

# Serotonin signaling is associated with lower amyloid- $\beta$ levels and plaques in transgenic mice and humans

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**Aggregation of amyloid- $\beta$  (A $\beta$ ) as toxic oligomers and amyloid plaques within the brain appears to be the pathogenic event that initiates Alzheimer's disease (AD) lesions. One therapeutic strategy has been to reduce A $\beta$  levels to limit its accumulation. Activation of certain neurotransmitter receptors can regulate A $\beta$  metabolism. We assessed the ability of serotonin signaling to alter brain A $\beta$  levels and plaques in a mouse model of AD and in humans. In mice, brain interstitial fluid (ISF) A $\beta$  levels were decreased by 25% following administration of several selective serotonin reuptake inhibitor (SSRI) antidepressant drugs. Similarly, direct infusion of serotonin into the hippocampus reduced ISF A $\beta$  levels. Serotonin-dependent reductions in A $\beta$  were reversed if mice were pretreated with inhibitors of the extracellular regulated kinase (ERK) signaling cascade. Chronic treatment with an SSRI, citalopram, caused a 50% reduction in brain plaque load in mice. To test whether serotonin signaling could impact A $\beta$  plaques in humans, we retrospectively compared brain amyloid load in cognitively normal elderly participants who were exposed to antidepressant drugs within the past 5 y to participants who were not. Antidepressant-treated participants had significantly less amyloid load as quantified by positron emission tomography (PET) imaging with Pittsburgh Compound B (PIB). Cumulative time of antidepressant use within the 5-y period preceding the scan correlated with less plaque load. These data suggest that serotonin signaling was associated with less A $\beta$  accumulation in cognitively normal individuals.**

microdialysis | selective serotonin reuptake inhibitor antidepressants | late-life depression

**A**myloid- $\beta$  (A $\beta$ ) dysregulation appears to initiate the pathogenesis of Alzheimer's disease (AD) with a cascade of downstream factors that exacerbate and propagate neuronal injury (1). A $\beta$  can accumulate as toxic plaques and soluble oligomers in the brains of individuals with AD a decade or more before the initial symptoms are identified (2). The concentration of A $\beta$  is a critical factor determining if and when it will aggregate into these toxic structures; high concentrations of A $\beta$  are more prone to convert from its normal soluble form into these multimeric conformations (3). A $\beta$  is formed within neurons by sequential cleavage of the amyloid precursor protein (APP) by two enzymes,  $\beta$ -secretase and then  $\gamma$ -secretase. Alternatively,  $\alpha$ -secretase can cleave APP within the A $\beta$  sequence, which precludes the peptide from being formed at all. The enzymes and mechanisms that produce A $\beta$  have been well characterized; however, the mechanisms that regulate A $\beta$  production and levels are only partly understood. Understanding the cellular processes that regulate A $\beta$  levels may provide greater insight into disease pathogenesis and suggest new avenues to treat or prevent AD.

Synaptic activity is one key regulator of brain A $\beta$  production. Depolarization and subsequent synaptic transmission causes A $\beta$  to be produced presynaptically and then secreted into the brain extracellular fluid or interstitial fluid (ISF) (4–6). Activation of postsynaptic receptors can also modulate A $\beta$  levels. For example, glutamate NMDA receptors activate intracellular signaling cascades that suppress A $\beta$  production (7). Similarly, muscarinic M1

acetylcholine receptors elevate cleavage of APP by  $\alpha$ -secretase, which lowers A $\beta$  production and levels (8, 9) (for review, see ref. 10). Chronic administration of M1 receptor agonists reduce brain A $\beta$  levels and plaque load (11–13). Several studies have also assessed the effect of serotonin receptors (5HT-Rs) on APP processing and A $\beta$  levels. Activation of select 5HT-Rs increases nonamyloidogenic processing of APP in vitro (14–16) and chronic administration of selective serotonin reuptake inhibitors (SSRIs) reduces brain A $\beta$  levels in mice (17–19).

Our current studies used animal models of AD to demonstrate that serotonin signaling, including through administration of SSRI antidepressants, rapidly reduced A $\beta$  production in vivo. Serotonin-mediated activation of extracellular regulated kinase (ERK) was necessary for this regulation. Human studies with PET imaging of amyloid plaques and retrospective analysis of antidepressant use suggested that serotonin signaling also was associated with less A $\beta$  accumulation in cognitively normal individuals.

## Results

**SSRIs Reduce ISF A $\beta$  Levels in a Mouse Model of AD.** We conducted studies in a mouse model of AD to better understand the relationship between serotonin signaling and A $\beta$  generation on an acute timescale in a physiological paradigm. A $\beta$  is primarily made within neurons and then secreted into the brain ISF as part of its normal metabolism. At least a portion of A $\beta$  within the ISF contributes to amyloid plaque growth (20) with higher concentrations of A $\beta$  being more likely to aggregate and deposit as plaques (3). Given that the ISF can be a source of toxic A $\beta$  species, we determined whether antidepressant drugs reduced A $\beta$  levels within this brain fluid. Dynamic changes in ISF A $\beta$  levels can be measured over several days using in vivo microdialysis (21). This technique permits the animals to be awake with freedom of movement during drug administration and sample collection.

