ALZHEIMER'S DISEASE

β-amyloid redirects norepinephrine signaling to activate the pathogenic GSK3β/tau cascade

Fang Zhang[1]*, Mary Gannon[1]*, Yunjia Chen[1], Shun Yan[2], Sixue Zhang[3], Wendy Feng[1], Jiahui Tao[1], Bingdong Sha[1], Zhenghui Liu[4], Takashi Saito[5†], Takaomi Saido[5], C. Dirk Keene[6], Kai Jiao[2], Erik D. Roberson[7], Huaxi Xu[8], Qin Wang[1‡]

The brain noradrenergic system is critical for normal cognition and is affected at early stages in Alzheimer's disease (AD). Here, we reveal a previously unappreciated direct role of norepinephrine signaling in connecting β-amyloid (Aβ) and tau, two key pathological components of AD pathogenesis. Our results show that Aβ oligomers bind to an allosteric site on α_{2A} adrenergic receptor (α_{2A}AR) to redirect norepinephrine-elicited signaling to glycogen synthase kinase 3β (GSK3β) activation and tau hyperphosphorylation. This norepinephrine-dependent mechanism sensitizes pathological GSK3β/tau activation in response to nanomolar accumulations of extracellular Aβ, which is 50- to 100-fold lower than the amount required to activate GSK3β by Aβ alone. The significance of our findings is supported by in vivo evidence in two mouse models, human tissue sample analysis, and longitudinal clinical data. Our study provides translational insights into mechanisms underlying Aβ proteotoxicity, which might have strong implications for the interpretation of Aβ clearance trial results and future drug design and for understanding the selective vulnerability of noradrenergic neurons in AD.

INTRODUCTION

Alzheimer's disease (AD) and related dementia affect nearly 50 million people globally, and there is currently no effective therapy to cure this devastating disease or to slow its progression. Strong genetic and experimental evidence indicates toxic β-amyloid (Aβ) peptides as a key driving factor of AD pathogenesis (*1*–*4*). However, the failure of multiple clinical trials that directly target Aβ in the brain suggests that simply reducing Aβ burden does not necessarily result in alleviation of cognitive impairment (*5*). The microtubule-associated protein tau is an essential mediator of Aβ toxicity (*6*, *7*). Hyperphosphorylated and aggregated tau disrupts neuronal functions and plasticity, and spreading of tau pathology positively correlates with cognitive impairment in AD (*8*–*10*). Yet, the molecular pathway from Aβ to tau pathology remains elusive, presenting a major gap in in-depth understanding of the pathological cascade of AD.

Brain locus coeruleus (LC) noradrenergic neurons are highly vulnerable in AD and degenerate at early stages of the disease (*11*–*13*). Noradrenergic degeneration often leads to compensatory changes (*12*–*14*) and enhanced responses to norepinephrine (NE) that likely underlie agitation, aggressive behaviors, and sleep disturbance in early AD (*14*–*16*). Whereas the noradrenergic system is well recognized as a sensitive target of Aβ and tau toxicity, our study reveals an unexpected direct etiological role of NE in AD pathogenesis. We report that Aβ oligomers at nanomolar concentrations hijack NE-elicited signaling through α_{2A} adrenergic receptor (α_{2A}AR) to activate glycogen synthase kinase 3β (GSK3β), resulting in tau hyperphosphorylation. GSK3β is a prominent tau kinase (*17*–*20*) and serves as an integral regulator in the development of AD pathophysiology and cognitive deficits (*21*–*23*). Thus, NE/α_{2A}AR directly mediates Aβ toxic effects. This NE-dependent mechanism markedly increases the response sensitivity of GSK3β/tau signaling to Aβ by nearly two orders of magnitude and provides a possible role for NE in failures of clinical trials targeting Aβ clearance. Given the enriched expression of α_{2A}AR in noradrenergic neurons, this mechanism may also render this neuronal population selectively vulnerable in AD. Our data obtained from human tissue samples and longitudinal clinical analysis, and two mouse models collectively support hyperactive noradrenergic signaling in AD as a critical element linking Aβ to the pathogenic GSK3β/tau cascade that ultimately leads to cognitive impairment.

RESULTS

α_{2A}AR activity is elevated in patients with AD and mouse models

α_{2A}AR is broadly expressed in both noradrenergic and non-noradrenergic neurons in the brain and controls both NE input and its resulting responses (*24*, *25*). As a member of the G protein–coupled receptor (GPCR) superfamily, α_{2A}AR activates heterotrimeric G proteins to trigger signal transduction. Our pharmacological characterization of α_{2A}AR in postmortem prefrontal cortex (table S1) revealed a significant increase ($P < 0.01$) in α_{2A}AR activity [Emax/Bmax, reflecting maximum G protein activation in response to NE per receptor (*26*)] in AD cases compared to nondemented, low pathology control subjects (Fig. 1A). Furthermore, our analysis of cases from the National Alzheimer's Coordinating Center (NACC) database (table S2) revealed that usage of clonidine, an α_{2A}AR activator, was associated with worsened cognitive function in patients with cognitive deficits, whereas it had no effect in subjects with normal cognition (Fig. 1B). The adverse effect of clonidine was stronger in patients with more severe dementia

[1]Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL 35294, USA. [2]Department of Genetics, University of Alabama at Birmingham, Birmingham, AL 35294, USA. [3]Department of Chemistry, Southern Research Institute, Birmingham, AL 35205, USA. [4]Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294, USA. [5]Laboratory for Proteolytic Neuroscience, RIKEN Center for Brain Science, Saitama 351-0198, Japan. [6]Department of Pathology, University of Washington, Seattle, WA 98104, USA. [7]Alzheimer's Disease Center, Center for Neurodegeneration and Experimental Therapeutics, Department of Neurology, University of Alabama at Birmingham, Birmingham, AL 35294, USA. [8]Neuroscience Initiative, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037, USA.
*These authors contributed equally to this work.
†Present address: Department of Neurocognitive Science, Nagoya City University Graduate School of Medical Science, Aichi 467-8601, Japan.
‡Corresponding author. Email: qinwang@uab.edu

