

Featured Article

# Established amyloid- $\beta$ pathology is unaffected by chronic treatment with the selective serotonin reuptake inhibitor paroxetine

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## Abstract

**Introduction:** Treatment with selective serotonin reuptake inhibitors has been suggested to mitigate amyloid- $\beta$  (A $\beta$ ) pathology in Alzheimer's disease, in addition to an antidepressant mechanism of action.

**Methods:** We investigated whether chronic treatment with paroxetine, a selective serotonin reuptake inhibitor, mitigates A $\beta$  pathology in plaque-bearing double-transgenic amyloid precursor protein (APP)<sub>swE</sub>/presenilin 1 (PS1) <sub>$\Delta$ E9</sub> mutants. In addition, we addressed whether serotonin depletion affects A $\beta$  pathology. Treatments were assessed by measurement of serotonin transporter occupancy and high-performance liquid chromatography. The effect of paroxetine on A $\beta$  pathology was evaluated by stereological plaque load estimation and A $\beta$ <sub>42</sub>/A $\beta$ <sub>40</sub> ratio by enzyme-linked immunosorbent assay.

**Results:** Contrary to our hypothesis, paroxetine therapy did not mitigate A $\beta$  pathology, and depletion of brain serotonin did not exacerbate A $\beta$  pathology. However, chronic paroxetine therapy increased mortality in APP<sub>swE</sub>/PS1 <sub>$\Delta$ E9</sub> transgenic mice.

**Discussion:** Our results question the ability of selective serotonin reuptake inhibitor therapy to ameliorate established A $\beta$  pathology. The severe adverse effect of paroxetine may discourage its use for disease-modifying purposes in Alzheimer's disease.

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## Keywords:

Alzheimer's disease; Cerebral amyloidosis; Neocortex; Monoamine; Serotonin; Selective serotonin reuptake inhibitor; SERT occupancy; [<sup>3</sup>H]DASB; Autoradiography; Transgenic mouse model; 5,7-dihydroxytryptamine; Stereology

## 1. Introduction

The serotonergic system degenerates in Alzheimer's disease (AD) along with the cholinergic and noradrenergic systems [1–3]. In patients with AD, the level of serotonin

(5-hydroxytryptamine [5-HT]) is significantly reduced in several cortical regions, in particular in the frontal and temporal cortex [4,5]. This pathological change has been suggested to contribute to the depressive symptoms that frequently precede the cognitive decline in patients with AD [6–8]. It is still being disputed whether antidepressive treatment of patients with AD with selective serotonin reuptake inhibitors (SSRIs) impacts on the decline of cognition in AD [9–12] or the conversion from mild cognitive impairment to AD [13]. In AD, the density of specific cortical

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5-HT receptors correlates positively to amyloid  $\beta$  (A $\beta$ ) pathology and negatively to cognitive performance [14], with a reduced density of the 5-HT<sub>4</sub>R and 5-HT<sub>6</sub>R being observed in mild cognitive impairment [15–17]. Evidence that SSRI treatment impacts on A $\beta$  accumulation comes from positron emission tomography studies, showing reduced uptake of Pittsburgh compound B, a proposed marker of A $\beta$  pathology, in the brains of individuals with a history of SSRI therapy, including citalopram and fluoxetine [18]. In addition, acute, successive administration of citalopram (30 mg) to healthy individuals with 2 hours interval is reported to diminish cerebrospinal fluid levels of A $\beta$  [19].