Two- to 3-mo-old presenilin-1 PS1APP transgenic mice (22) were implanted with unilateral microdialysis probes in the hippocampus. At this age, this mouse model of AD does not yet contain insoluble A $\beta$  deposits, thus allowing us to study normal cellular pathways that affect A $\beta$  metabolism. Basal ISF A $\beta$  concentrations were determined in each mouse over a 6-h period followed by i.p. administration of one of several SSRIs; fluoxetine, desvenlafaxine, and citalopram. Compared with vehicle-treated mice, all SSRIs reduced ISF A $\beta$  levels almost immediately following drug administration with significant decreases starting 12–14 h after treatment (Fig. 1 *A* and *B*). The effect of SSRIs was long lived with ISF A $\beta$

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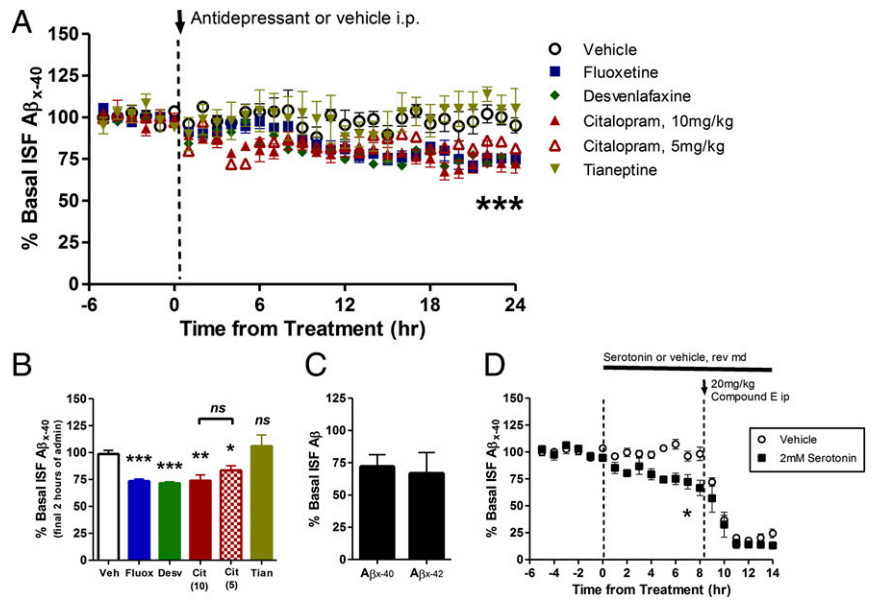
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**Fig. 1.** SSRIs reduce ISF A $\beta$  levels in vivo. Two- to 3-mo-old PS1APP hemizygous mice were administered vehicle (PBS) or one of several antidepressants by i.p. injection: fluoxetine 10 mg/kg, desvenlafaxine 30 mg/kg, citalopram 5 mg/kg and 10 mg/kg, tianeptine 20 mg/kg ( $n = 5-8$  per group). (A) As assessed by in vivo microdialysis, SSRIs reduced ISF A $\beta_{x-40}$  levels significantly beginning between 10 and 14 h after administration. (B) Twenty-four hours after administration, fluoxetine and desvenlafaxine reduced A $\beta_{x-40}$  levels to  $73.4 \pm 2.0\%$  ( $P < 0.0001$ ;  $n = 6$ ) and  $71.6 \pm 1.2\%$  ( $P = 0.001$ ;  $n = 6$ ), respectively, compared with baseline levels in each mouse. Doses of 10 mg/kg and 5 mg/kg citalopram reduced ISF A $\beta_{x-40}$  to  $74.0 \pm 5.4\%$  ( $P = 0.004$ ;  $n = 8$ ) and  $83.5 \pm 4.2\%$  ( $P = 0.02$ ;  $n = 6$ ) of baseline levels, respectively. ISF A $\beta$  levels did not change significantly in tianeptine and vehicle-treated mice ( $n = 5$  per group). (C) PS1APP mice treated with 10 mg/kg citalopram had similar reductions in ISF A $\beta_{x-40}$  and A $\beta_{x-42}$  levels;  $71.9 \pm 9.4\%$  and  $66.7 \pm 16.3\%$  of baseline levels, respectively, by 24 h posttreatment ( $n = 5$ ). (D) PS1APP mice were treated with vehicle (artificial CSF, aCSF) or 2 mM serotonin directly to the hippocampus by reverse microdialysis for 14 h. By 8 h of administration, serotonin reduced ISF A $\beta_{x-40}$  levels to  $66.7 \pm 7.2\%$  of baseline ( $P = 0.003$ ,  $n = 5$  per group). After 8 h, mice were administered a  $\gamma$ -secretase inhibitor, Compound E (20 mg/kg i.p.) to assess A $\beta_{x-40}$  half-life. Data presented as mean  $\pm$  SEM.