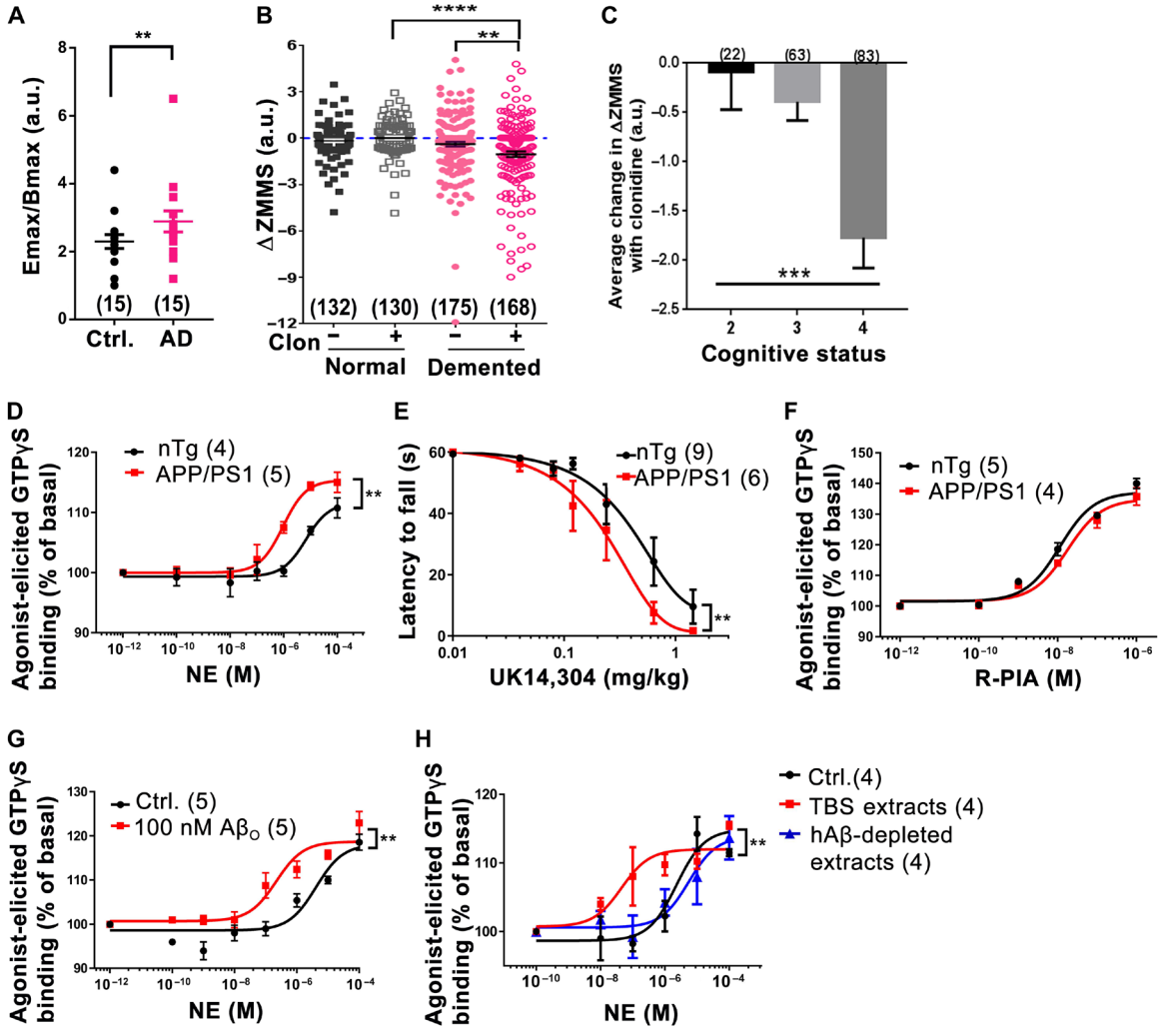

Fig. 1. α_{2A}AR function is enhanced in patients with AD and animal models. (**A**) Membrane homogenates were prepared from postmortem prefrontal cortical tissues of patients with AD and control subjects. Bmax reflects α_{2A}AR density. Emax reflects maximum α_{2A}AR-mediated G protein activation in response to NE (applied with propranolol and prazosin to selectively activate α_{2A}AR). For each set of experiments, an AD subject and a control subject were analyzed in parallel. $^{**}P < 0.01$ by paired *t* test. (**B**) Changes in the adjusted *z* score for the mini-mental state (ZMMS) examination during the time period with or without clonidine usage were analyzed. ΔZMMS reflects average change in ZMMS in a year. $^{**}P < 0.01$; $^{****}P < 0.0001$, by post hoc Sidak's multiple comparisons test. (**C**) Average changes in the adjusted ZMMS score in patients with cognitive deficits during the time period with clonidine usage. Cognitive status code: 2, impaired not mild cognitive impairment (MCI); 3, MCI; and 4, dementia. $^{***}P < 0.001$ by one-way ANOVA. (**D**) Brain homogenates were prepared from nontransgenic (nTg) or APP/PS1 littermates at 7.5 months of age. G protein activation was measured in response to NE (with prazosin and propranolol to selectively activate α_{2A}AR). $^{**}P < 0.01$ by two-way ANOVA. (**E**) Sedation was measured by rotarod test in response to an α_{2A}AR activator, UK14,304, in nTg, or APP/PS1 littermates at 7.5 to 8 months of age. $^{**}P < 0.01$ by two-way ANOVA. (**F**) G protein activation in response to an A1R-selective activator, (R)-N6-(1-Methyl-2-phenylethyl)adenosine (R-PIA), in brain homogenates prepared from nTg or APP/PS1 littermates at 7.5 months of age. (**G**) α_{2A}AR-mediated G protein activation in WT mouse brain homogenates in the presence or absence of $A\beta_O$ (100 nM, monomer equivalent). $^{**}P < 0.01$ by two-way ANOVA. (**H**) α_{2A}AR-mediated G protein activation in WT mouse brain homogenates in the presence of human TBS extracts with or without Aβ depletion. $^{**}P < 0.01$, TBS extracts versus control by two-way ANOVA. All data are shown as means ± SEM. a.u., arbitrary units; GTP-γ-S, guanosine 5′-*O*-(3′-thiotriphosphate).

(Fig. 1C). Combined, these data suggest that α_{2A}AR signaling is hyperactive in patients with AD, and activation of this receptor is detrimental to cognitive function.

We recapitulated the AD-related increase in α_{2A}AR activity in mouse models. We first compared APP(*Swe*)/PS1ΔE9 (APP/PS1) transgenic mice (*27*) and their age-matched nontransgenic littermates at 7.5 months of age, well after initiation of Aβ deposition. There was a leftward shift of the ex vivo NE dose-response curve that indicates enhanced efficiency of α_{2A}AR-mediated G protein activation in the brains of APP/PS1 mice compared to their age-matched nontransgenic littermates (Fig. 1D). In addition, the α_{2A}AR-elicited sedation response was potentiated in 7.5-month-old APP/PS1 mice (Fig. 1E), suggesting that the increase in G protein activation efficiency by α_{2A}AR indeed results in enhancement of in vivo α_{2A}AR function in experimental AD. G protein activation by another Gi/o-coupled receptor, the adenosine A1 receptor, is not altered in APP/PS1 mice (Fig. 1F), suggesting specific enhancement of α_{2A}AR activity in these mice. Similarly, we observed enhanced efficiency of α_{2A}AR-mediated G protein activation in an independent AD mouse model, $App^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ knock-in (APP-KI) (*28*), at 7.5 months of age

compared to age-matched wild-type (WT) controls (fig. S1). There was no change in α_{2A}AR density in transgenic or KI mice compared to their respective controls (fig. S2).

We found no difference in α_{2A}AR-mediated G protein activation nor α_{2A}AR density between APP/PS1 and nontransgenic mice at 5 weeks of age before the development of Aβ-related pathology (fig. S3), suggesting that the change in α_{2A}AR response efficiency in 7.5-month-old transgenic mice is likely attributed to Aβ accumulation in the brain. In support of this notion, addition of Aβ42 oligomers ($A\beta_O$; 100 nM, monomer equivalent; fig. S4), which are the primary toxic species in AD, sufficiently increased the efficiency of NE to induce G protein activation through α_{2A}AR (Fig. 1G). Furthermore, tris-buffered saline (TBS) extracts from AD prefrontal cortex (AD-TBS extracts), which contain soluble Aβ oligomers (*29*), also increased the efficiency of α_{2A}AR-mediated G protein activation (Fig. 1H and fig. S5).

$A\beta_O$ acts as an allosteric ligand of α_{2A}AR

The direct effect of $A\beta_O$ on α_{2A}AR function motivated us to test whether $A\beta_O$ could bind to α_{2A}AR directly. Flow cytometry assays showed binding of $A\beta_O$, but not Aβ42 monomers, to cells expressing α_{2A}AR (Fig. 2, A to C). Bound $A\beta_O$ was colocalized with α_{2A}AR on the cell surface (Fig. 2D). Binding of $A\beta_O$ to immunopurified α_{2A}AR showed a saturable curve with a dissociation constant (K_d) less than 30 nM (monomer equivalent concentration of total Aβ42 peptide, Fig. 2E). We could not detect substantial binding of $A\beta_O$ to two other GPCRs, α_{2C}AR (another α_2AR subtype), and adenosine A1 receptor (Fig. 2F and fig. S6), demonstrating the specificity of the $A\beta_O$-α_{2A}AR interaction.

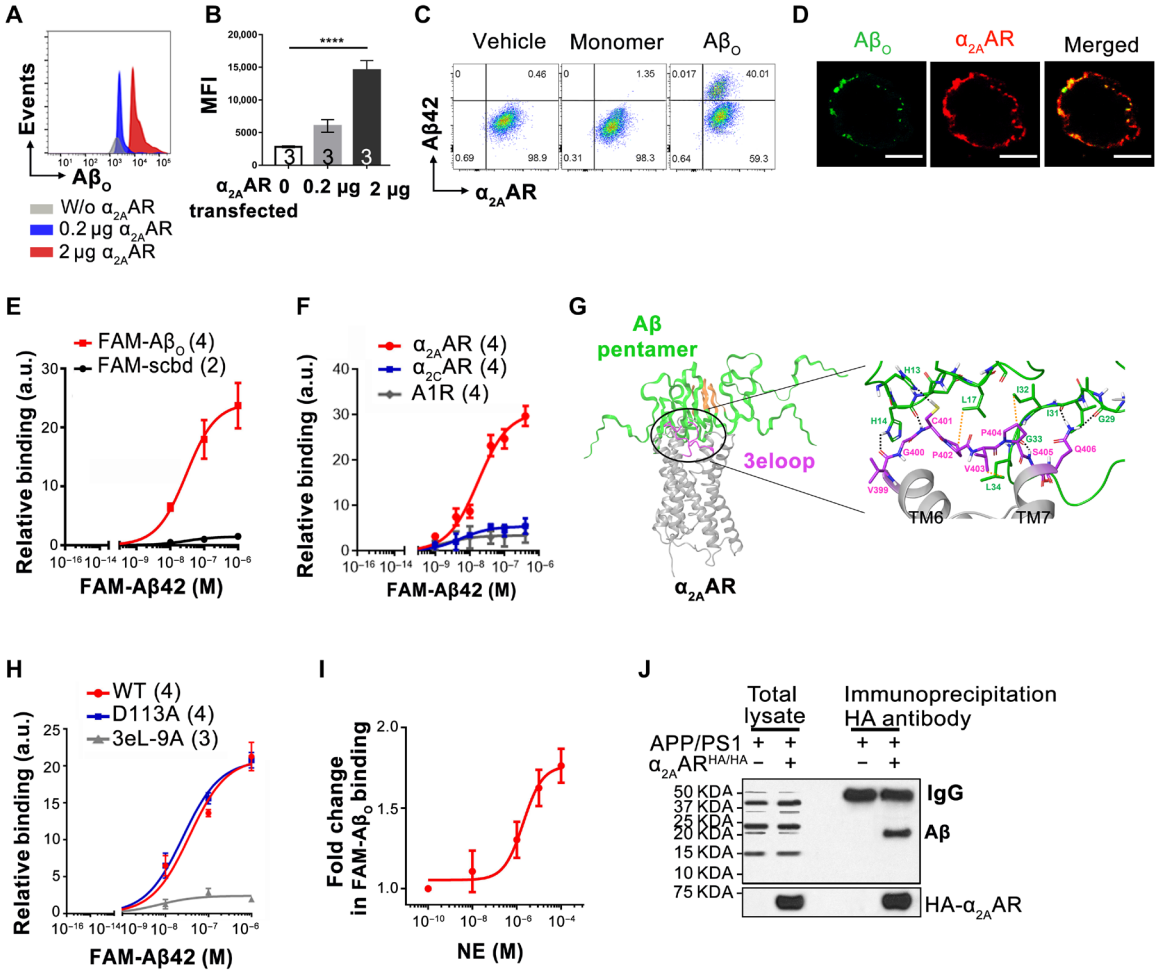

Fig. 2. $A\beta_O$ binds to an allosteric site of α_{2A}AR with nanomolar affinity. (**A** and **B**) Human embryonic kidney (HEK) cells transfected with the empty vector or hemagglutinin (HA)–tagged α_{2A}AR were incubated with AβO for 30 min. AβO bound to the surface of cells was detected by flow cytometry assays. ****$P < 0.0001$ by one-way ANOVA in (B). (**C**) Flow cytometry assays were performed with cells expressing HA-α_{2A}AR after incubation with vehicle, monomer, or oligomer Aβ. (**D**) $A\beta_O$ and HA-α_{2A}AR were detected by immunocytochemistry. Scale bars, 5 μm. (**E**) HA-α_{2A}AR was immunopurified from HEK cells and incubated with increasing amounts of 5-Carboxyfluorescein (FAM)–labeled $A\beta_O$ or scrambled (scbd) Aβ42 peptide. (**F**) Saturation binding curves of FAM-$A\beta_O$ to different receptors expressed on the surface of intact HEK cells. (**G**) The docked Aβo-α_{2A}AR complex model. Green, Aβ pentamer with hydrophobic C termini of monomers indicated in orange. Purple, the 3eL of α_{2A}AR. Dashed black lines and orange lines indicate hydrogen bonds and hydrophobic contacts, respectively. (**H**) Binding of FAM-$A\beta_O$ to WT or α_{2A}AR mutants, as indicated, expressed on the cell surface. (**I**) Binding of FAM-$A\beta_O$ (20 nM, monomer equivalent) to immunoisolated α_{2A}AR in the presence of increasing concentrations of NE. All data are shown as means ± SEM. (**J**) Total brain lysates prepared from APP/PS1 or APP/PS1,$\alpha_{2A}AR^{HA/HA}$ mice were subjected to coimmunoprecipitation assays using an HA antibody. The $\alpha_{2A}AR^{HA/HA}$ allele harbors an HA tag at the N terminus of the endogenous α_{2A}AR locus. APP/PS1 mouse brains were used as a negative control. Representative blots from multiple experiments are shown. IgG, immunoglobulin G; IP, immunoprecipitation.

