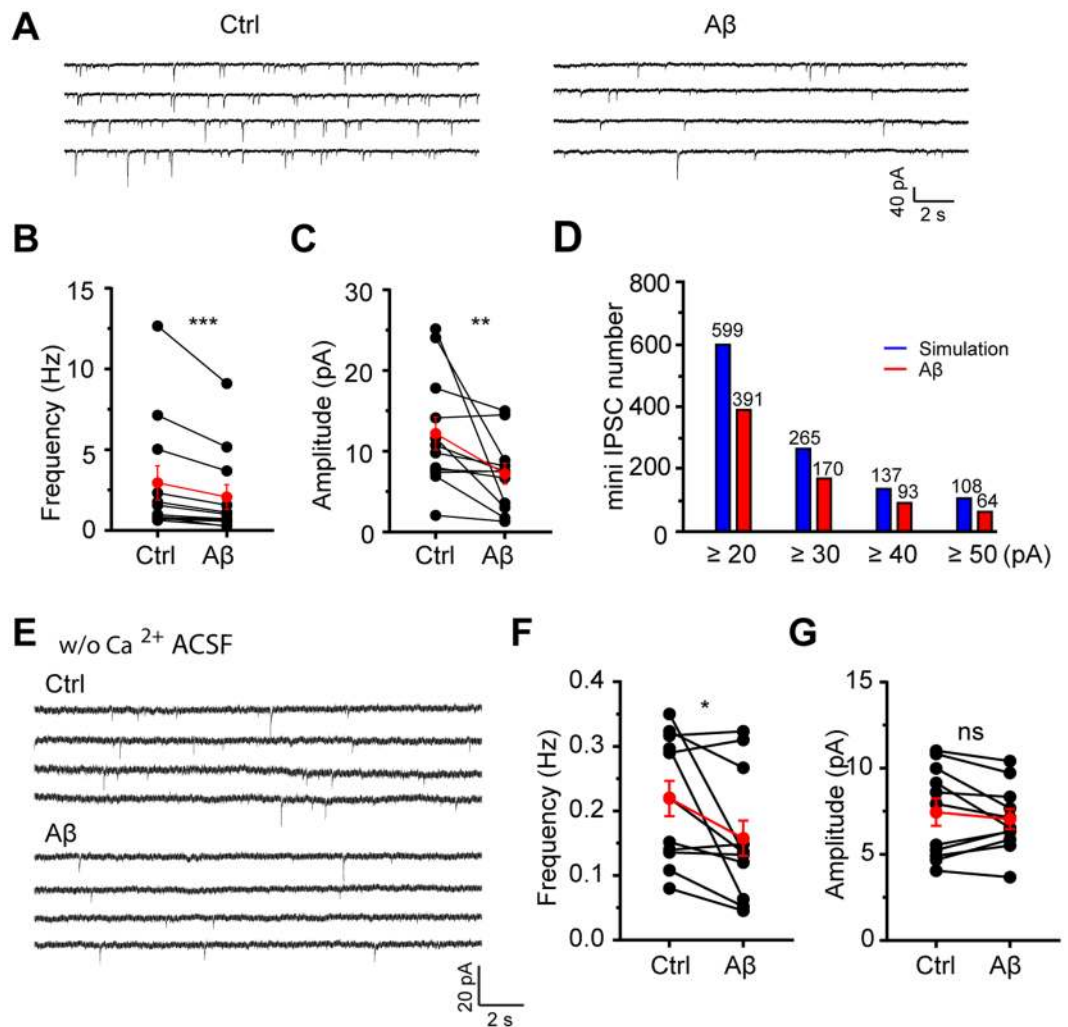


Figure 2. A β disrupts inhibitory input selectively by perturbing presynaptic GABA release in ACC. (A) Example traces of mini IPSCs of excitatory pyramidal cells in ACC before and after A β application; (B) quantification of mini IPSCs frequency before and after A β application; (C) quantification of mini IPSCs amplitude before and after A β application; (D) numbers of large-amplitude mini IPSCs in the simulated and A β conditions. The simulation was done based on the decreased percentages of mini IPSCs frequency and amplitude shown in (B) and (C); (E) example traces of mini IPSCs of excitatory pyramidal cells in ACC before and after A β application recorded in calcium-free perfusing ACSF; (F) quantification of mini IPSCs frequency before and after A β application in calcium-free perfusing ACSF; (G) quantification of mini IPSCs amplitude before and after A β application in calcium-free perfusing ACSF; Wilcoxon signed-rank test (* $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$).