levels remaining reduced by 25% at 24 h after drug administration. Citalopram was administered at two doses that roughly equate to high and low doses prescribed to human patients for depression (23). Citalopram at 5 mg/kg and 10 mg/kg reduced ISF A $\beta$  by 16 and 26%, respectively (Fig. 1A and B), both of which were significantly different from vehicle-treated mice, but not statistically different from each other. In contrast, ISF A $\beta$  levels did not change appreciably in vehicle-treated mice compared with the basal A $\beta$  level in each mouse. To determine whether the reduction in ISF A $\beta$  levels was due to either the antidepressant effects of the SSRIs on mood or their action on serotonin receptors, we administered an antidepressant drug that does not act through serotonin receptors. Tianeptine, a non-SSRI antidepressant, had no effect on ISF A $\beta$  levels over a 24-h period (Fig. 1A and B). A subset of PS1APP mice was treated with citalopram during longer ISF sample collections to measure ISF A $\beta_{x-40}$  and A $\beta_{x-42}$  at the same time. Citalopram depressed both species of A $\beta$  to the same extent (Fig. 1C).

**Serotonin Reduces ISF A $\beta$  Levels Without Altering A $\beta$  Elimination Half-life.** We hypothesized that serotonin signaling was responsible for reduced ISF A $\beta$  levels in SSRI-treated mice. To this end, we continually infused serotonin directly into the hippocampus by adding the compound to the microdialysis probe perfusion buffer (reverse microdialysis). Serotonin reduced ISF A $\beta$  levels by 35% over an 8-h period compared with vehicle-treated mice (Fig. 1D). After 8 h of treatment, animals were administered a potent  $\gamma$ -secretase inhibitor, Compound E (20 mg/kg i.p.), to rapidly block A $\beta$  production. This approach allows us to assess the elimination rate of existing endogenous A $\beta$  within the brain ISF. Compound E caused ISF A $\beta$  levels to drop rapidly in both vehicle- and serotonin-treated mice (Fig. 1D). In both groups, although the starting concentrations were different, the calculated elimination half-life of ISF A $\beta$  was the same,  $\sim 1$  h (Fig. S1A-C). This suggests that an alteration in A $\beta$  elimination was not responsible for depressed A $\beta$  levels in serotonin or SSRI-treated mice.

**Serotonergic Regulation of ISF A $\beta$  Does Not Require Action Potentials.** Serotonin G protein coupled receptors (GPCRs) activate signaling pathways that can mediate both excitatory and inhibitory synaptic transmission primarily by modulating a neuron's release of, or response to, neurotransmitters. The 5HT-R $_3$  subtype, however, is an ionotropic cation channel that directly depolarizes neurons. To determine whether action potentials are

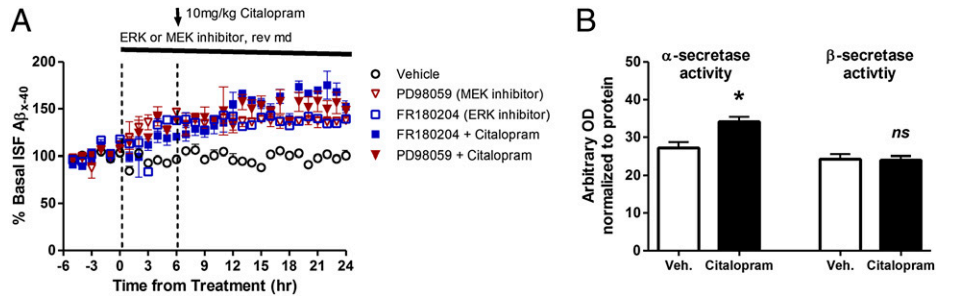
required for 5HT-mediated regulation of A $\beta$ , we first infused a sodium channel blocker, tetrodotoxin (TTX), into the hippocampus by reverse microdialysis. TTX dramatically reduces neuronal activity and ISF A $\beta$  levels in vivo (5). After 15 h of TTX infusion, when A $\beta$  levels reached a new steady-state level, mice were coadministered serotonin to the hippocampus (Fig. S1D). Serotonin caused an additional 35% decrease in ISF A $\beta$  compared with TTX-treatment alone, suggesting that action potentials are not required for serotonergic regulation of A $\beta$  production. Instead, serotonin likely is activating a signaling cascade that alters the processing of APP into A $\beta$ .