We next determined the nature of $A\beta_O$ binding to α_{2A}AR. Our in silico docking result indicated that $A\beta_O$ likely binds to the third extracellular loop (3eL) of α_{2A}AR (Fig. 2G). Alanine mutations of nine amino acids (amino acids 399 to 406) in the 3eL (3eL-9A) abolished $A\beta_O$ binding to the receptor (Fig. 2H and fig. S7) without affecting binding of an orthosteric ligand, RX821002 (fig. S8). Conversely, a mutation at D113 of α_{2A}AR (D113A) that eliminates binding of orthosteric ligands (fig. S8) (*30*) did not alter binding of $A\beta_O$ to α_{2A}AR (Fig. 2H). These data demonstrate that $A\beta_O$ binds to an allosteric site of α_{2A}AR involving the 3eL. Furthermore, NE enhanced the binding affinity of $A\beta_O$ to α_{2A}AR in a dose-dependent and saturable manner (Fig. 2I). These results, along with the fact shown above that $A\beta_O$ enhanced the potency of NE (reflecting its binding affinity to the receptor) to elicit α_{2A}AR-mediated G protein activation, clearly demonstrate the reciprocal nature of the regulation between a GPCR orthosteric ligand (NE) and an allosteric ligand ($A\beta_O$). Moreover, our data suggest that $A\beta_O$ actions can be sensitized by an endogenous neurotransmitter (NE). To examine the endogenous interaction between $A\beta_O$ and α_{2A}AR, we crossbred the APP/PS1 line with the HA-tagged α_{2A}AR KI line ($\alpha_{2A}AR^{HA/HA}$) that we generated previously (*31*) to acquire APP/PS1,$\alpha_{2A}AR^{HA/HA}$ mice. Using these mice, we detected stable complex formation between the endogenous α_{2A}AR and $A\beta_O$ in the brain (Fig. 2J).

Aβo binding to α_{2A}AR redirects receptor signaling to activate the GSK3β/tau cascade

Binding of an allosteric ligand to a GPCR often alters signaling through the same receptor (*32*, *33*). We therefore searched for $A\beta_O$-induced changes in α_{2A}AR signaling using protein kinase arrays. Among the kinases tested, we found a change in GSK3β phosphorylation at Ser^9 (indicating an increase in activity) ($P < 0.05$; Fig. 3A and fig. S9). In cultured primary neurons, we observed a significant reduction ($P < 0.01$) in GSK3β phosphorylation at Ser^9 only in cells cotreated with $A\beta_O$ and NE but not in cells treated with either agent alone (Fig. 3, B and C). When coapplied with clonidine, $A\beta_O$ sufficiently induced GSK3β dephosphorylation/activation at a concentration of 20 nM (monomer equivalent; Fig. 3, B and D). This amount is less than 1% of the concentration required for GSK3β activation in neurons by $A\beta_O$ alone (*34*, *35*). Furthermore, naturally secreted oligomeric Aβ at nanomolar concentrations (*36*) also induced GSK3β dephosphorylation/activation in neurons in the presence of clonidine (fig. S10). Concurrent with GSK3β activation in cells cotreated with NE and $A\beta_O$, we observed a significant increase ($P < 0.01$) in tau hyperphosphorylation at AD-relevant sites, Ser^{202} and Thr^{205}, detected by AT8 antibody (Figs. 3E and 4, A and C, and fig. S11). This change in tau could not be detected in cells where GSK3β expression was suppressed by small interfering RNAs (siRNAs) (Fig. 3E and fig. S11), suggesting that GSK3β activation is required for tau hyperphosphorylation in response to NE and $A\beta_O$ cotreatment. Together, these data demonstrate that Aβ aberrantly redirects NE-induced α_{2A}AR signaling to GSK3β activation and subsequent tau hyperphosphorylation. This NE/α_{2A}AR-dependent pathway can increase the response sensitivity of GSK3β/tau signaling to Aβ by two orders of magnitude.

We further examined the role of endogenous α_{2A}AR activation by NE in Aβ-induced GSK3β/tau signaling in vivo. $A\beta_O$ (100 pmol, monomer equivalent) or vehicle was microinjected bilaterally into the dorsal hippocampus of WT C57BL/6 mice, which then received treatment with either saline, idazoxan (an α_{2A}AR blocker), or lithium (a GSK3β blocker). $A\beta_O$ injection induced a decrease in GSK3β phosphorylation and a concurrent increase in tau hyperphosphorylation (detected by AT8 antibody) in dorsal hippocampi when compared to vehicle sham controls (Fig. 3, F to H). $A\beta_O$-induced tau hyperphosphorylation was diminished by lithium treatment (Fig. 3, F and H), indicating an essential role of GSK3β in this process. When α_{2A}AR was blocked by idazoxan, $A\beta_O$-induced changes in GSK3β and tau were abolished (Fig. 3, F to H). These data suggest that endogenous α_{2A}AR activation is required for Aβ-induced GSK3β/tau signaling in vivo, providing strong evidence for an essential role of noradrenergic signaling in Aβ-induced tau hyperphosphorylation.

To validate that $A\beta_O$ acts through allosteric binding to α_{2A}AR to activate the GSK3β/tau signaling, we performed experiments with cells expressing the 3eL-9A mutant α_{2A}AR, which cannot interact with $A\beta_O$ (Fig. 4A). Mutations in the 3eL did not affect binding of orthosteric ligands to α_{2A}AR (fig. S8), and this mutant receptor was still able to mediate G protein signaling in response to NE (Fig. 4D). However, in cells expressing the 3eL-9A mutant receptor, cotreatment with $A\beta_O$ and NE failed to alter either GSK3β or tau phosphorylation (Fig. 4, A to C). These data suggest that the allosteric binding of $A\beta_O$ to the 3eL of α_{2A}AR is required for activation of the pathogenic GSK3β/tau cascade. Collectively, our data reveal a previously unappreciated molecular mechanism, namely, hijacking NE/α_{2A}AR signaling, that enables nanomolar concentrations of extracellular Aβ to activate the pathogenic GSK3β/tau cascade and that this pathway can be effectively blocked by α_{2A}AR inhibitors (fig. S12).

Blockade of α_{2A}AR reduces GSK3β activation and tau hyperphosphorylation and ameliorates AD-related pathological and cognitive deficits

Our results reveal an NE/α_{2A}AR-dependent mechanism connecting Aβ to the pathogenic GSK3β/tau cascade. We therefore hypothesized that blocking α_{2A}AR in the presence of Aβ pathology would have therapeutic potential. To test this, we treated APP/PS1 mice with idazoxan for 8 weeks starting at 7.5 to 8 months of age when Aβ plaques were present, and α_{2A}AR activity was enhanced. Compared to saline treatment, idazoxan reversed hyperactivation of GSK3β in APP/PS1 mouse brains (Fig. 5, A and B), providing additional support for the critical role of α_{2A}AR in mediating Aβ-induced GSK3β activation in vivo. In the cerebral cortex of idazoxan-treated APP/PS1 mice, the extent of Aβ load was lower than that in saline-treated mice (Fig. 5, C and D), indicating that blocking α_{2A}AR slows the progression of Aβ pathology. This effect likely results both from the reduction in GSK3β activity, given that GSK3β can promote Aβ generation (*37*), and from regulation of sorting-related receptor with A repeats (SorLA)–mediated APP trafficking, as we previously reported (*38*). Similarly, idazoxan treatment of APP-KI mice also reduced Aβ plaque load (fig. S13) and GSK3β activation (fig. S14) in the brain. We further examined another important feature of AD pathology, microglial activation (*39*). Idazoxan treatment decreased the density of Iba-1–positive microglial cells (Fig. 5, E and F), suggesting reduction of neuroinflammation.

In the cerebral cortex of APP/PS1 mice, we detected accumulations of pretangle phospho-tau clusters (detected by AT8 antibody) in areas with Aβ accumulation (detected by an Aβ antibody) (Fig. 5G), and the intensity of AT8 staining positively correlated with the size of Aβ depositions (Fig. 5H), consistent with the idea that the Aβ plaque environment promotes pathological changes in tau (*40*). In idazoxan-treated APP/PS1 mice, the appearance and intensity of AT8-positive signals were markedly reduced compared to those in