Interestingly, studies in transgenic mouse models of AD have suggested that SSRI treatment has major impact on A $\beta$  pathology [18–20]. In the APP<sub>swc</sub>/PS1 $\Delta$ E9 (amyloid precursor protein [APP]/presenilin 1 [PS1]) transgenic mouse, which is a well-established model of A $\beta$  pathology [21], 4 months treatment with citalopram (8 mg/kg/day *per os*) from 3 months of age results in ~50% lower A $\beta$  plaque load in the neocortex at 7 months of age [18]. In 6-month-old APP/PS1 mice, 4 weeks of treatment with citalopram (10 mg/kg/day *i.p.*) impairs initial A $\beta$  plaque formation and growth [18]. Five months treatment with paroxetine (5 mg/kg/day *i.p.*) from 5 months of age results in lower A $\beta$  levels at 10 months of age in the hippocampus of 3  $\times$  Triple-Tg mice [20], a model of combined A $\beta$  and tau pathology. In addition, 5-week treatment with fluoxetine (10 mg/kg/day *i.p.*) is suggested to reduce A $\beta$  load in 18-month-old APP/PS1 mice [22], at an age when these mice show a reduction in 5-HT in the neocortex [23,24]. Finally, infusion of 5-HT into the hippocampus of APP/PS1 mice has been reported to reduce interstitial levels of A $\beta$  [18], by mechanisms involving stimulation of the 5-HT<sub>4</sub>R and 5-HT<sub>6</sub>R and increased processing of APP via the nonamyloidogenic route [25,26].

Importantly, there are still no preclinical studies, where plaque-bearing APP/PS1 mice have been chronically treated with doses of SSRI, resulting in  $\approx$ 80% occupancy of the serotonin transporter (SERT), which is considered therapeutic for the treatment of depression [27]. Therefore,

we tested the hypothesis that chronic treatment of plaque-bearing 9-month-old APP/PS1 mice for 9 months with paroxetine (5 or 10 mg/kg/day *per os*) would mitigate A $\beta$  pathology and improve behavior (locomotion, social memory). We also tested the hypothesis that a neurotoxin-induced loss of cortical 5-HT at 9 months of age would impact on A $\beta$  pathology at 12 months. The results of this study question the proposed beneficial effect of SSRI therapy and 5-HT on A $\beta$  pathology in mice with manifest A $\beta$  pathology.

## 2. Methods

### 2.1. Paroxetine treatment and neurotoxin injection

Paroxetine: APP<sub>swc</sub>/PS1 $\Delta$ E9 (APP/PS1) and littermate wild-type (Wt) mice [21] were bred on a B6C3 background in house or at Taconic A/S, Denmark [28]. Paroxetine (Seroxat oral solution 2 mg/mL, GSKline) was administered in the drinking water in a dose of 10 mg/kg/day or 5 mg/kg/day (Table 1) to 9-month-old male APP/PS1 and Wt mice. APP/PS1 and Wt controls received normal drinking water. Treatment efficacy was assessed by autoradiographic measurement of the SERT occupancy using [<sup>3</sup>H]3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile, as detailed in Supplementary Methods.

Neurotoxin: To destroy 5-HT fibers and neurons, 5,7-dihydroxytryptophan (5,7-DHT) was stereotaxically injected into the lateral ventricles of 9-month-old male APP/PS1 and Wt mice (Table 1), as described elsewhere [31], but injecting a total dose of 80  $\mu$ g 5,7-DHT. Mice with unsuccessful lesions, as determined by the measurement of 5-HT in neocortical tissues, were excluded from statistical analysis (Danish Veterinary & Food Administration: J.no. 2007/562-50, J.no. 2012-15-2935-00,023, J.no. 2012-DY-2934-00,008).

Tissue processing: See Supplementary Methods.

### 2.2. Behavior

The behavior of 18-month-old mice was assessed using the open field, the elevated plus maze, and the social

Table 1  
Study design

| Experimental group                        | Treatment/intervention  | Genotype |             | Treatment period (months) | Behavioral analysis  |
|---|-------------------------|----------|-------------|---------------------------|--|
|   |                         | Wt (n)   | APP/PS1 (n) |                           |  |
| Nine months paroxetine treatment          | Vehicle                 | 20       | 14          | 9–18 months               | Open field, elevated plus maze, social interaction, social memory* Y-maze <sup>†</sup> |
|   | Paroxetine 10 mg/kg/day | 15       | 5           |                           |  |
|   | Vehicle                 | 15       | 7           | 9–18 months               | Open field, elevated plus maze, social interaction, social memory Y-maze <sup>‡</sup>  |
|   | Paroxetine 5 mg/kg/day  | 11       | 7           |                           |  |
| Three months paroxetine treatment         | Vehicle                 | 6        | 10          | 9–12 months               | Not done   |
|   | Paroxetine 10 mg/kg/day | 6        | 7           |                           |  |
| Three months 5,7-DHT-induced loss of 5-HT | Sham                    | 6        | 6           | 9–12 months               | Open field, social interaction, social memory Y-maze <sup>‡</sup>                      |
|   | 5,7-DHT                 | 6        | 6           |                           |  |