**ERK Is Required for Serotonin-Dependent Depression of ISF A $\beta$ .** ERK, a mitogen-activated protein kinase (MAPK), has been shown to suppress A $\beta$  production in vitro and in vivo by altering secretase activity (24, 25). Extracellular ligands bind to a GPCR on the cell surface, which leads to sequential activation of Raf, MEK (MAPK kinase), and then ERK. Activated phospho-ERK (pERK) can remain in the cytoplasm to phosphorylate proteins or translocate to the nucleus to alter transcription. We infused inhibitors of two different steps of this signaling cascade, PD98059 and FR180204, to block MEK and ERK, respectively, in vivo. Each inhibitor alone significantly increased ISF A $\beta$  levels by 40%, suggesting basal activity of this signaling pathway suppresses A $\beta$  generation (Fig. 2A). After 6 h of MEK or ERK inhibitor treatment, a subset of mice were then coadministered citalopram. Inhibition of MEK or ERK completely blocked the effect of the SSRI on ISF A $\beta$  levels.

A separate cohort of 3-mo-old PS1APP mice were administered vehicle or citalopram (10 mg/kg) and then killed 8 h later. Total ERK2 (Fig. S2) and ERK1 levels did not change within the hippocampus; however, activated pERK2 levels significantly increased in the presence of citalopram (Fig. S2). Phosphorylated MEK1/2 (pMEK) had a trend for an increase in SSRI-treated mice ( $P = 0.103$ ). As assessed by enzymatic cleavage assays,  $\alpha$ -secretase activity was significantly higher in citalopram-treated mice; however,  $\beta$ -secretase activity was unchanged (Fig. 2B).

ERK is activated by a wide range of extracellular signals; however, its targets will depend on the cellular context in which it is activated; thus, ERK activation through various receptors can have very precise and distinct effects within a cell (26). Consequently, not all ligands that activate ERK will have the same outcome. We tested several receptors, TrkB receptors, adrenergic neurotransmitter receptors, and NMDA glutamate receptors that are known to

**Fig. 2.** ERK-dependent changes in A $\beta$  metabolism. (A) Young PS1APP mice were administered vehicle (aCSF), a MEK inhibitor (PD98059, 100  $\mu$ M), or an ERK inhibitor (FR180204, 100  $\mu$ M) by reverse microdialysis ( $n = 6-8$  per group). At 24 h from the beginning of treatment, the MEK and ERK inhibitors alone significantly increased ISF A $\beta$  levels by  $37.7 \pm 3.9\%$  ( $P = 0.001$ ) and  $39.4 \pm 1.6\%$  ( $P < 0.0001$ ) compared with baseline levels. Coadministration of citalopram (10 mg/kg i.p.) with either of these inhibitors blocked the SSRI-dependent reduction in ISF A $\beta$ . ISF A $\beta$  levels were not significantly different between inhibitor-treated and inhibitor plus citalopram-treated mice. (B) Citalopram increased  $\alpha$ -secretase enzymatic activity by  $25 \pm 4.9\%$  ( $P = 0.01$ ) within the hippocampus with no change in  $\beta$ -secretase activity ( $n = 8$  per group). Data presented as mean  $\pm$  SEM.



modulate ERK activity, for their ability to alter ISF A $\beta$  levels in vivo (27–30). Treatment with BDNF, a TrkB receptor ligand, or norepinephrine, a similar neurotransmitter to serotonin, did not change ISF A $\beta$  levels after direct administration to the hippocampus (Fig. S3). In contrast, infusion of NMDA, which can also activate ERK (29), significantly lowered ISF A $\beta$  levels by over 50% (Fig. S3); this reduction was blocked by ERK and MEK inhibitors (31).

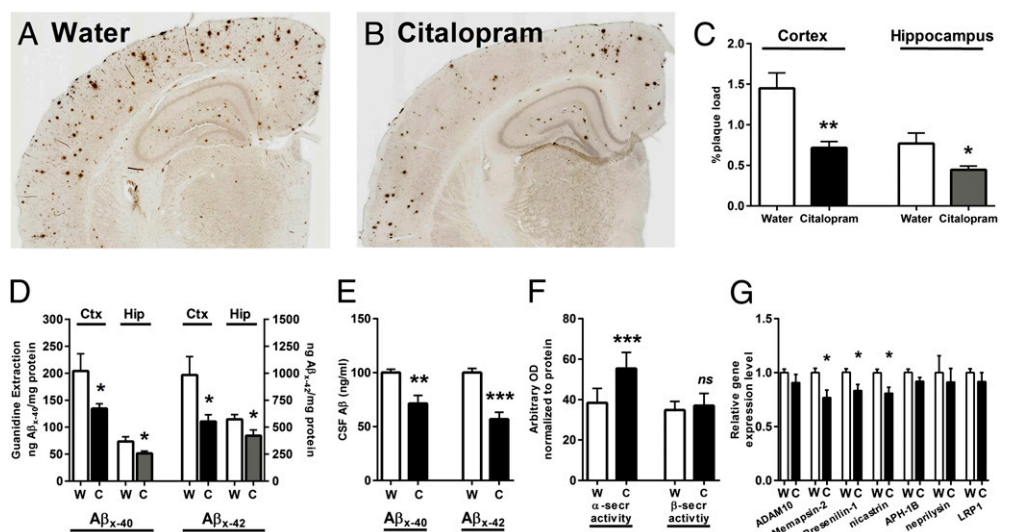
**Chronic Administration of Citalopram Reduces A $\beta$  and Plaque Burden in a Mouse Model of AD.** Given that SSRIs acutely reduce ISF A $\beta$  levels, we determined whether chronic administration would impact plaque burden in the PS1APP transgenic mice. Beginning at 3 mo of age, an age before A $\beta$  deposition in this mouse model, littermate females were administered vehicle (water) or citalopram (8 mg/kg/day) in drinking water for 4 mo. As assessed histologically, citalopram significantly reduced plaque burden within the brains of these mice compared with littermate controls that drank only water (Fig. 3A and B). Cortical and hippocampal plaque load was significantly reduced by 62 and 50%, respectively (Fig. 3C). The contralateral brain regions were processed for biochemical analysis of A $\beta$  protein levels. Insoluble A $\beta_{40}$  and A $\beta_{42}$  extracted with 5 M guanidine were significantly reduced (Fig. 3D); however PBS-soluble and Triton X-100-soluble A $\beta$  levels did not change significantly in citalopram-treated mice (Fig. S4). Whereas PBS extraction of whole brain tissue will include A $\beta$  within the ISF, it may also include A $\beta$  loosely associated to membrane or from intracellular locales that are