together with A β . With GABA $_A$ receptors blocked by BMI, the frequency of APs evoked by serial currents injection ($p = 0.5344$ compared with Ctrl, repeated-measures two-way ANOVA, $n = 12$ cells; Supplemental Fig. 4A,B) and the input resistance (203.89 ± 19.64 M Ω , $n = 11$ cells, $p = 0.2869$ compared with ctrl: 210.57 ± 19.55 M Ω ; paired t test; Supplemental Fig. 4A,B) were not changed after A β administration, suggesting GABAergic inhibitory input is involved in the A β -induced hyperexcitability of pyramidal cells in ACC.

Taken together, all the above results indicate that A β promotes pyramidal cell excitation by disrupting presynaptic GABAergic inhibitory input.

Inhibitory input from FS interneurons is preferentially disrupted by A β . The predominant interneuron subtype in the neocortex is the FS interneurons, which preferentially targets the soma and the axonal initial segment of pyramidal cells to control AP output and synchronization^{32–34}. In contrast, non-FS interneurons primarily target the dendrites to control the efficacy and plasticity of excitatory inputs onto pyramidal cells^{32–34}. To test if A β uniformly disrupts inhibitory inputs from both FS and non-FS interneurons, we simultaneously recorded interneurons and pyramidal cells to specifically detect unitary inhibitory postsynaptic currents (uIPSCs) from either FS or non-FS interneurons to pyramidal cells. A 500 ms current was injected into cells to evoke the cell types (Fig. 3A,C). A brief current was injected into interneuron to trigger single AP and evoke uIPSCs in pyramidal cells. As previously reported⁴³, the amplitude of uIPSCs from FS interneurons to pyramidal