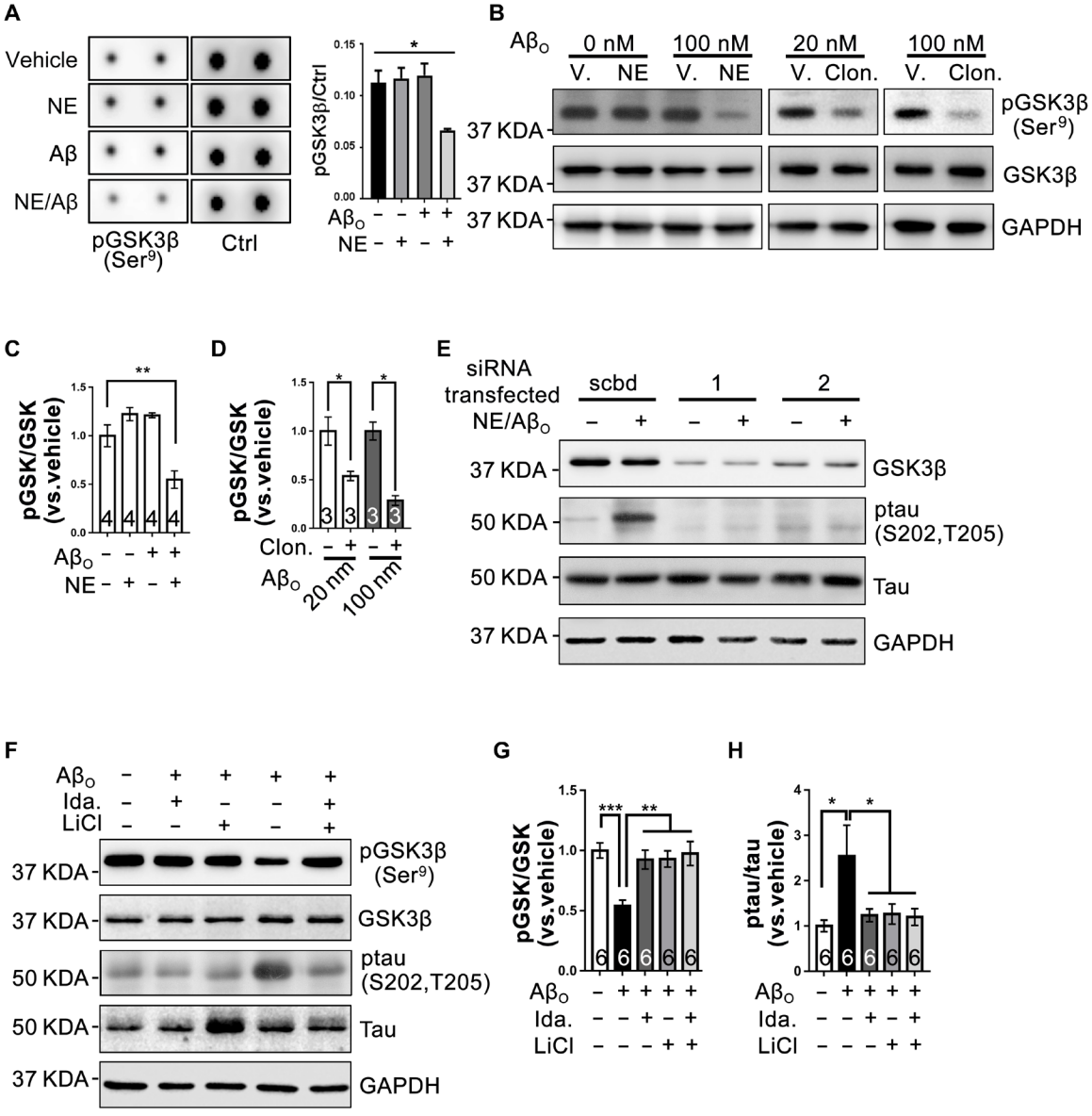

Fig. 3. $A\beta_O$ redirects α_{2A}AR signaling to activation of the GSK3β/tau cascade. (**A**) Representative blots and quantitation of protein kinase arrays. Array blots were incubated with lysates from Neuro2A cells expressing WT α_{2A}AR with treatment as indicated. NE (400 nM) was applied with prazosin and propranolol to selectively activate α_{2A}AR. Ctrl, positive controls for array blotting. *$P < 0.05$ by one-way ANOVA. (**B**) Primary cortical neurons (14 days in vitro) were stimulated as indicated for 30 min. V, vehicle; Clon, clonidine (1 μM). Representative Western blots of phospho-GSK3β (pGSK3β), total GSK3β, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). (**C** and **D**) Quantitation of changes in the ratio of pGSK3β to GSK3β. **$P < 0.01$ by one-way ANOVA Tukey's multiple comparisons. *$P < 0.05$ by unpaired *t* test. (**E**) Neuro2A cells were cotransfected with WT α_{2A}AR and a siRNA against GSK3β or scrambled (scbd) siRNA. Representative blots of tau phosphorylation are shown. (**F** to **H**) Mice that received bilateral intrahippocampal injection of $A\beta_O$ (100 pmol, monomer equivalent, each side) or vehicle were treated intraperitoneally with saline, idazoxan (3 mg/kg), lithium (300 mg/kg), or idazoxan and lithium. Twenty-four hours later, hippocampal lysates were analyzed by Western blot. Representative blots (F) and quantitation (G and H) of GSK3β and tau phosphorylation are shown. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ by one-way ANOVA Tukey's multiple comparisons. All data are shown as means ± SEM.

saline-treated littermates (Fig. 5, G to I). Furthermore, idazoxan treatment reduced tau hyperphosphorylation induced by the same amount of Aβ deposition (Fig. 5, H and I), suggesting that blockade of α_{2A}AR effectively alleviates Aβ-induced tau pathology. In APP-KI mice, idazoxan treatment also reduced tau hyperphosphorylation in the brain (fig. S14).

Reduced Aβ pathology and tau hyperphosphorylation would result in enhanced preservation of cognitive function. Saline-treated APP/PS1 mice showed clear deficits in the Morris water maze task when compared to their age-matched nontransgenic littermate controls, whereas idazoxan-treated APP/PS1 mice performed significantly better ($P < 0.01$; Fig. 6, A and B). Despite the presence of a substantial Aβ burden (Fig. 5C), idazoxan-treated APP/PS1 mice behaved similarly to their nontransgenic littermates on the last day of training (Fig. 6, A and B). Idazoxan treatment did not alter the baseline activity or anxiety (figs. S15 and S16). Cognitive changes in APP-KI mice were also mitigated by idazoxan treatment. Compared to age-matched WT controls, APP-KI mice showed reduced latency to the

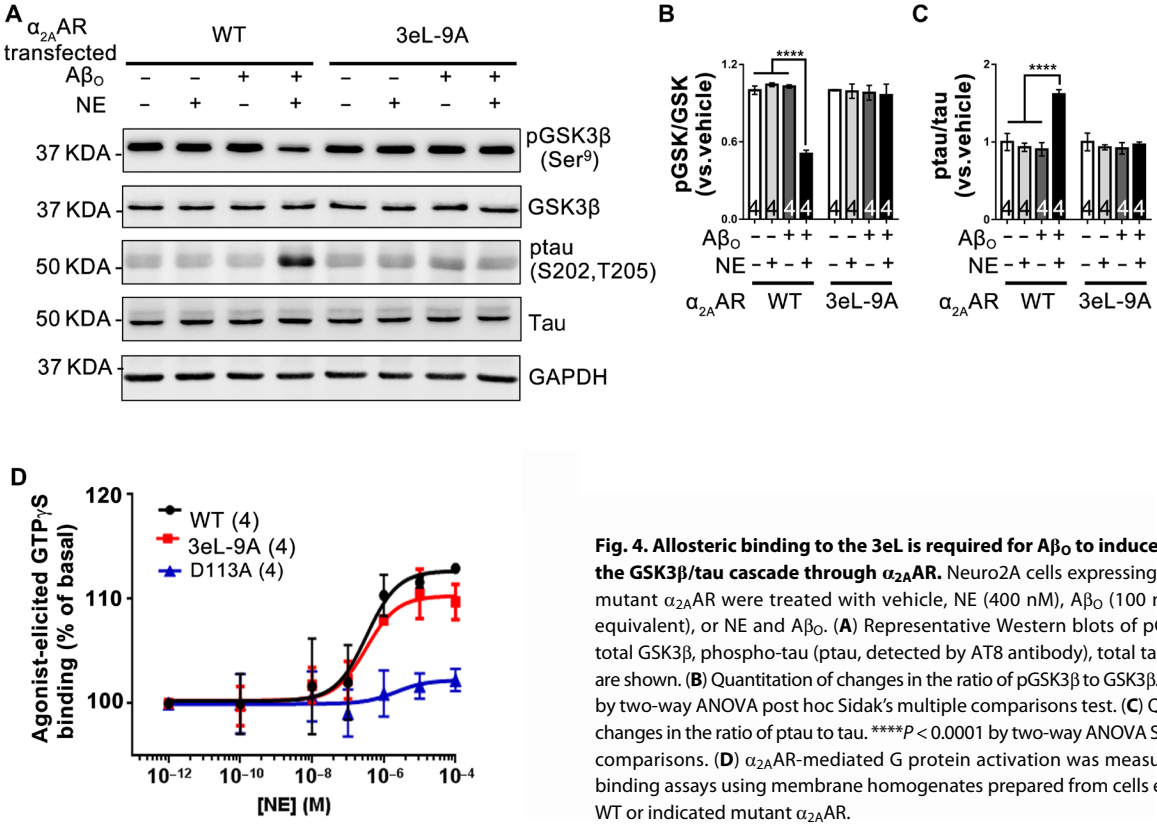

Fig. 4. Allosteric binding to the 3eL is required for $A\beta_O$ to induce activation of the GSK3β/tau cascade through α_{2A}AR. Neuro2A cells expressing WT or 3eL-9A mutant α_{2A}AR were treated with vehicle, NE (400 nM), $A\beta_O$ (100 nM, monomer equivalent), or NE and $A\beta_O$. (**A**) Representative Western blots of pGSK3β at Ser^9, total GSK3β, phospho-tau (ptau, detected by AT8 antibody), total tau, and GAPDH are shown. (**B**) Quantitation of changes in the ratio of pGSK3β to GSK3β. ****$P < 0.0001$ by two-way ANOVA post hoc Sidak's multiple comparisons test. (**C**) Quantitation of changes in the ratio of ptau to tau. ****$P < 0.0001$ by two-way ANOVA Sidak's multiple comparisons. (**D**) α_{2A}AR-mediated G protein activation was measured by GTPγS binding assays using membrane homogenates prepared from cells expressing the WT or indicated mutant α_{2A}AR.

dark on day 2 in passive avoidance task (Fig. 6C), which was normalized by idazoxan treatment (Fig. 6D). These behavioral changes were not due to acute drug effects, but are interpreted as a result of the reduction in GSK3β/tau signaling by idazoxan treatment because a 1-week drug washout period was incorporated before testing the mice. These data collectively demonstrate that blocking NE signaling through α_{2A}AR is an effective strategy to ameliorate pathological and cognitive deficits associated with Aβ.