exposed even during the gentle PBS extraction process. That PBS extraction and microdialysis do not measure the same pools of A $\beta$  likely accounts for the differential effects seen on A $\beta$ . A $\beta_{40}$  and A $\beta_{42}$  levels within the cerebrospinal fluid (CSF), a truly soluble compartment similar to ISF, were significantly reduced by 28 and 55%, respectively, in these mice (Fig. 3E). Interestingly, the reduction of CSF A $\beta_{42}$  levels was significantly greater than A $\beta_{40}$  ( $P = 0.0004$ ).

To be sure SSRIs acutely reduce A $\beta$  levels in young and aged mice to a similar extent, we administered vehicle or citalopram (10 mg/kg) to 12-mo-old PS1APP mice (this cohort of mice was naive to SSRI treatment before the study). Citalopram reduced ISF A $\beta_{40}$  levels by 25% compared with vehicle (Fig. S5), which was not statistically different from young PS1APP mice treated with the same dose of citalopram (Fig. 1A).

Chronic citalopram treatment significantly increased  $\alpha$ -secretase cleavage activity; however, it had no effect on  $\beta$ -secretase activity (Fig. 3F). Similarly, these mice also had a trend for an increase in APP C-terminal fragment- $\alpha$  (CTF- $\alpha$ ) levels ( $P = 0.014$ ) but no change in CTF- $\beta$  levels (Fig. S6). Citalopram did not, however, alter the mRNA of one putative  $\alpha$ -secretase, ADAM10 (Fig. 3G), suggesting that a posttranslational event by ERK is likely responsible for altering  $\alpha$ -secretase activity. Memapsin-2 ( $\beta$ -secretase) mRNA levels were significantly reduced in citalopram-treated mice; however, given that  $\beta$ -secretase activity and APP CTF- $\beta$  levels were not significantly different, the relevance of the transcriptional change in this setting is unknown. Two com-

**Fig. 3.** Chronic SSRI administration reduces plaque load in PS1APP transgenic mice. Beginning at 3 mo of age, PS1APP hemizygous mice were treated with water or citalopram (8 mg/kg/day) in drinking water for 4 mo ( $n = 10$  per group). Representative images of cortex and hippocampus stained for A $\beta$  plaques in (A) water and (B) citalopram-treated mice. (C) Quantification of plaque load in the hippocampus and cortex was performed blinded. Surface area covered by plaques was reduced in citalopram-treated mice to  $57.9 \pm 6.1\%$  ( $P = 0.03$ ) and  $49.5 \pm 6.2\%$  ( $P = 0.004$ ) of mean levels in water-treated mice. (D) Guanidine-extracted A $\beta_{x-40}$  and A $\beta_{x-42}$  in the hippocampus and cortex was significantly reduced in citalopram-treated mice. (E) A $\beta_{x-40}$  and A $\beta_{x-42}$  levels within the CSF of citalopram-treated mice were reduced to  $71.4 \pm 7.5\%$  ( $P = 0.02$ ) and  $49.5 \pm 6.2\%$  ( $P < 0.0001$ ) compared with mean levels in water-treated mice. (F)  $\alpha$ -Secretase enzymatic activity was significantly increased in chronic citalopram mice ( $P < 0.001$ ); however,  $\beta$ -secretase activity was unchanged. (G) Quantitative PCR showed that mRNA levels of ADAM10 did not change significantly but that memapsin-2 ( $P = 0.01$ ) and two components of the  $\gamma$ -secretase complex, presenilin-1 and nicastrin, were significantly reduced in citalopram-treated mice ( $P = 0.02$  and  $P = 0.01$ ;  $n = 9-10$  per group). APH-1B, neprilysin, and LRP1 mRNA levels did not change following citalopram treatment. Values normalized to mean level in water-treated mice. Data presented as mean  $\pm$  SEM.



ponents of the  $\gamma$ -secretase complex, presenilin-1 (PS1) and nicastrin, had significantly reduced mRNA levels in SSRI-treated mice, which is consistent with the depression in A $\beta$  levels. Levels of mRNA for APH-1B, another component of  $\gamma$ -secretase, however, did not change. Transcripts for other proteins known to alter A $\beta$  metabolism, neprilysin and low-density lipoprotein receptor-1 (LRP1), also did not change following citalopram treatment.