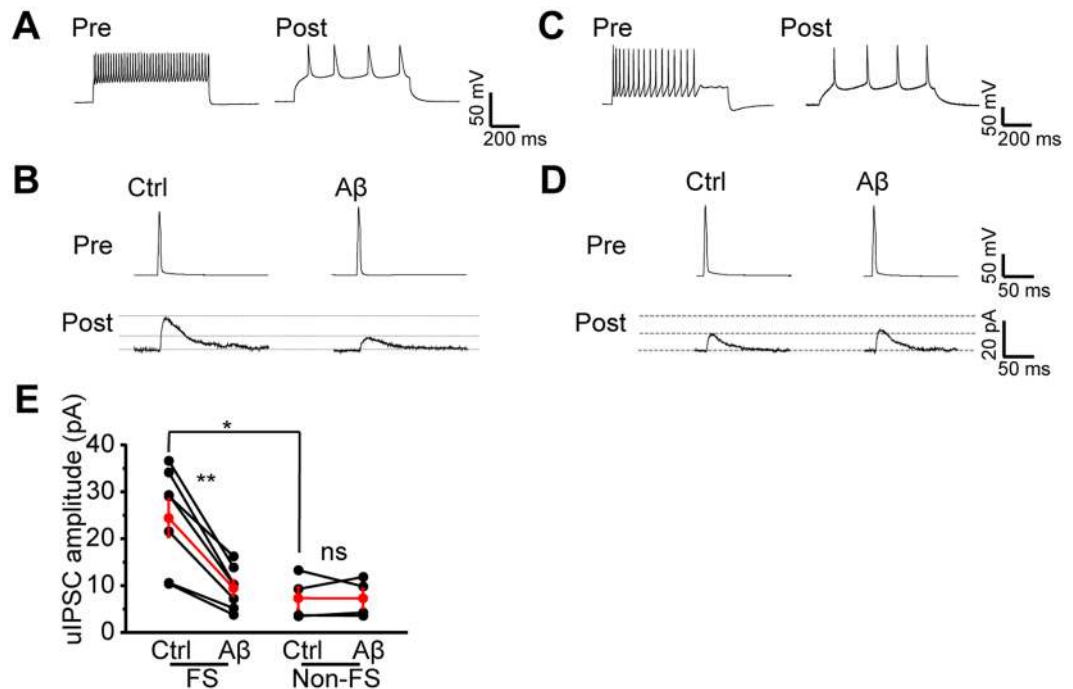


Figure 3. A β disrupts inhibitory synaptic connection from FS but not non-FS interneurons to pyramidal cells. (A) Example AP traces of a pair of nearby FS interneuron and excitatory neuron simultaneously recorded; (B) example traces of uIPSCs triggered by brief current injection to the FS interneuron and recorded in the pyramidal neuron before and after A β application; (C) example AP traces of a pair of nearby non-FS interneuron and excitatory neuron simultaneously recorded; (D) example traces of uIPSCs triggered by brief current injection to the non-FS interneuron and recorded in the pyramidal neuron before and after A β application; (E) quantification of uIPSCs amplitude from FS and non-FS interneurons before and after A β application. (* $p < 0.05$, ** $p < 0.01$).

cells was more robust than that from non-FS interneurons to pyramidal cells (FS: 24.53 ± 4.04 pA, $n = 7$ pairs of cells, $p = 0.015$ compared with non-FS: 7.43 ± 2.37 pA, $n = 4$ pairs of cells, independent t test; Fig. 3B,D,E). A β application caused a dramatic decrease of uIPSCs amplitude from FS interneurons (A β : 9.57 ± 1.71 pA, $n = 7$ pairs of cells, $p = 0.002$ compared with Ctrl: 24.53 ± 4.04 pA, paired t test; Fig. 3B,E) but not non-FS interneurons (A β : 7.37 ± 2.04 pA, $n = 4$ pairs of cells, $p = 0.969$ compared with Ctrl: 7.43 ± 2.37 pA, paired t test; Fig. 3C,E), suggesting that GABAergic inhibitory input from FS interneurons is preferentially disrupted by A β .

To examine if presynaptic GABA release from FS interneurons is affected by A β application, we monitored the paired pulse ratio (PPR) of uIPSCs. We found that the PPR of FS (A β : 0.87 ± 0.03 , $n = 6$ pairs of cells, $p = 0.004$ compared with Ctrl: 0.74 ± 0.02 , paired t test; Fig. 4A–C) but not non-FS (A β : 0.79 ± 0.02 , $n = 4$ pairs of cells, $p = 0.979$ compared with Ctrl: 0.79 ± 0.02 , paired t test; Fig. 4A–C) interneurons was significantly increased after A β administration, suggesting that presynaptic GABA release perturbation counts for the disrupted inhibitory input from FS interneurons.

Excessive activation of D1 receptor is involved in A β -induced disruption of inhibitory input and Inhibition of D1 receptor can restore E/I balance.

GABA release from the axonal terminal of FS interneurons is regulated by dopamine through activating D1 receptor³⁵. The dopaminergic neurons are related to A β -induced pathological processes during early onset of Alzheimer's disease⁴⁴. Moreover, dopamine release can be promoted by nanomolar A β in frontal cortex³⁸ and D1 receptor participates in A β -induced epileptic activity³⁹. To examine if a dopamine-dependent signaling pathway is involved in A β -induced inhibitory input disruption and E/I imbalance, we applied 10 μ M D1 receptors antagonist SCH23390 (SCH) with A β into perfusing ACSF. Interestingly, inhibition of D1 receptor largely ameliorated A β -induced disruption of inhibitory input from FS interneurons (A β + SCH: 42.65 ± 7.19 pA, $n = 7$ pairs of cells, $p = 0.393$ compared with Ctrl: 44.19 ± 8.05 pA, paired t test; Fig. 5A–C). More importantly, D1 receptor antagonist also reversed A β -induced hyperexcitability of excitatory pyramidal cells ($p = 0.1519$ compared with Ctrl, repeated-measures two-way ANOVA, $n = 10$ cells; Fig. 5D,E) and increase of input resistance (A β + SCH: 175.62 ± 9.15 M Ω , $n = 12$ cells, $p = 0.8479$ compared with Ctrl: 176.48 ± 8.67 M Ω , paired t test; Fig. 5F,G). As a control, SCH23390 itself did not change basal excitatory synaptic transmission (sp EPSCs frequency: 4.66 ± 0.54 Hz, $n = 4$ cells, $p = 0.5190$ compared with Ctrl: 4.43 ± 0.35 Hz; sp EPSCs amplitude: 6.08 ± 1.09 pA, $n = 4$ cells, $p = 0.5190$ compared with Ctrl: 6.78 ± 1.37 pA; paired t test; Supplemental Fig. 5). These results suggest that D1 receptor is involved in A β -induced disruption of inhibitory input and E/I imbalance.