DISCUSSION

Our current study provides strong evidence for an essential role of noradrenergic signaling in Aβ proteotoxicity. We show that $A\beta_O$ can hijack NE-elicited signaling through α_{2A}AR to induce activation of the pathogenic GSK3β/tau cascade (fig. S12), resulting in tau hyperphosphorylation and accelerated cognitive decline. This NE/α_{2A}AR-dependent mechanism allows $A\beta_O$ to induce GSK3β activation at a concentration as low as 1% of that required for GSK3β activation by $A\beta_O$ alone and thus could be engaged in early stages of AD when Aβ concentrations are low. Normal physiological concentrations of Aβ are in picomolar ranges (*41*, *42*), and oligomers start to form when Aβ concentrations reach a critical aggregation concentration of 90 nM (*43*). At this concentration, $A\beta_O$ can sufficiently induce the pathogenic GSK3β/tau cascade through the mechanism identified here, providing a route for Aβ to initiate the disease process. We thus speculate that the noradrenergic system, α_{2A}AR in particular, could play a critical role in establishing the Aβ-dependent tipping point at which hyperphosphorylation of tau accelerates. Interventions targeting this NE/α_{2A}AR-dependent mechanism of Aβ action would be helpful in slowing or even halting the transition from normal physiology to the earliest stages of disease. Our study may also inform interpretation of failed clinical trials targeting Aβ. Considering that Aβ in human AD brains can reach micromolar concentrations (*44*), it would be difficult to reduce Aβ to below nanomolar concentrations to prevent activation of the α_{2A}AR/GSK3β/tau cascade.

We show that α_{2A}AR activity is enhanced in AD, which would further sensitize neurons to Aβ-induced tau pathology and cognitive dysfunction. This notion aligns with results reported here from unbiased epidemiological analysis of the NACC database and preclinical studies using two mouse models of experimental AD. Given that α_{2A}AR is highly expressed in LC noradrenergic neurons, the Aβ/α_{2A}AR/GSK3β/tau cascade also provides a possible mechanism for these neurons to be exquisitely vulnerable in AD.

Our preclinical studies demonstrate that pharmacologically blocking endogenous NE/α_{2A}AR signaling can effectively reduce activation of the GSK3β/tau cascade, resulting in mitigation of behavioral deficits. α_{2A}AR blockers such as idazoxan have been developed for use in other disorders, and repurposing these drugs could be a potentially effective, readily available strategy for AD treatment. In addition, our data suggest that the Aβ-α_{2A}AR interaction represents an attractive disease-specific therapeutic target for AD because the α_{2A}AR/GSK3β/tau cascade can only be activated in the presence of Aβ oligomers. Directly targeting the Aβ-α_{2A}AR interface would not interfere with normal α_{2A}AR functions and therefore be less likely to result in complications associated with an extended dosing period necessary for AD treatment. Furthermore, from a pharmacological perspective, simultaneously targeting both the orthosteric and allosteric binding sites would create a synergistic effect on receptor-mediated

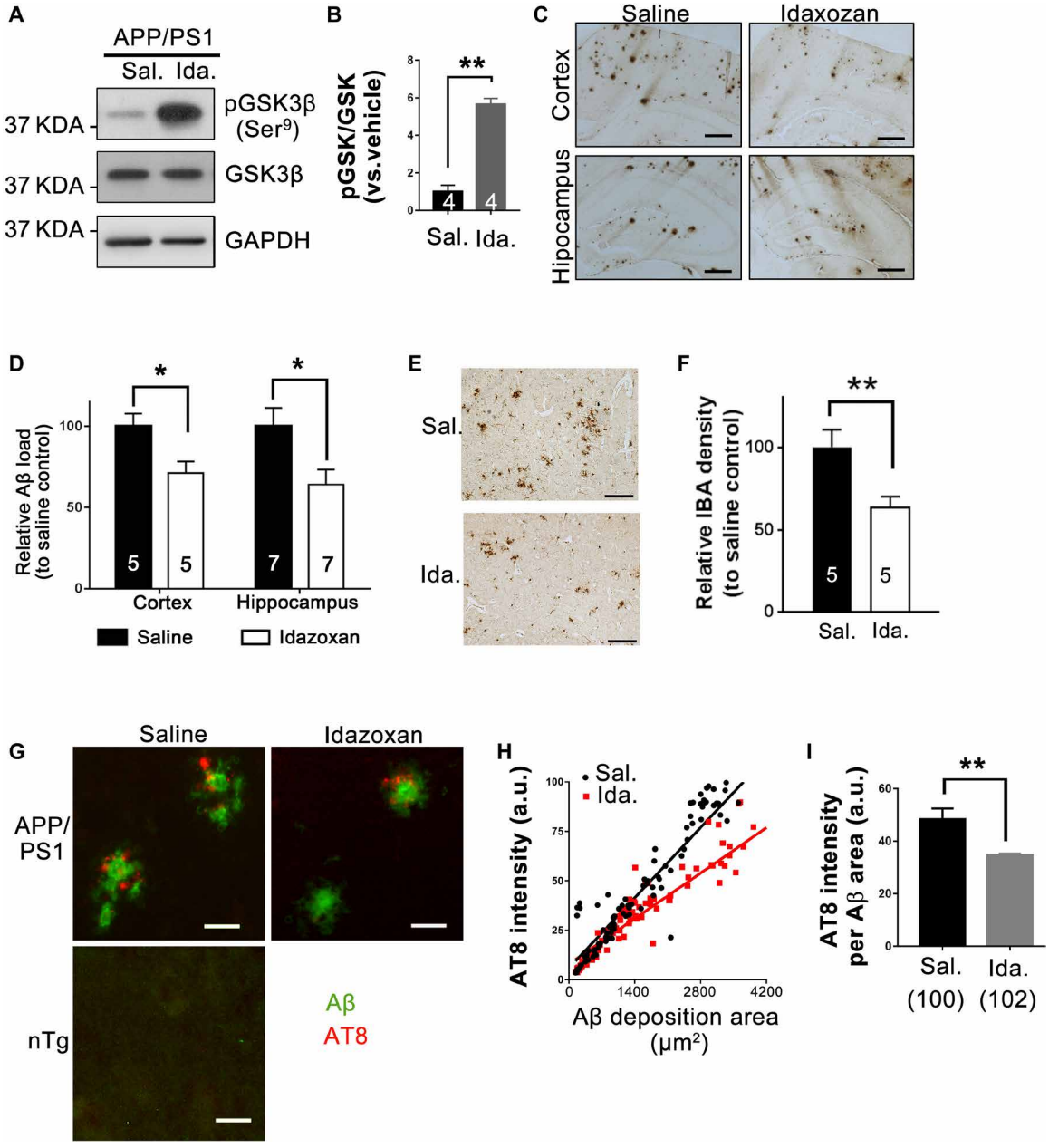

Fig. 5. Blocking α_{2A}AR in AD model mice with profound Aβ pathology reduces GSK3β activity, amyloid pathology, and tau hyperphosphorylation. Eight-month-old APP/PS1 and nontransgenic (nTg) littermate mice were treated with saline or idazoxan for 8 weeks, followed by a 1-week washout period. (**A**) Representative Western blots and (**B**) quantitation of phospho-GSK3β (pGSK3β) at Ser^9 and total GSK3β in total cortical lysates. $^{**}P < 0.01$ by unpaired Student's *t* test. (**C**) Representative images and (**D**) quantitation of Aβ plaques (detected by 6E10 antibody) in the cerebral cortex and hippocampus of *APP/PS1* mice subjected to treatments indicated. Scale bars, 500 μm. $^{*}P < 0.05$ by unpaired *t* test. (**E**) Representative images and (**F**) quantitation of microglial cells (detected by Iba-1 antibody) in the cerebral cortex of *APP/PS1* mice subjected to treatments were indicated. Scale bars, 100 μm. $^{**}P < 0.01$ by unpaired *t* test. (**G**) Representative images of AT8 (for hyperphosphorylated tau) and an Aβ antibody staining in the cortex of nTg and APP/PS1 mice after the indicated treatment. Scale bars, 20 μm. (**H**) Quantitation of the intensity of AT8 signals plotted against the area of Aβ accumulations in the cortex. $r^2 = 0.84$. Slope values for saline and idazoxan groups are 34.29 (±1.519) and 22.98 (±1.017), respectively. (**I**) Relative AT8 intensity normalized against the corresponding area of Aβ depositions. $^{**}P < 0.01$ by unpaired *t* test.

responses (*45*). Thus, the combined use of α_{2A}AR blockers (targeting the orthosteric site) and drugs that reduce Aβ load (decreasing the allosteric binding of Aβ to α_{2A}AR) may lead to an enhanced therapeutic effect.