Abbreviations: 5,7-DHT, 5,7-dihydroxytryptophan; APP, amyloid precursor protein; 5-HT, 5-hydroxytryptamine; PS1, presenilin 1.

\*Results reported in Olesen et al. [29].

<sup>†</sup>Results reported in Olesen et al. [30].

<sup>‡</sup>Unpublished results.

interaction tests [29]. The 5,7-DHT-treated mice were assessed using the open field test [32]. All tests were performed in the light phase of 24-hr light/dark cycle. For details, see [Supplementary Methods](#).

### 2.3. High-performance liquid chromatography for 5-HT

Monoamine levels were assessed in perchlorate extracts of the dissected tissues using high-performance liquid chromatography with electrochemical detection [24,32]. The detection limit for 5-HT was 30 fmol on column. The detection level in tissues was 0.25 pmol/mg wet weight.

### 2.4. Immunohistochemistry for human A $\beta$

Cryostat and vibratome sections were demasked free floating in 70% formic acid diluted in H<sub>2</sub>O for 15 min at room temperature, followed by staining for human A $\beta$  using biotinylated monoclonal mouse-anti-A $\beta$  immunoglobulin G1 (6E10, Biosite), which was detected by horse radish peroxidase-Streptavidin and developed with diaminobenzidine. Sections were mounted on glass-slides, dehydrated, and coverslipped from xylene in Depex. Paraffin sections were demasked in Tris-ethylenediaminetetraacetic acid-glucose-Buffer at 480 Watts for 15 min and stained as mentioned previously. The protocol for SERT is given in [Supplementary Methods](#).

### 2.5. Enzyme-linked immunosorbent assay for human A $\beta$

Samples were processed according to the manufacturers protocol (Invitrogen), whereafter the A $\beta$  was measured using Invitrogen A $\beta$ <sub>42</sub> Human enzyme-linked immunosorbent assay Kit (KHB3441) and A $\beta$ <sub>40</sub> Human enzyme-linked immunosorbent assay Kit (KHB3482), as described in [Supplementary Materials](#). Data expressed in pg/mg protein were used for calculation of the A $\beta$ <sub>42</sub>/A $\beta$ <sub>40</sub> ratio.

### 2.6. Quantification of A $\beta$ plaque load

The A $\beta$  plaque load was estimated in the neocortex by the use of stereological grid counting, using the new CAST software (Visiopharm), by an observer who was blinded to the treatment of the mice. The estimation was performed in 6.2  $\pm$  1.5 (mean  $\pm$  standard deviation) sections per mouse in the 18-month-old APP/PS1 mice. For details, see [Supplementary Methods](#). The percentage of neocortex covered by plaques in individual sections was calculated as: A $\beta$  plaque load (%) = No. of A $\beta$ <sup>+</sup> plaques touching a cross (in a grid)/No. of crosses in neocortex  $\times$  100%. Plaque density was given by the number of plaques per area, and plaque size by the number of crosses hitting individual plaques. Data on A $\beta$  plaque load are presented in dot diagrams, and plaque density and plaque size in bar graphs. The coefficient of error (CE) of the estimate of individual mice was used to calculate a mean CE for

the group. The CE values and the coefficient of variation were calculated as done previously [33].