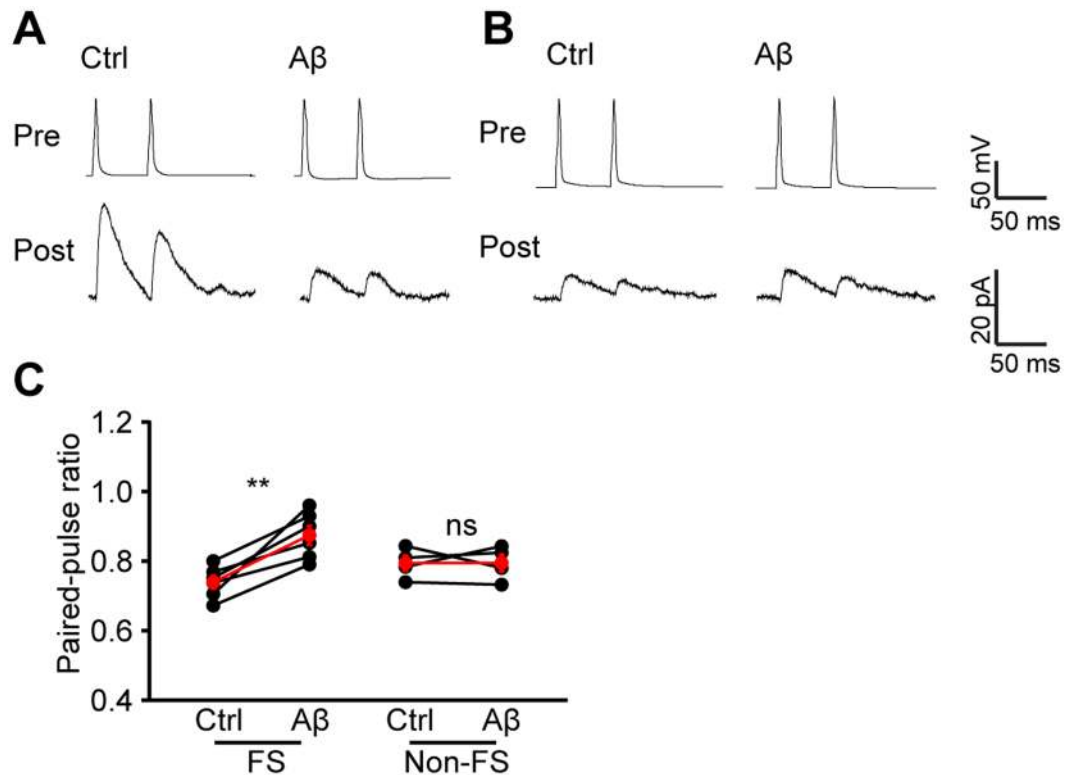


Figure 4. A β disrupts GABA release from FS but not non-FS interneurons. (A) example traces of uIPSCs triggered by brief paired currents injections with 50 ms interval to the same FS interneuron and recorded in the same pyramidal neuron as in Fig. 3 A,B before and after A β application; (B) example traces of uIPSCs triggered by paired brief current injections with 50 ms interval to the same non-FS interneuron and recorded in the same pyramidal neuron as in Fig. 3 C,D before and after A β application; (C) quantification of PPRs from FS and non-FS interneurons before and after A β application. (** $p < 0.01$).

To further confirm that D1 receptor plays a critical role in A β -induced disruption of inhibitory input and E/I imbalance, we applied a D1 receptor agonist to see if activating D1 receptor can mimic and occlude A β -induced disruption of inhibitory input from FS interneurons. Indeed, application of the D1 receptor agonist SKF 38393 (SKF) dramatically decreased uIPSCs amplitude to about 47% (47.26%, $n = 4$ pairs of cells, $p = 0.0015$ compared with Ctrl, paired t test; Supplemental Fig. 6), and application of A β after SKF administration failed to further decrease uIPSCs amplitude (44.74%, $n = 4$ pairs of cells, $p = 0.2466$ compared with SKF; $p = 0.0003$ compared with Ctrl; Supplemental Fig. 6), confirming A β and D1 receptor function in the same signaling pathway to disrupt inhibitory input and cause E/I imbalance.