We are aware of limitations of our study. Our analysis of the clonidine effect on cognition in human patients is not a controlled study, and the sample size is relatively small. Nonetheless, our unbiased epidemiological analysis of the longitudinal clinical data supports the notion that chronic α_{2A}AR activation exacerbates AD disease progression in human patients. It would also be useful to analyze the effect of α_{2A}AR blockers in patients. Unfortunately, the sample size of subjects using other α_{2A}AR agonists or antagonists in the

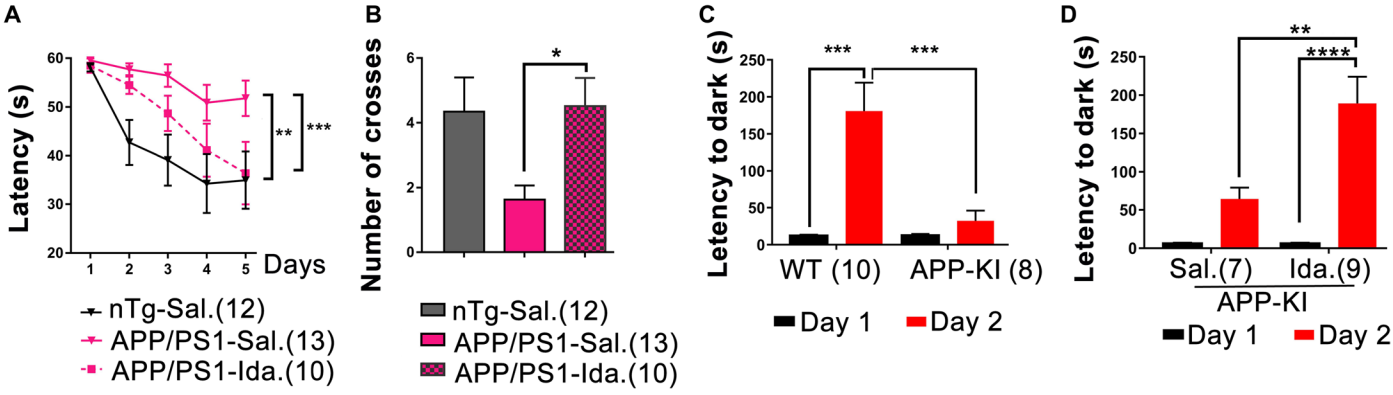

Fig. 6. Blocking α_{2A}AR in AD model mice with profound Aβ pathology ameliorates cognitive deficits. (**A**) Measurement of escape latency on each day in Morris water maze tests in APP/PS1 and nTg mice. ***$P < 0.001$, saline-treated APP/PS1 versus nTg mice; **$P < 0.01$, saline-treated versus idazoxan-treated APP/PS1 mice by two-way ANOVA. (**B**) Quantitation of the number of crosses of the target quadrant in probe trial. *$P < 0.05$ by one-way ANOVA post hoc Tukey's multiple comparisons test. (**C**) Measurement of escape latency to the dark side in passive avoidance tests in 8-month-old WT and APP-KI mice. ***$P < 0.001$ by two-way ANOVA Tukey's multiple comparisons test. (**D**) Measurement of escape latency to the dark side in passive avoidance tests in 8-month-old APP-KI mice treated with saline or idazoxan. **$P < 0.01$ and ****$P < 0.0001$ by two-way ANOVA Tukey's multiple comparisons test. All data are shown as means ± SEM.

NACC database is too small for statistical tests. Another limitation of the study is that a homology model of α_{2A}AR was used in the in silico docking study, as the α_{2A}AR crystal structure is not available. A precise view of the structural base of the Aβ-α_{2A}AR interaction would be necessary for the design of compounds to disrupt the interaction interface.

In summary, our current study demonstrates that Aβ rewires NE signaling to induce activation of the pathogenic GSK3β/tau pathway, providing new insights into mechanisms underlying Aβ proteotoxicity, which have strong implications for the interpretation of Aβ clearance trial results and future drug design.

MATERIALS AND METHODS

Study design

The overall goal of our study is to address the potential role of the brain noradrenergic system in AD pathogenesis. We focused on α_{2A}AR, a key component of the noradrenergic system. We first examined the potential disease relevance of this receptor to AD using human tissue samples and longitudinal clinical data and then recapitulated the AD-related increase in α_{2A}AR activity in two independent AD mouse models. Next, we determined properties of Aβ oligomer ($A\beta_O$) binding to α_{2A}AR using combined biochemical, cell biological, pharmacological, and computational methods. We then investigated the biological consequence of the $A\beta_O$-α_{2A}AR interaction on intracellular signaling in neuronal cells and in the brain. Last, we performed preclinical studies to explore the therapeutic potential of blocking α_{2A}AR in ameliorating AD-related pathological and cognitive deficits. Throughout the study, we exploited an interdisciplinary approach, used multiple technical controls, and included both technical replicates and biological repeats in our assays. Littermate mice were randomly assigned to different treatment groups. Experimenters were blinded with phenotypes or treatments in animal studies. Sample sizes were determined on the basis of previous experience with similar studies. The number of samples indicated in the figure legends reflects independent biological repeats. Conclusions were drawn on the basis of careful statistical analyses.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 7.0 software. In general, Student's *t* test was performed to determine differences between two groups, and one-way or two-way analysis of variance (ANOVA) was performed to determine variations in multiple groups with one or two variances. For Fig. 1A, age- and gender-matched controls and AD samples were paired and analyzed in parallel, and paired *t* test was performed to determine the difference between the two groups. For Fig. 1C, one-way ANOVA was performed, and for the rest of the panels in Fig. 1, two-way ANOVA was used. For Fig. 3 (C, G, and H), one-way ANOVA was performed, and post hoc Tukey's multiple comparisons were used to determine the difference between two groups. For Fig. 4 (B and C), two-way ANOVA and post hoc Sidak's multiple comparisons were performed. For Fig. 6 (A, C, and D), two-way ANOVA and post hoc Tukey's multiple comparisons were performed. For all statistical tests, $P < 0.05$ was considered statistically significant. Data are presented as means ± SEM. Nonlinear regression curve fit for saturation binding and dose-response curves was also performed using GraphPad Prism.

SUPPLEMENTARY MATERIALS

stm.sciencemag.org/cgi/content/full/12/526/eaay6931/DC1

Materials and Methods

Fig. S1. α_{2A}AR-mediated G protein activation in brain homogenates prepared from WT or APP-KI mice at 7.5 months of age.

Fig. S2. α_{2A}AR density measured by radioligand-binding assays in AD models.

Fig. S3. α_{2A}AR-mediated G protein activation and receptor density tested in brain homogenates prepared from nTg or APP/PS1 mice at 5 weeks of age.

Fig. S4. Profiling of Aβ42 peptide oligomerization by fluorescent size-exclusion chromatography.

Fig. S5. α_{2A}AR-mediated G protein activation in WT mouse brain homogenates in the presence of human TBS extracts with or without Aβ depletion.

Fig. S6. Cell-surface expression of HA-tagged receptors tested by fluorescence-activated cell sorting.

Fig. S7. $A\beta_O$ was detected on the surface of cells expressing WT but not 3eL-9A mutant α_{2A}AR.

Fig. S8. Binding of an orthosteric ligand to WT or mutant α_{2A}ARs.

Fig. S9. Full blots of the AKT pathway phosphorylation arrays.

Fig. S10. Naturally secreted oligomeric Aβ induced GSK3β dephosphorylation/activation in neurons in the presence of clonidine.

Fig. S11. Quantitation of tau phosphorylation and GSK3β expression.

Fig. S12. Proposed model of Aβ hijacking NE signaling through α_{2A}AR to induce activation of GSK3β/tau cascade.

Fig. S13. Idazoxan treatment reduces Aβ pathology in APP-KI mouse brains.
Fig. S14. Idazoxan treatment reduces GSK3β activity and tau hyperphosphorylation in APP-KI mouse brains.
Fig. S15. Open-field and elevated zero maze tests in nTg and APP/PS1 mice.
Fig. S16. Open-field and elevated zero maze tests in APP-KI mice.
Table S1. Information of human samples used in Fig. 1A.
Table S2. Extracted data used in Fig. 1 (B and C).
Table S3. Information of antibodies used in this study.
Data file S1. Raw data.
References (*27*–*29*, *31*, *38*, *46*–*65*)

View/request a protocol for this paper from *Bio-protocol*.