### 2.7. Statistical methods

Data sets were tested for normality of distribution. Bartlett's test was used to check the equality of variances. Binary logistic regression was used to assess the effects of genotype, age, and treatment on mouse survival. Statistically significant differences between groups were evaluated using unpaired, two-tailed Student *t* tests, one-way analysis of variance (ANOVA), or repeated measures two-way ANOVA, followed by least significant difference post hoc tests. Analyses were performed using GraphPad software (Prism 4.0b) for Macintosh or XLSTAT (version 2010.3.06) for Windows. In one data set, one outlier  $>2$  SDs from the mean was excluded. Statistical difference is indicated as \**P* < .05, \*\**P* < .01, \*\*\**P* < .001, and \*\*\*\**P* < .0001.

## 3. Results

### 3.1. 5-HT is reduced in aging APP/PS1 mice

The 18-month-old male APP/PS1 mice showed a reduction in 5-HT levels of  $\approx 40\%$  in frontal cortex compared with littermate Wt mice (*P* < .01, Student *t* test) (Fig. 1). This reduction is within the range previously reported for this AD model [23] and in line with the reduction in 5-HT levels observed in frontal cortex of AD patients [5,6].

### 3.2. Chronic paroxetine treatment reduces the survival of APP/PS1 mice

Individually caged 9-month-old male APP/PS1 and Wt mice were treated *per os* with paroxetine (Seroxat oral solution 2 mg/mL, GSKline) for up to 9 months, whereas vehicle-

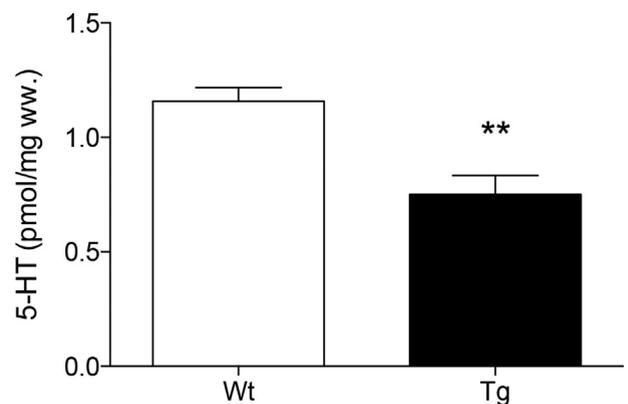


Fig. 1. 5-HT levels (pmol/mg wet weight) are reduced in frontal cortex of old APP/PS1 mice. HPLC measurement of 5-HT in the frontal cortex of 18-month-old male APP/PS1 Tg and Wt mice. Data are expressed as mean  $\pm$  SEM. Student *t* test; Tg, *n* = 6; Wt, *n* = 5. \*\**P* < .01. Abbreviations: 5-HT, 5-hydroxytryptamine; APP, amyloid precursor protein; HPLC, high-performance liquid chromatography; SEM, standard error of the mean; PS1, presenilin 1; ww, wet weight of dissected brain tissue.

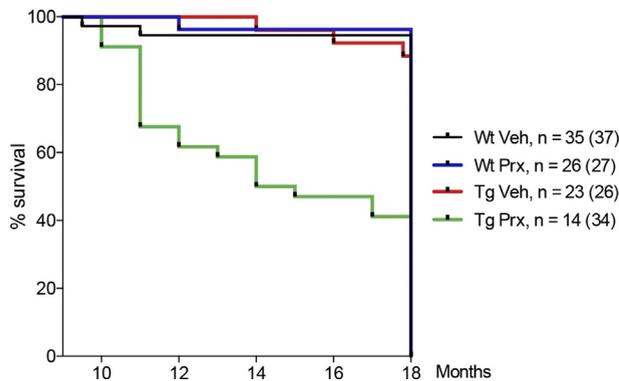


Fig. 2. Peroral paroxetine treatment reduces the survival of aging APP/PS1 mice. The survival was recorded for mice treated with paroxetine (prx) or vehicle (veh) for 9 months and shown in a Kaplan-Meier diagram. The APP/PS1 mice treated with paroxetine had a lower survival than all other groups after 9 months of treatment. Numbers of mice before and after treatment are shown, with the number before initiation of treatment indicated in brackets. Abbreviations: APP, amyloid precursor protein; PS1, presenilin 1.