Thus, taken together, our results show that A β promotes dopamine release from dopaminergic (DAergic) axons in ACC, and the excessive dopamine overactivates D1 receptors of FS interneurons which dramatically inhibits GABA release and then leads to E/I imbalance in ACC (Fig. 6A,B).

Discussion

The present study demonstrates that 50 nM A β leads to hyperexcitability of excitatory pyramidal cells in ACC through specifically disrupting inhibitory input from FS interneurons. Moreover, the perturbation of presynaptic GABA release is the main cause of this inhibitory input disruption. Interestingly, this study also illustrates that excessive dopamine action on D1 receptor on FS interneurons plays a key role in the A β -induced perturbation of inhibitory innervation and hyperexcitability of pyramidal cells.

A β is progressively accumulated during AD development, and A β accumulation-induced disruption of neuronal signaling is the best correlation with neuropathology in AD patients²⁻⁴. Many studies suggest that A β functions in a concentration-dependent manner, low concentrations of A β (pM to nM) can induce presynaptic calcium level increases⁴⁵ and also promote neuronal excitability and plasticity^{12,13,46}, whereas high concentration of A β (μ M) depresses excitatory and inhibitory synaptic transmission or plasticity^{41,47,48}. Nanomolar A β concentrations (1–100 nM) are more likely to aggregate into the small oligomers and fibrils and are believed to be most pathologically relevant in AD patients and in AD animal models⁴⁹. In this study, acute application of 50 nM A β on mouse ACC is shown to disrupt E/I balance and lead to hyperexcitability of pyramidal cells.

Many mechanisms underlying neuronal hyperexcitation have been found in *in vitro* A β exposure paradigms as well as in A β overexpression AD animal models. For example, in cultures of primary mouse hippocampus pyramidal cells, upregulation of α 7-nAChRs is necessary for production of chronic A β -induced neuronal hyperexcitation¹². In transgenic *Drosophila* line that overexpresses a secreted form of the toxic human A β ₁₋₄₂,