REFERENCES AND NOTES

1. D. J. Selkoe, J. Hardy, The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol. Med.* **8**, 595–608 (2016).
2. S. A. Small, K. Duff, Linking Aβ and tau in late-onset Alzheimer's disease: A dual pathway hypothesis. *Neuron* **60**, 534–542 (2008).
3. J. A. Hardy, G. A. Higgins, Alzheimer's disease: The amyloid cascade hypothesis. *Science* **256**, 184–185 (1992).
4. J. C. Polanco, C. Li, L.-G. Bodea, R. Martinez-Marmol, F. A. Meunier, J. Götz, Amyloid-β and tau complexity—Towards improved biomarkers and targeted therapies. *Nat. Rev. Neurol.* **14**, 22–39 (2018).
5. J. Cao, J. Hou, J. Ping, D. Cai, Advances in developing novel therapeutic strategies for Alzheimer's disease. *Mol. Neurodegener.* **13**, 64 (2018).
6. E. D. Roberson, K. Scearce-Levie, J. J. Palop, F. Yan, I. H. Cheng, T. Wu, H. Gerstein, G.-Q. Yu, L. Mucke, Reducing endogenous tau ameliorates amyloid β-induced deficits in an Alzheimer's disease mouse model. *Science* **316**, 750–754 (2007).
7. I.-C. Stancu, B. Vasconcelos, D. Terwel, I. Dewachter, Models of β-amyloid induced tau-pathology: The long and "folded" road to understand the mechanism. *Mol. Neurodegener.* **9**, 51 (2014).
8. M. Morris, S. Maeda, K. Vossel, L. Mucke, The many faces of tau. *Neuron* **70**, 410–426 (2011).
9. G. Šimić, M. Babić Leko, S. Wray, C. Harrington, I. Delalle, N. Jovanov-Milošević, D. Bažadona, L. Buée, R. De Silva, G. Di Giovanni, C. Wischik, P. R. Hof, Tau protein hyperphosphorylation and aggregation in Alzheimer's disease and other tauopathies, and possible neuroprotective strategies. *Biomol. Ther.* **6**, 6 (2016).
10. D. R. Thal, M. Holzer, U. Rüb, G. Waldmann, S. Günzel, D. Zedlick, R. Schober, Alzheimer-related τ-pathology in the perforant path target zone and in the hippocampal stratum oriens and radiatum correlates with onset and degree of dementia. *Exp. Neurol.* **163**, 98–110 (2000).
11. J. A. Ross, B. A. S. Reyes, E. J. Van Bockstaele, Amyloid beta peptides, locus coeruleus-norepinephrine system and dense core vesicles. *Brain Res.* **1702**, 46–53 (2019).
12. M. Gannon, P. Che, Y. Chen, K. Jiao, E. D. Roberson, Q. Wang, Noradrenergic dysfunction in Alzheimer's disease. *Front. Neurosci.* **9**, 220 (2015).
13. M. Gannon, Q. Wang, Complex noradrenergic dysfunction in Alzheimer's disease: Low norepinephrine input is not always to blame. *Brain Res.* **1702**, 12–16 (2018).
14. M. A. Raskind, E. R. Peskind, Neurobiologic bases of noncognitive behavioral problems in Alzheimer disease. *Alzheimer Dis. Assoc. Disord.* **8** (suppl. 3), 54–60 (1994).
15. N. Herrmann, K. L. Lanctôt, L. R. Khan, The role of norepinephrine in the behavioral and psychological symptoms of dementia. *J. Neuropsychiatr. Clin. Neurosci.* **16**, 261–276 (2004).
16. L. Y. Wang, J. B. Shofer, K. Rohde, K. L. Hart, D. J. Hoff, Y. H. McFall, M. A. Raskind, E. R. Peskind, Prazosin for the treatment of behavioral symptoms in patients with Alzheimer disease with agitation and aggression. *Am. J. Geriatr. Psychiatry* **17**, 744–751 (2009).
17. S. Lovestone, C. H. Reynolds, D. Latimer, D. R. Davis, B. H. Anderton, J.-M. Gallo, D. Hanger, S. Mulot, B. Marquardt, S. Stabel, J. R. Woodgett, C. C. J. Miller, Alzheimer's disease-like phosphorylation of the microtubule-associated protein tau by glycogen synthase kinase-3 in transfected mammalian cells. *Curr. Biol.* **4**, 1077–1086 (1994).
18. M. Hong, D. C. R. Chen, P. S. Klein, V. M.-Y. Lee, Lithium reduces tau phosphorylation by inhibition of glycogen synthase kinase-3. *J. Biol. Chem.* **272**, 25326–25332 (1997).
19. J. R. Muñoz-Montaño, F. J. Moreno, J. Avila, J. Díaz-Nido, Lithium inhibits Alzheimer's disease-like tau protein phosphorylation in neurons. *FEBS Lett.* **411**, 183–188 (1997).
20. P. J. Dolan, G. V. W. Johnson, The role of tau kinases in Alzheimer's disease. *Curr. Opin. Drug Discov. Devel.* **13**, 595–603 (2010).
21. C. Hooper, R. Killick, S. Lovestone, The GSK3 hypothesis of Alzheimer's disease. *J. Neurochem.* **104**, 1433–1439 (2008).
22. A. Takashima, GSK-3 is essential in the pathogenesis of Alzheimer's disease. *J. Alzheimer's Dis.* **9**, 309–317 (2006).
23. M. Maqbool, M. Mobashir, N. Hoda, Pivotal role of glycogen synthase kinase-3: A therapeutic target for Alzheimer's disease. *Eur. J. Med. Chem.* **107**, 63–81 (2016).
24. L. Hein, Adrenoceptors and signal transduction in neurons. *Cell Tissue Res.* **326**, 541–551 (2006).
25. C. Cottingham, H. Chen, Y. Chen, Y. Peng, Q. Wang, Genetic variations of α_2-adrenergic receptors illuminate the diversity of receptor functions. *Curr. Top. Membr.* **67**, 161–190 (2011).
26. R. B. Clark, B. J. Knoll, *G Protein Pathways*, R. Iyengar, J. D. Hildebrandt, Eds. (Academic Press, 2002), vol. 343, pp. 506–529.
27. J. L. Jankowsky, H. H. Slunt, T. Ratovitski, N. A. Jenkins, N. G. Copeland, D. R. Borchelt, Co-expression of multiple transgenes in mouse CNS: A comparison of strategies. *Biomol. Eng.* **17**, 157–165 (2001).
28. T. Saito, Y. Matsuba, N. Mihira, J. Takano, P. Nilsson, S. Itohara, N. Iwata, T. C. Saido, Single *App* knock-in mouse models of Alzheimer's disease. *Nat. Neurosci.* **17**, 661–663 (2014).
29. G. M. Shankar, S. Li, T. H. Mehta, A. Garcia-Munoz, N. E. Shepardson, I. Smith, F. M. Brett, M. A. Farrell, M. J. Rowan, C. A. Lemere, C. M. Regan, D. M. Walsh, B. L. Sabatini, D. J. Selkoe, Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat. Med.* **14**, 837–842 (2008).
30. C. D. Wang, M. A. Buck, C. M. Fraser, Site-directed mutagenesis of alpha 2A-adrenergic receptors: Identification of amino acids involved in ligand binding and receptor activation by agonists. *Mol. Pharmacol.* **40**, 168–179 (1991).
31. R. Lu, Y. Li, Y. Zhang, Y. Chen, A. D. Shields, D. G. Winder, T. Angelotti, K. Jiao, L. E. Limbird, Y. Zhou, Q. Wang, Epitope-tagged receptor knock-in mice reveal that differential desensitization of α_2-adrenergic responses is because of ligand-selective internalization. *J. Biol. Chem.* **284**, 13233–13243 (2009).
32. L. T. May, K. Leach, P. M. Sexton, A. Christopoulos, Allosteric modulation of G protein-coupled receptors. *Annu. Rev. Pharmacol. Toxicol.* **47**, 1–51 (2007).
33. D. J. Foster, P. J. Conn, Allosteric modulation of GPCRs: New insights and potential utility for treatment of schizophrenia and other CNS disorders. *Neuron* **94**, 431–446 (2017).
34. L. Kirouac, A. J. Rajic, D. H. Cribbs, J. Padmanabhan, Activation of Ras-ERK signaling and GSK-3 by amyloid precursor protein and amyloid beta facilitates neurodegeneration in Alzheimer's disease. *eNeuro* **4**, ENEURO.0149-16.2017 (2017).
35. J. Jo, D. J. Whitcomb, K. M. Olsen, T. L. Kerrigan, S.-C. Lo, G. Bru-Mercier, B. Dickinson, S. Scullion, M. Sheng, G. Collingridge, K. Cho, $A\beta_{1-42}$ inhibition of LTP is mediated by a signaling pathway involving caspase-3, Akt1 and GSK-3β. *Nat. Neurosci.* **14**, 545–547 (2011).
36. D. M. Walsh, I. Klyubin, J. V. Fadeeva, W. K. Cullen, R. Anwyl, M. S. Wolfe, M. J. Rowan, D. J. Selkoe, Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* **416**, 535–539 (2002).
37. P. T. T. Ly, Y. Wu, H. Zou, R. Wang, W. Zhou, A. Kinoshita, M. Zhang, Y. Yang, F. Cai, J. Woodgett, W. Song, Inhibition of GSK3β-mediated BACE1 expression reduces Alzheimer-associated phenotypes. *J. Clin. Invest.* **123**, 224–235 (2013).
38. Y. Chen, Y. Peng, P. Che, M. Gannon, Y. Liu, L. Li, G. Bu, T. van Groen, K. Jiao, Q. Wang, α_{2A} adrenergic receptor promotes amyloidogenesis through disrupting APP-SorLA interaction. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 17296–17301 (2014).
39. A. G. Efthymiou, A. M. Goate, Late onset Alzheimer's disease genetics implicates microglial pathways in disease risk. *Mol. Neurodegener.* **12**, 43 (2017).
40. Z. He, J. L. Guo, J. D. McBride, S. Narasimhan, H. Kim, L. Changolkar, B. Zhang, R. J. Gathagan, C. Yue, C. Dengler, A. Stieber, M. Nitla, D. A. Coulter, T. Abel, K. R. Brunden, J. Q. Trojanowski, V. M.-Y. Lee, Amyloid-β plaques enhance Alzheimer's brain tau-seeded pathologies by facilitating neuritic plaque tau aggregation. *Nat. Med.* **24**, 29–38 (2018).
41. J. R. Cirrito, P. C. May, M. A. O'Dell, J. W. Taylor, M. Parsadanian, J. W. Cramer, J. E. Audia, J. S. Nissen, K. R. Bales, S. M. Paul, R. B. DeMattos, D. M. Holtzman, In vivo assessment of brain interstitial fluid with microdialysis reveals plaque-associated changes in amyloid-β metabolism and half-life. *J. Neurosci.* **23**, 8844–8853 (2003).
42. M. Ramsden, A. C. Nyborg, M. P. Murphy, L. Chang, F. Z. Stanczyk, T. E. Golde, C. J. Pike, Androgens modulate β-amyloid levels in male rat brain. *J. Neurochem.* **87**, 1052–1055 (2003).
43. M. Novo, S. Freire, W. Al-Soufi, Critical aggregation concentration for the formation of early amyloid-β (1–42) oligomers. *Sci. Rep.* **8**, 1783 (2018).
44. A. E. Roher, C. L. Esh, T. A. Kokjohn, E. M. Castaño, G. D. Van Vickle, W. M. Kalback, R. L. Patton, D. C. Luehrs, I. D. Daugs, Y.-M. Kuo, M. R. Emmerling, H. Soares, J. F. Quinn, J. Kaye, D. J. Connor, N. B. Silverberg, C. H. Adler, J. D. Seward, T. G. Beach, M. N. Sabbagh, Amyloid beta peptides in human plasma and tissues and their significance for Alzheimer's disease. *Alzheimers Dement.* **5**, 18–29 (2009).
45. C. J. Wenthur, P. R. Gentry, T. P. Mathews, C. W. Lindsley, Drugs for allosteric sites on receptors. *Annu. Rev. Pharmacol. Toxicol.* **54**, 165–184 (2014).
46. C. Cottingham, Y. Chen, K. Jiao, Q. Wang, The antidepressant desipramine is an arrestin-biased ligand at the α_{2A}-adrenergic receptor driving receptor down-regulation in vitro and in vivo. *J. Biol. Chem.* **286**, 36063–36075 (2011).
47. Y. Chen, Y. Liu, C. Cottingham, L. McMahon, K. Jiao, P. Greengard, Q. Wang, Neurabin scaffolding of adenosine receptor and RGS4 regulates anti-seizure effect of endogenous adenosine. *J. Neurosci.* **32**, 2683–2695 (2012).