**Effect of Antidepressant Drugs on Amyloid Plaque Load in Humans.** Given that SSRIs reduced A $\beta$  production and plaque load in mouse models, we hypothesized that individuals with a history of antidepressant drug use may have reduced A $\beta$  plaques. A total of 186 cognitively normal participants were recruited into a study for imaging A $\beta$  plaques in late life by PET imaging (32, 33). Participants underwent PET A $\beta$  imaging with Pittsburgh Compound B (PIB) and past use of antidepressant drugs was also ascertained (Table 1). PIB is a radioligand that binds to amyloid plaques and enables quantification of cortical plaque load in the living human brain using PET imaging (34) (*SI Materials and Methods* for more detail). The majority of antidepressant drugs used by participants were SSRIs, with a rank order of sertraline, fluoxetine, and citalopram (equal), escitalopram, and various other SSRI antidepressants (Table S1). Eight participants had used more than one antidepressant, three were uncertain about the exact antidepressant they had received (“not sure of the name”), and three had been on a combination of antidepressants. Participants who received any antidepressant medication within the past 5 y were considered as “treated” (mean exposure = 34.5 mo). These treated individuals had significantly lower mean cortical binding potential (MCBP) ( $0.06 \pm 0.023$ ) by PET PIB imaging compared with participants who reported no antidepressant medication use in the past 5 y (MCBP =  $0.13 \pm 0.019$ ; Wilcoxon rank sum test  $P = 0.01$ ) (Fig. 4A). Participants in the treated and untreated groups (Table 1) did not differ in age, sex, education, or ApoE4 allele status. A  $t$  test for the Spearman rank correlation found a significant negative correlation between duration of antidepressant treatment over the 5 y before PIB scan and MCBP ( $\rho = -0.298$ ,  $P = 0.02$ ). Fig. 4B shows a normalized A $\beta$  PET subtraction image of PET scans of participants who were not treated with antidepressants within the past 5 y minus those of participants who were treated within the past 5 y, demonstrating that the increased A $\beta$  found in the untreated participants occurred in the same brain regions previously identified in AD (32, 34).

## Discussion

Serotonin signaling acutely reduced brain A $\beta$  levels and chronically reduced A $\beta$  plaques in a mouse model of AD. The dose of each SSRI administered in mice was within the range of what would be prescribed to human patients to treat depression (taking into account species differences in metabolism) (23). These findings were supported by the observation that cerebral amyloid levels, as depicted by PIB, showed an inverse relationship with chronic use of SSRIs in cognitively normal humans. These findings are preliminary and unreplicated. Should future studies support these data, a randomized controlled clinical trial

will be needed to determine whether there is a beneficial effect of SSRIs on cerebral amyloid burden in humans.

**Model of Serotonergic Regulation of A $\beta$  Metabolism.** Synaptic activity is known to regulate A $\beta$  levels through a presynaptic mechanism that is dependent on APP endocytosis and cleavage (4, 5) and through neurotransmitter-activated second messenger signaling pathways that alter APP processing into A $\beta$  (7, 11, 12, 31). Activation of serotonin receptors on the cell surface initiates the ERK signaling cascade, which is required for changes in A $\beta$  generation within this mechanism (Fig. 5). There are seven families of 5HT-Rs with at least 15 unique subtypes. A subset of these receptors have been shown to activate ERK (for review, ref. 35); however, to date it is unknown which particular receptors underlie the change in A $\beta$  metabolism. Once activated, pERK can act within the cytoplasm to phosphorylate proteins and alter their function or translocate to the nucleus to alter transcription of a wide range of genes. We demonstrated that both acute and chronic citalopram treatment increased  $\alpha$ -secretase activity, likely through a posttranslational modification, which was responsible for the depression in A $\beta$  levels demonstrated here. Although  $\beta$ -secretase mRNA levels were reduced after chronic citalopram treatment, neither enzymatic activity nor CTF- $\beta$  levels were different, suggesting this was a transcriptional change that did not manifest past translation. Levels of several mRNAs relating to the  $\gamma$ -secretase complex were slightly, but significantly, depressed by citalopram treatment, suggesting that lower  $\gamma$ -secretase activity could have contributed to a reduction in A $\beta$  levels and plaques. Analysis of APP-CTFs, however, suggests that changes to  $\gamma$ -secretase activity, at most, may play a small role in the reduction of A $\beta$ .