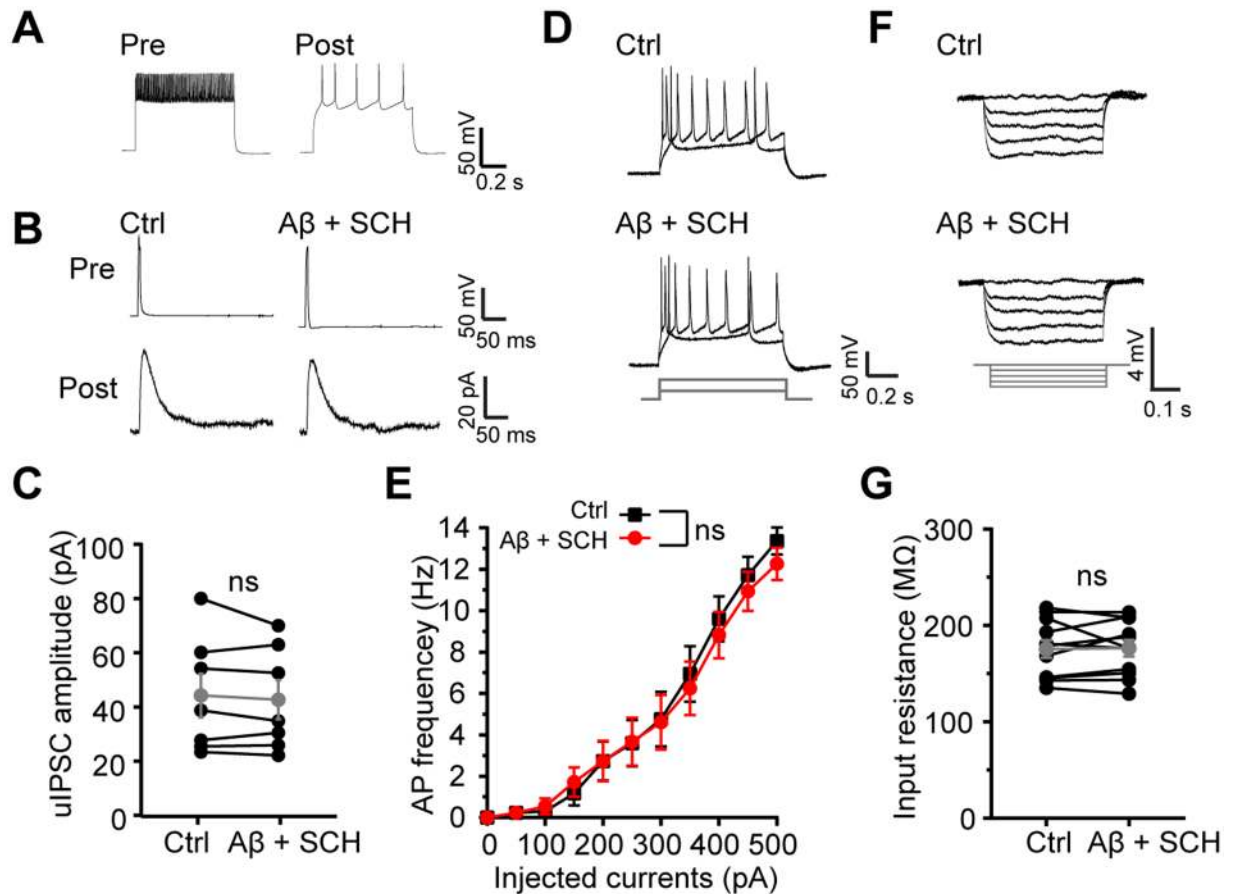


Figure 5. D1 receptor is involved in A β -induced disruption of inhibitory input and hyperexcitability of pyramidal cells. (A) Example AP traces of a pair of nearby FS interneuron and excitatory neuron simultaneously recorded; (B) example traces of uIPSCs triggered by brief current injection to the FS interneuron and recorded in the pyramidal neuron before and after A β + SCH23390 application; (C) quantification of uIPSCs amplitude from FS interneurons before and after A β + SCH23390 application; (D) examples of action potential traces induced by 150 pA and 400 pA current injections before and after A β + SCH23390 application; (E) quantification of the APs frequencies at different current injections before and after A β + SCH23390 application; (F) examples of membrane potential responses induced by serial currents injection from -20 pA to 0 pA before and after A β + SCH23390 application; (G) quantification of cellular input resistance before and after A β + SCH23390 application.

selective degradation of the highly conserved A-type K $^{+}$ channel, Kv4 leads to an increased AP firing and neuronal hyperactivity¹³. In addition, impairment of E/I balance has also been found in APP-overexpressed AD mouse models^{50,51} and in *in vitro* A β exposure⁵⁰. Moreover, the decreased level of the interneuron specific PV cell-predominant voltage-gated sodium channel subunit Nav1.1 leads to decreased inhibitory synaptic activity and enhanced hypersynchrony, memory deficits and premature mortality⁵¹. In our study, we found that disruption of inhibitory input from FS interneurons is responsible for the E/I imbalance caused by acute A β exposure in ACC, and perturbation of presynaptic GABA release leads to the inhibitory input disruption and E/I imbalance.

Inhibitory interneurons can be classified into the Ca $^{2+}$ -binding protein parvalbumin (PV), the neuropeptide somatostatin (SST) and the ionotropic serotonin receptor 5HT3a (5HT3aR) subtypes based on neuronal markers, they can also be classified into FS and non-FS subtypes based on AP firing patterns⁵². The PV FS group accounts for $\sim 40\%$ of GABAergic neurons and are the major source of perisomatic inhibition onto excitatory pyramidal cells^{32,52}. Our results are in line with previous studies to support that FS interneurons play a pivotal role in A β -induced E/I imbalance and hyperexcitability of pyramidal cells.

Enriched dopamine innervation of frontal cortex has been implicated both in the modulation of normal cognitive processes such as working memory formation and in many neurobiological diseases including age-related memory decline and schizophrenia⁵³. Dopamine regulates the recurrent excitatory transmission between pyramidal cells³⁶, it also depresses inhibitory input from FS interneurons and enhances inhibitory input from non-FS interneurons³⁵. Excessive dopamine innervation of ACC FS PV interneurons has been suggested to disrupt E/I balance in schizophrenia³⁷. In AD, A β can promote dopamine release in frontal cortex through activating $\alpha 7$ -nAChRs³⁸, and D1 receptor is also involved in A β -induced epileptic activity in mice³⁹. Here we found that