48. M. Jin, N. Shepardson, T. Yang, G. Chen, D. Walsh, D. J. Selkoe, Soluble amyloid β-protein dimers isolated from Alzheimer cortex directly induce tau hyperphosphorylation and neuritic degeneration. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 5819–5824 (2011).
49. S. D. Shirk, M. B. Mitchell, L. W. Shaughnessy, J. C. Sherman, J. J. Locascio, S. Weintraub, A. Atri, A web-based normative calculator for the uniform data set (UDS) neuropsychological test battery. *Alzheimers Res. Ther.* **3**, 32 (2011).
50. D. M. Rosenbaum, C. Zhang, J. A. Lyons, R. Holl, D. Aragao, D. H. Arlow, S. G. Rasmussen, H.-J. Choi, B. T. DeVree, R. K. Sunahara, P. S. Chae, S. H. Gellman, R. O. Dror, D. E. Shaw, W. I. Weis, M. Caffrey, P. Gmeiner, B. K. Kobilka, Structure and function of an irreversible agonist-β_2 adrenoceptor complex. *Nature* **469**, 236–240 (2011).
51. L. Tran, N. Basdevant, C. Prévost, T. Ha-Duong, Structure of ring-shaped $A\beta_{42}$ oligomers determined by conformational selection. *Sci. Rep.* **6**, 21429 (2016).
52. S. Tomaselli, V. Esposito, P. Vangone, N. A. J. van Nuland, A. M. J. J. Bonvin, R. Guerrini, T. Tancredi, P. A. Temussi, D. Picone, The α-to-β conformational transition of Alzheimer's Aβ-(1-42) peptide in aqueous media is reversible: A step by step conformational analysis suggests the location of beta conformation seeding. *ChemBioChem* **7**, 257–267 (2006).
53. *Small-Molecule Drug Discovery Suite 2015–1* (Schrödinger, LLC, New York, NY, 2015).
54. D. Kozakov, R. Brenke, S. R. Comeau, S. Vajda, PIPER: An FFT-based protein docking program with pairwise potentials. *Proteins* **65**, 392–406 (2006).
55. G.-Y. Chuang, D. Kozakov, R. Brenke, S. R. Comeau, S. Vajda, DARS (decoys as the reference state) potentials for protein-protein docking. *Biophys. J.* **95**, 4217–4227 (2008).
56. R. Lu, Y. Chen, C. Cottingham, N. Peng, K. Jiao, L. E. Limbird, J. M. Wyss, Q. Wang, Enhanced hypotensive, bradycardic, and hypnotic responses to α_2-adrenergic agonists in spinophilin-null mice are accompanied by increased G protein coupling to the α_{2A}-adrenergic receptor. *Mol. Pharmacol.* **78**, 279–286 (2010).
57. J. González-Maeso, R. Rodríguez-Puertas, A. M. Gabilondo, J. J. Meana, Characterization of receptor-mediated [^{35}S]GTPγS binding to cortical membranes from postmortem human brain. *Eur. J. Pharmacol.* **390**, 25–36 (2000).
58. F. Zhang, M. Gannon, Y. Chen, L. Zhou, K. Jiao, Q. Wang, The amyloid precursor protein modulates α_{2A}-adrenergic receptor endocytosis and signaling through disrupting arrestin 3 recruitment. *FASEB J.* **31**, 4434–4446 (2017).
59. J. L. Jankowsky, D. J. Fadale, J. Anderson, G. M. Xu, V. Gonzales, N. A. Jenkins, N. G. Copeland, M. K. Lee, L. H. Younkin, S. L. Wagner, S. G. Younkin, D. R. Borchelt, Mutant presenilins specifically elevate the levels of the 42 residue β-amyloid peptide in vivo: Evidence for augmentation of a 42-specific γ secretase. *Hum. Mol. Genet.* **13**, 159–170 (2004).
60. Y. Liu, C. Harmelink, Y. Peng, Y. Chen, Q. Wang, K. Jiao, CHD7 interacts with BMP R-SMADs to epigenetically regulate cardiogenesis in mice. *Hum. Mol. Genet.* **23**, 2145–2156 (2014).
61. C. Cottingham, X. Li, Q. Wang, Noradrenergic antidepressant responses to desipramine in vivo are reciprocally regulated by arrestin3 and spinophilin. *Neuropharmacology* **62**, 2354–2362 (2012).
62. T. L. Lewis, D. Cao, H. Lu, R. A. Mans, Y. R. Su, L. Jungbauer, M. F. Linton, S. Fazio, M. J. LaDu, L. Li, Overexpression of human apolipoprotein A-I preserves cognitive function and attenuates neuroinflammation and cerebral amyloid angiopathy in a mouse model of Alzheimer disease. *J. Biol. Chem.* **285**, 36958–36968 (2010).
63. T. van Groen, I. Kadish, S. A. Funke, D. Bartnik, D. Willbold, Treatment with D3 removes amyloid deposits, reduces inflammation, and improves cognition in aged AβPP/PS1 double transgenic mice. *J. Alzheimer's Dis.* **34**, 609–620 (2013).
64. C. J. Yuskaitis, M. A. Mines, M. K. King, J. D. Sweatt, C. A. Miller, R. S. Jope, Lithium ameliorates altered glycogen synthase kinase-3 and behavior in a mouse model of fragile X syndrome. *Biochem. Pharmacol.* **79**, 632–646 (2010).
65. D. M. Walsh, I. Klyubin, J. V. Fadeeva, W. K. Cullen, R. Anwyl, M. S. Wolfe, M. J. Rowan, D. J. Selkoe, Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* **416**, 535–539 (2002).

Acknowledgments: We thank Y. Peng, I. Mendoza, and U. Nur for technical support. **Funding:** This work is funded by NIA/NIH grants AG056815 (to Q.W.) and 064664 (to Q.W.) and NIMH/NIH grant MH081917 (to Q.W.). Autopsy materials used in this study were obtained from the University of Washington Neuropathology Core, which is supported by the Alzheimer's Disease Research Center (AG05136), the Adult Changes in Thought Study (AG006781), and the Morris K. Udall Center of Excellence for Parkinson's Disease Research (NS062684). The NACC database is funded by NIA/NIH Grant U01 AG016976. NACC data are contributed by the NIA-funded ADCs: P30 AG019610 (E. Reiman), P30 AG013846 (N. Kowall), P50 AG008702 (S. Small), P50 AG025688 (A. Levey), P50 AG047266 (T. Golde), P30 AG010133 (A. Saykin), P50 AG005146 (M. Albert), P50 AG005134 (B. Hyman), P50 AG016574 (R. Petersen), P50 AG005138 (M. Sano), P30 AG008051 (T. Wisniewski), P30 AG013854 (M. M. Mesulam), P30 AG008017 (J. Kaye), P30 AG010161 (D. Bennett), P50 AG047366 (V. Henderson), P30 AG010129 (C. DeCarli), P50 AG016573 (F. LaFerla), P50 AG005131 (J. Brewer), P50 AG023501 (B. Miller), P30 AG035982 (R. Swerdlow), P30 AG028383 (L. Van Eldik), P30 AG053760 (H. Paulson), P30 AG010124 (J. Trojanowski), P50 AG005133 (O. Lopez), P50 AG005142 (H. Chui), P30 AG012300 (R. Rosenberg), P30 AG049638 (S. Craft), P50 AG005136 (T. Grabowski), P50 AG033514 (S. Asthana), P50 AG005681 (J. Morris), and P50 AG047270 (S. Strittmatter). **Author contributions:** Q.W., K.J., E.D.R., H.X., and B.S. conceived, designed, and/or planned the experiments. M.G. analyzed receptor densities and activities in human and animal samples. F.Z. performed amyloid binding and signaling experiments. Y.C. analyzed the clinical data. M.G. treated the mice and performed behavioral tests. M.G., F.Z., S.Y., and W.F. performed pathological analyses of the mice. S.Z. performed in silico modeling. J.T. analyzed amyloid oligomerization. Z.L. performed flow cytometry assays. Q.W., K.J., B.S., F.Z., M.G., Y.C., S.Z., and J.T. analyzed the data. C.D.K. provided human tissue samples. T. Saito and T. Saido generated the APP-KI line and provided suggestions. Q.W., K.J., F.Z., M.G., Y.C., S.Z., J.T., E.D.R., H.X., and C.D.K. prepared the manuscript. **Competing interests:** E.D.R. has served on scientific advisory boards for Biogen, AVROBIO, AGTC, and Novartis. Q.W., M.G., F.Z., Y.C., K.J., S.Y., W.F., B.S., J.T., Z.L., S.Z., C.D.K., H.X., T. Saito, and T. Saido declare that they have no competing interests. **Data and materials availability:** All data associated with this study are present in the paper or the Supplementary Materials. The APP-KI line is available from the RIKEN Center for Brain Science under a material transfer agreement with the institute, and the human postmortem tissues are available from University of Washington under a material transfer agreement with the university.

Submitted 10 July 2019
Accepted 14 November 2019
Published 15 January 2020
10.1126/scitranslmed.aay6931

Citation: F. Zhang, M. Gannon, Y. Chen, S. Yan, S. Zhang, W. Feng, J. Tao, B. Sha, Z. Liu, T. Saito, T. Saido, C. D. Keene, K. Jiao, E. D. Roberson, H. Xu, Q. Wang, β-amyloid redirects norepinephrine signaling to activate the pathogenic GSK3β/tau cascade. *Sci. Transl. Med.* **12**, eaay6931 (2020).

Science Translational Medicine

#-amyloid redirects norepinephrine signaling to activate the pathogenic GSK3#/tau cascade

Fang Zhang, Mary Gannon, Yunjia Chen, Shun Yan, Sixue Zhang, Wendy Feng, Jiahui Tao, Bingdong Sha, Zhenghui Liu, Takashi Saito, Takaomi Saido, C. Dirk Keene, Kai Jiao, Erik D. Roberson, Huaxi Xu, and Qin Wang

Sci. Transl. Med. **12** (526), eaay6931. DOI: 10.1126/scitranslmed.aay6931

The noradrenergic link

Norepinephrine (NE) and the noradrenergic system play a main role in cognition and noradrenergic changes have been documented in patients with Alzheimer's disease (AD). However, the role of NE in AD has not been completely elucidated. Here, Zhang *et al.* show that #-amyloid oligomers are allosteric ligands of the #2A adrenergic receptor (#2AAR) and modulate NE signaling, redirecting the pathway toward GSK3# activation and subsequent tau hyperphosphorylation. Blocking #2AAR reduced tau phosphorylation and ameliorated pathological and cognitive abnormalities in AD mouse models.

Science Translational Medicine (ISSN 1946-6242) is published by the American Association for the Advancement of Science. 1200 New York Avenue NW, Washington, DC 20005. The title *Science Translational Medicine* is a registered trademark of AAAS.