**Selectivity of ERK-Dependent Regulation of A $\beta$  Generation.** ERK activation and activity are promiscuous; however, both are highly regulated and can have very precise biological responses, in large part due to cellular context, scaffold proteins, and compartmentalization where it is activated (for review, ref. 26). ERK activation has been shown to modulate APP processing and A $\beta$  generation (24, 25, 36). M1 muscarinic acetylcholine receptors activate the ERK signaling pathway (37), which is at least in part responsible for their regulation of APP processing (38). Similar to our current data with serotonin, M1-mediated down-regulation of A $\beta$  acts by increasing  $\alpha$ -secretase cleavage of APP (8, 9). Only certain receptors that activate ERK appear to alter APP processing; serotonin and NMDA receptors regulate A $\beta$  metabolism in an ERK-dependent fashion; however, in the current study norepinephrine and TrkB receptors did not influence A $\beta$ . The particular cellular cues, cofactors, and scaffold proteins that underlie this downstream substrate specificity are still unknown.

**Depression as a Risk Factor for AD and the Influence of Antidepressants.** The relationship between major depressive disorder (MDD) and AD is controversial, with several studies reporting contradictory findings. The most consistent observation is that depression itself increases risk of AD (39–41). Interpretation of many MDD/AD risk studies, however, are complicated in that

**Table 1. Demographics for participants**

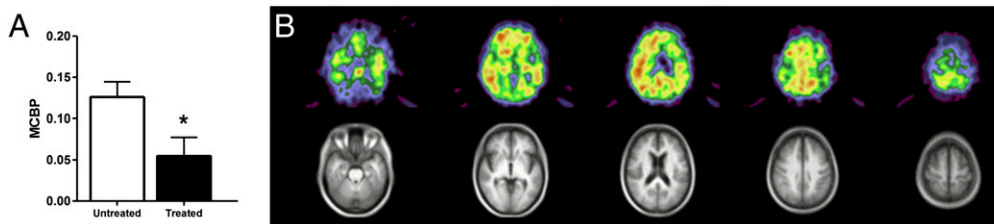
	No treatment, $n = 134$	Treatment, $n = 52$	Statistics	$P$
Female $n$ (%)	93 (69.4)	35 (67.3)	0.08*	0.78
ApoE4 positive $n$ (%)	40 (29.9)	17 (32.7)	0.14*	0.71
Age mean (SD)	69.46 (8.2)	69.69 (7.1)	-0.18 <sup>†</sup>	0.86
Education mean (SD)	15.59 (2.6)	15.27 (2.3)	0.79 <sup>†</sup>	0.43
MMSE mean (SD)	29.01 (1.2)	28.57 (1.8)	1.53 <sup>†</sup>	0.13
MCBP mean (SD)	0.13 (0.2)	0.05 (0.2)	-2.44 <sup>‡</sup>	0.02

MCBP, mean cortical binding potential; MMSE, mini mental status examination.

\* $\chi^2$  test,  $df = 1$ .

<sup>†</sup> $t$  test.

<sup>‡</sup>Wilcoxon rank-sum test.



**Fig. 4.** Antidepressant use is associated with less cortical amyloid in human participants. (A) Mean cortical binding potential of PIB (MCBP) in participants who had taken antidepressants within the past 5 y “treated” versus those who had not been treated “untreated.” Mean exposure time for the treated group = 34.5 mo (SD 23.6). MCBP in untreated participants was  $0.13 \pm 0.22$  ( $n = 134$ ; mean  $\pm$  SD) versus  $0.06 \pm 0.20$  ( $n = 43$ ;  $P = 0.01$ ). (B) Normalized A $\beta$  PET subtraction image of untreated participants, minus treated participants, showing the pattern of cortical rim A $\beta$  plaque accumulation typical of AD.

incipient or early AD can present clinically as apparent late-life depression in some cases (42). Recently, several important prospective studies have demonstrated that depression is a predisposing factor for the development of incident dementia (43–45). MDD is also accompanied by an increased risk of comorbid conditions that independently increase risk for AD, such as vascular disease, diabetes, lower cognitive reserve, and stress (39, 46). Therefore, antidepressant use could potentially reduce amyloid load, but not necessarily translate into an observed decrease in risk for AD, particularly in depressed subjects.