treated mice received normal drinking water (Table 1). Paroxetine treatment at 10 mg/kg/day *per os* reduced the survival of APP/PS1 mice (Fig. 2). Binary logistic regression showed that the effect of treatment, genotype, and age on premature death was statistically significant ( $\chi^2 [3] = 166.2, P < .001$ ). The model explained 27.8% of the variance in premature deaths (Nagelkerke  $R^2$ ), and correctly classified 88.3% of cases. The independent factors treatment (Wald score = 39.3;  $P < .001$ ), genotype (Wald score = 46.6;  $P < .001$ ), and age (Wald score = 41.2;  $P < .001$ ) added significantly to the model and increased the likelihood of death by 4.8, 5.6, and 1.3, respectively. Because of the reduced survival rate, the dose of paroxetine was reduced to 5 mg/kg/day (Table 1), resulting in paroxetine serum levels of 40–50 nmol/L. Binary logistic regression of the effects of genotype, age, and 5 mg/kg/day paroxetine treatment was significant ( $\chi^2 [3] = 56.7, P < .001$ ), with the independent factors age (Wald score = 41.2;  $P < .001$ ) and paroxetine (Wald score = 5.3,  $P < .05$ ), but

not genotype (Wald score = 0.0;  $P > .05$ ), adding significantly to the model. Age and paroxetine treatment at 5 mg/kg/day *per os* increased the likelihood of premature death by 2.0 and 0.2, respectively.

### 3.3. Clinically relevant occupancy is achieved by oral paroxetine treatment

To ensure that all mice, including those treated with 5 mg/kg/day *per os*, were treated with a clinically relevant dose of paroxetine, we assessed the SERT occupancy in 18-month-old Wt mice treated with paroxetine in a dose of 5 mg/kg/day for 3 months. Vehicle-treated mice showed a high specific [ $^3$ H]3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzotrile binding in all brain regions analyzed, except the cerebellum (data not shown). In neocortex, the occupancy of SERT in paroxetine-treated mice was 86% (Fig. 3). These results confirmed that the APP/PS1 and Wt mice were treated with paroxetine doses considered therapeutic in humans [27].

### 3.4. Long-term paroxetine treatment has no effect on established $A\beta$ pathology

The effect of the 9-month oral SSRI treatment was examined by comparing the  $A\beta$  plaque load in 18-month-old paroxetine- and vehicle-treated APP/PS1 mice. The  $A\beta$  plaque load was estimated by the use of a stereological point counting technique. The plaque load in the neocortex showed to be comparable in paroxetine- and vehicle-treated mice (Student  $t$  test: Veh,  $18.0 \pm 0.7\%$  (mean  $\pm$  standard error of the mean) vs. Prx,  $16.5 \pm 1.2\%$ ,  $P = .27$ ) (Fig. 4A and 4B). The mean CE and coefficient of variation of the estimated plaque loads are shown in Table 2. In addition, no difference was noted in plaque density ( $P = .66$ , Student  $t$  test), and plaque size ( $P > .05$ , all comparisons, Student  $t$  test), between vehicle- and paroxetine-treated mice (Fig. 4C and 4D). The majority of the plaques (>99%) were smaller than  $7.0 \times 10^3 \mu\text{m}^2$ , a few ranged from  $7.0$ – $13.9 \times 10^3 \mu\text{m}^2$  (0.5%), and almost no plaques were larger than  $14.0 \times 10^3 \mu\text{m}^2$  (Fig. 4D).