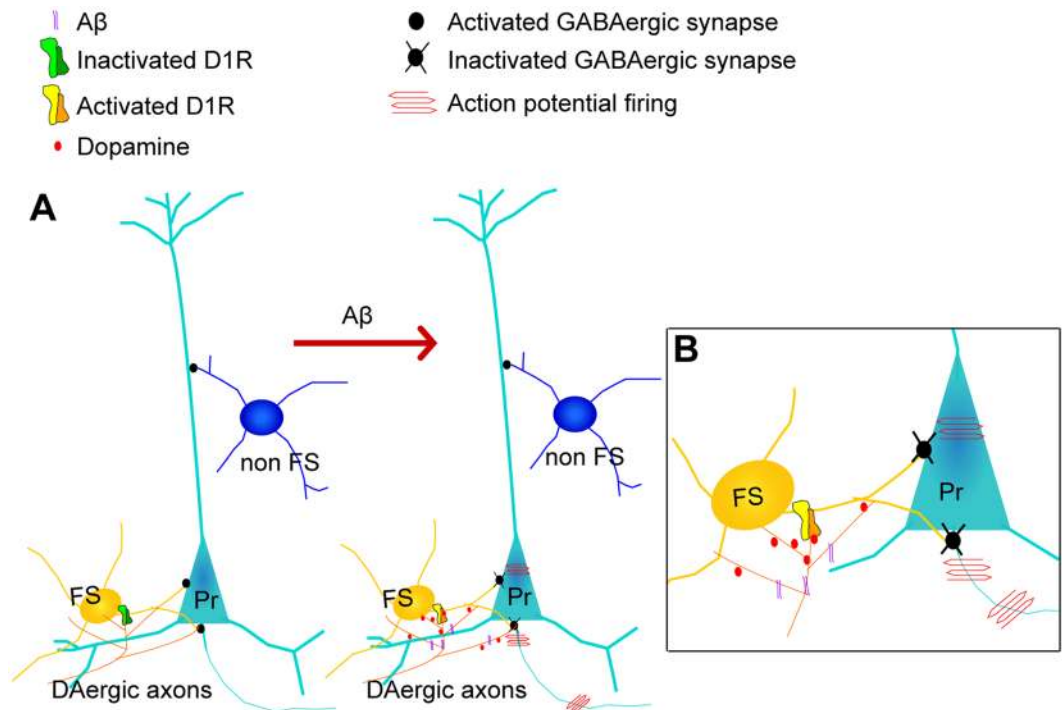


Figure 6. Working model: $A\beta$ induces hyperexcitability of excitatory pyramidal cells through D1 receptor-dependent disruption of GABAergic inhibitory input from FS interneurons. (A) In this model based on our results, we propose that low concentration of 50 nM $A\beta$ promotes dopamine release from dopaminergic (DAergic) axons in ACC, dopamine in turn excessively activate D1 receptors on FS interneurons which dramatically inhibit GABA release from FS interneurons. These sequential events ultimately lead to E/I imbalance and hyperexcitability of excitatory pyramidal cells in ACC; (B) Zoom-in image shows this process.

excessive D1 receptor activation is involved in $A\beta$ -induced E/I imbalance in ACC, highlighting pathological roles the dopamine-D1 receptor signaling pathway plays in AD.

ACC plays a pivotal role in memory, attention and emotion^{17–19}. ACC also represents one of most vulnerable areas and “epicenters” during the pathological course of AD^{23–26}. Here our results illustrate $A\beta$ can cause hyperexcitability of pyramidal cells through D1 receptor-dependent disruption of inhibitory input from FS interneurons in ACC. This indicates that $A\beta$ -induced dysfunction of excitatory, inhibitory and neuromodulatory circuitries in some key areas are the critical pathological mechanisms underlying cognitive decline in AD.

Methods

All animal experiments in this study were approved by Medical Research Ethics Committee of Nanjing Medical University and performed in accordance with the approved guidelines and regulations.

$A\beta_{1-42}$ preparation. $A\beta_{1-42}$ and scrambled peptides were purchased from ChinaPeptides Company in Shanghai, China. Soluble peptide solution was prepared as previously⁴¹. In brief, the peptide was dissolved in dimethyl sulfoxide at a concentration of 10 mM and then diluted 100 times into phosphate-buffered saline (PBS). After that, it was vortexed for 30 min at room temperature and centrifuged 15,000 g at 4 °C for 1 h. The supernatant was immediately aliquoted and stored at –20 °C. Aliquots were diluted into perfusing ACSF to a final concentration of 50 nM.