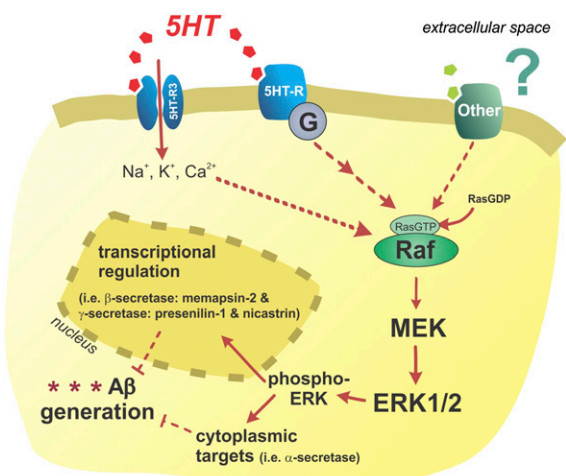
Several studies have found that SSRIs reduce risk of AD in depressed individuals. A recent retrospective study of over 1.4 million people in Denmark over a 10-y period suggested that depressed individuals who were treated only once, or for only a short period, had a greater risk of AD than those who had chronic antidepressant use (47); chronic antidepressant use lowered the risk toward that of the general population. Given that AD pathology develops over many years, we postulate that a short-term A $\beta$ -lowering effect from SSRIs may not be sufficient to influence AD risk but that chronic administration of a SSRI may reduce A $\beta$  plaque burden. Consistent with this, in our retrospective study there was an inverse correlation between the amount of time on antidepressants and the degree of amyloid burden as detected by PIB imaging ( $P = 0.02$ ).

**Antidepressants Correlate with Reduced Amyloid Burden in Cognitively Normal Participants.** One strength of the current study is that

participants with a history of antidepressant use did not differ in age, sex, and ApoE status from participants with no antidepressant exposure. Increasing age and ApoE genetic background are the strongest known risk factors for AD and plaque load (33, 48); thus it was critical that these parameters were controlled. A limitation of the current study is that, because participants were excluded if their clinical dementia rating (CDR) was greater than zero, antidepressant-treated participants with potential dementia or mild cognitive impairment would have been excluded, producing a selection bias. Another limitation of this retrospective human study is that the participants with a history of antidepressant exposure also had histories of depression or depressive symptoms sufficient to warrant the prescription of antidepressant medication. It was not possible to use retrospective data to separate out the effects of antidepressant exposure from depression. However, given the mouse data demonstrating a direct effect of SSRIs on A $\beta$  levels and on reducing A $\beta$  plaque load, and the negative correlation between length of time on antidepressants and amyloid PIB binding in our human participants, it would appear possible that antidepressants may influence the development of A $\beta$  plaques in humans.

**Targeting Serotonin Signaling as a Treatment for AD.** A $\beta$  accumulation and deposition in the AD brain can begin 10 y before the appearance of the first symptoms (2, 32). The concept of “preclinical AD” indicates that AD pathologies are present but AD symptoms are not (49, 50). Several anti-A $\beta$  therapeutic strategies are being pursued to treat AD; however, it is likely that treatment will need to begin during the preclinical phase to prevent or limit plaque accumulation to be beneficial in reducing the risk of developing AD (51, 52). Consequently, if anti-A $\beta$  therapies may be used for years or decades, then very safe compounds will likely be necessary. SSRIs are one of the safest neuroactive classes of compounds approved by the Food and Drug Administration. Whereas SSRIs do have side effects, they are generally well tolerated, even with chronic use. In our current mouse studies, acute treatment with several SSRIs (fluoxetine, desvenlafaxine, and citalopram) reduced ISF A $\beta$  levels by 25% (Fig. 1A) and chronic administration of citalopram reduced brain and CSF A $\beta_{40}$  and A $\beta_{42}$  levels by 30–50% (Fig. 3). In *in vivo* model systems, similar magnitudes of A $\beta$  reductions are sufficient to prevent or halt A $\beta$  plaque accumulation (53, 54).

Our data suggest a potential causal mechanism for antidepressant reduction of A $\beta$  in mice and an association with decreased plaque burden in humans. A direct causal relation between antidepressants and plaque reduction in humans remains to be demonstrated, however.



**Fig. 5.** Model of serotonergic regulation of A $\beta$  metabolism. Serotonin receptors are activated on the cell surface, which initiates a signaling cascade that leads to ERK phosphorylation and activation. ERK appears to increase  $\alpha$ -secretase cleavage and may reduce  $\gamma$ -secretase cleavage of APP. The particular serotonin receptor subtypes responsible for ERK activation in this paradigm are unknown. Not all receptors or ligands that activate ERK will alter APP processing, however similar to serotonin, NMDA receptors can also modulate APP processing through the ERK pathway.

**Materials and Methods**

All participants ( $n = 186$ ) were assessed with the clinical dementia rating (CDR) scale (55), with all human participants scoring CDR = 0 (cognitively normal). All participants were assessed for ApoE genotype, were screened to rule out severe or unstable medical disorders or known primary neurological disorders, received a PIB PET scan (29) (see *SI Materials and Methods* for more details), and received a structured interview to assess lifetime use of antidepressants

(56). This structured interview, adapted from the National Institute for Mental Health (NIMH) Life Chart method (57), incorporated retrospective assessment of cumulative antidepressant use, which we previously demonstrated had an intraclass correlation coefficient of 0.92, when interviews were separated by a mean interval of 3 y (56). All assessment and imaging procedures were approved by Washington University's Human Research Protection Office. Written consent was obtained from each participant.

For statistical analyses, microdialysis, tissue extraction, ELISAs, quantitative PCR, other biochemical assays, and PIB imaging, see *SI Materials and Methods*.

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