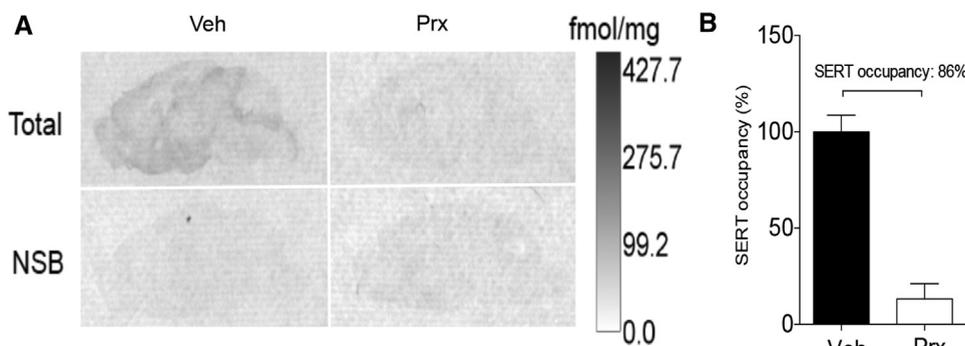


Fig. 3. Assessment of SERT occupancy by [ $^3$ H]DASB radioligand binding. (A) Autoradiograms showing the binding of the [ $^3$ H]DASB radioligand in sagittal brain sections from Wt mice administered paroxetine in the drinking water (5 mg/kg/day) or vehicle from 15 to 18 months of age. Nonspecific binding (NSB) is shown at the bottom. (B) Graphic presentation showing that the SERT occupancy (%) of the paroxetine-treated Wt mice (5 mg/kg/day) is 86% in the neocortex. Data are expressed as mean  $\pm$  SEM.  $n = 4$ /group. Abbreviations: [ $^3$ H]DASB, [ $^3$ H]3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzotrile; SEM, standard error of the mean.