Brain slice preparation. Acute Coronal brain slices were prepared as previously^{40,41}. Briefly, 2–3 weeks old CD1 male mice were deeply anesthetized with avertin and decapitated. The brains were immediately removed and cut into 350 μ m coronal slices using a vibrating blade microtome in ice-cold ACSF containing (in mM) 126 NaCl, 3 KCl, 1.25 KH_2PO_4 , 1.3 $MgSO_4$, 3.2 $CaCl_2$, 26 $NaHCO_3$ and 10 glucose, bubbled continuously with Carbogen (95% O_2 /5% CO_2) at pH 7.4. Fresh slices were incubated in a chamber with continuously carbo-generated ACSF at 34 °C and recovered for at least 1 h before recording.

Whole-cell electrophysiological recording and immunostaining and confocal scanning. For recording of APs from pyramidal cells, whole-cell recordings in the voltage-clamp mode were made as previously^{40,41} with patch pipettes containing (in mM): 130 potassium-gluconate, 6 KCl, 2 $MgCl_2$, 0.2 EGTA, 10 HEPES, 2.5 Na2ATP, 0.5 Na2GTP, 10 potassium-phosphocreatine and 0.5% neurobiotin for morphological reconstruction (Vector Lab) (pH 7.25 and 295 mOsm kg^{-1}). 800 ms depolarizing current steps from –20 pA to 500 pA with 5 pA interval were injected to induce serial APs to study neuronal intrinsic properties like cellular excitability and input resistance before and 5–10 mins after $A\beta$ administration into perfusing ACSF. Input

resistance was calculated as 800 ms -10 pA current injection-evoked steady membrane potential divided by the injected current. Mini IPSCs were recorded with patch pipettes containing (in mM): 135 KCl, 2 MgCl₂, 0.1 EGTA, 10 HEPES, 2 Na₂ATP, 0.2 Na₂GTP with 1 μ M TTX and AMPA receptor antagonist NBQX (10 μ M, Tocris) in perfusing ACSF. For recording of uIPSCs from interneurons to pyramidal cells, multiple-channels patches of at least 1 interneuron and 1–3 pyramidal cells were made and whole cell recordings were formed with internal solution (in mM): 130 mM potassium-gluconate, 6 KCl, 2 MgCl₂, 0.2 EGTA, 10 HEPES, 2.5 Na₂ATP, 0.5 Na₂GTP, 10 potassium-phosphocreatine and 0.5% neurobiotin. A brief of current (5ms) was injected to induce single AP in interneurons under current clamping configuration and uIPSCs were simultaneously recorded from postsynaptic pyramidal cells with membrane potential clamped around EPSC reversal potential 0–10 mV. Paired-pulse ratio (PPR) of uIPSCs was recorded with paired-currents injections with 50 ms inter-pulse interval. The PPR was calculated as the ratio of the second IPSC amplitude to the first. 15 sweeps of paired EPSCs before and 5–10 mins after A β application were averaged and calculated separately. Synaptic responses collected with an Axopatch-700B amplifier (Molecular Devices, Palo Alto, CA), filtered at 2 kHz and digitized at 5–10 kHz. Data were analyzed using Clampfit 9.2 (Molecular Devices, Palo Alto, CA).

Some brain slices after recording were fixed in 4% formaldehyde overnight and stained with nuclear dye DAPI and streptavidin (Invitrogen) for visualizing recorded cells. Images were scanned by Olympus FV1000 confocal microscope.

Drugs. SCH23390, SKF 38393 and BMI were purchased from Tocris. NBQX were purchased from Sigma-Aldrich. These agents were prepared in either distilled water or DMSO and immediately stored in aliquots at -20°C . The aliquot was diluted directly in the perfusing ACSF during experiments.

Statistical analysis. All values were described as mean \pm SEM. Statistical significance was assessed using repeated-measures two-way ANOVA, paired-sample *t* test, independent-sample *t* test (two populations) or Wilcoxon signed-rank test. Differences were considered significant when $p \leq 0.05$ (*0.05, **0.01, *** $p < 0.001$).

Data availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

Most of the experiments and data analysis in this study were performed by S.R., W.Y. and J.-Z.Y. C.J., J.-J.Y., J.-M.Y. and S.Y. contributed with some data analysis, biochemicals purchase and experiments organizing. Z.C. supervised this project. S.R. wrote the manuscript.

Additional Information

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