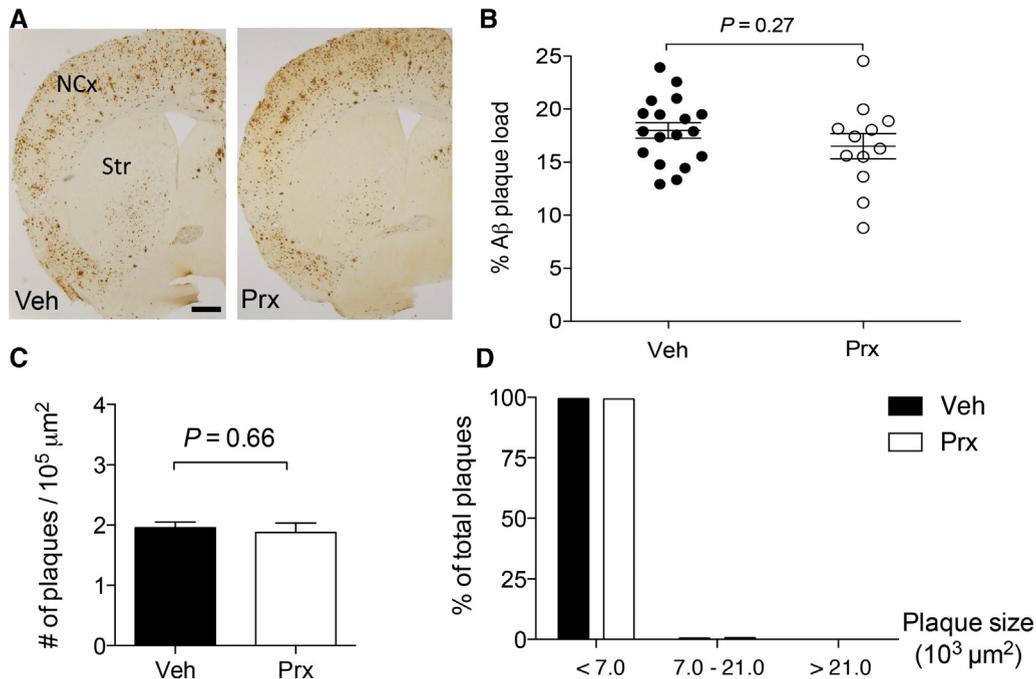


Fig. 4. No effect on A $\beta$  pathology in 18-month-old APP/PS1 mice treated with paroxetine for 9 months. (A) Immunohistochemistry of coronal section for the human A $\beta$  using the 6E10 antibody suggesting that the distribution and density of plaques is comparable in the neocortex of 18-month-old APP/PS1 mice treated with vehicle or paroxetine for 9 months. Bar: 200  $\mu\text{m}$ . (B) Dot-diagram showing the % A $\beta$  plaque load in APP/PS1 mice treated with vehicle or paroxetine for 9 months. The horizontal line indicates the mean and the error bars, the SEM. Statistical comparison shows no difference in % A $\beta$  plaque load between groups (Student  $t$  test,  $P = .27$ ). (C) Bar diagram showing the density of plaques in APP/PS1 treated with paroxetine ( $n = 12$ ) or vehicle ( $n = 18$ ). Student  $t$  test;  $P = .66$ . (D) Bar diagram showing the size distribution in % of plaques counted in APP/PS1 mice treated with paroxetine ( $n = 12$ ) or vehicle ( $n = 18$ ). Student  $t$  test;  $P < .05$ , all comparisons. Abbreviations: A $\beta$ , amyloid- $\beta$ ; APP, amyloid precursor protein; NCx, neocortex; SEM, standard error of the mean; Str, Striatum; Veh, vehicle; Prx, paroxetine; PS1, presenilin 1.

To evaluate if paroxetine treatment might reduce plaque load compared with baseline at the time when the paroxetine treatment was initiated, groups of 3- and 9-month-old male APP/PS1 mice were processed in parallel with the 18-month-old APP/PS1 and Wt mice. As previously shown for female APP/PS1 mice [28], the plaque load in the male APP/PS1 mice increased steadily from 3 months ( $0.04 \pm 0.04\%$ ) through 9 months ( $8.1 \pm 0.7\%$ ), to 18 months of age ( $19.8 \pm 0.9\%$ ) [One-way ANOVA:  $F(2,28) = 110.1$ ,  $P < .0001$ ]. The plaque load in the paroxetine-treated 18-month-old APP/PS1 mice was thereby statistically higher than that in 9-month-old APP/PS1 mice (Student  $t$  test  $16.5 \pm 1.2\%$  vs.  $8.1 \pm 0.7\%$ ,  $P < .0001$ ).

Table 2  
Precision of stereological estimates of the % A $\beta$  plaque load in paroxetine- and vehicle-treated 18-month-old APP/PS1 transgenics

| Treatment  | $n$ | % A $\beta$ plaque load |      |      |
|------------|-----|-------------------------|------|------|
|            |     | Mean $\pm$ SEM          | CE   | CV   |
| Vehicle    | 18  | $18.0 \pm 0.7$          | 0.07 | 0.17 |
| Paroxetine | 12  | $16.5 \pm 1.2$          | 0.06 | 0.24 |

Abbreviations: SEM, standard error of the mean; A $\beta$ , amyloid- $\beta$ ; CE, coefficient of error; CV, coefficient of variation; APP, amyloid precursor protein; PS1, presenilin 1.

NOTE. The CE and CV are calculated as in West et al. [29].

In conclusion, 9-month oral paroxetine treatment (10 and 5 mg/kg/day) does not mitigate A $\beta$  pathology in the neocortex of 18-month-old male APP/PS1 mice.

### 3.5. Paroxetine treatment impacts mouse behavior in both APP/PS1 and Wt mice

Effects of paroxetine treatment (5 mg/kg/day) were evaluated using the open field test, the elevated plus maze test, and the social interaction test. Repeated measures two-way ANOVA showed no significant effect of genotype in the open field ( $F[1,33] = 0.76$ ,  $P > .05$ ), no genotype by treatment interaction ( $F[1,33] = 0.18$ ,  $P > .05$ ), and only a tendency toward an effect of the paroxetine treatment ( $F[1,33] = 3.22$ ,  $P = .08$ ). Still, there was a significant effect of the paroxetine treatment that reduced locomotion given by the number of horizontal entries in both Wt and APP/PS1 mice (data not shown) ( $F[5,165] = 2.86$ ,  $P = .02$ ). No significant differences were observed in any parameters in the elevated plus maze. There was an effect of treatment on social interaction (two-way ANOVA:  $F[1,31] = 4.70$ ,  $P = .04$ ). However, comparison with vehicle- and paroxetine-treated mice showed no effect of treatment on individual behaviors, such as total contact time, and latency. There was no effect of genotype on any parameters, and no significant effect was observed in the social memory test (data not shown).

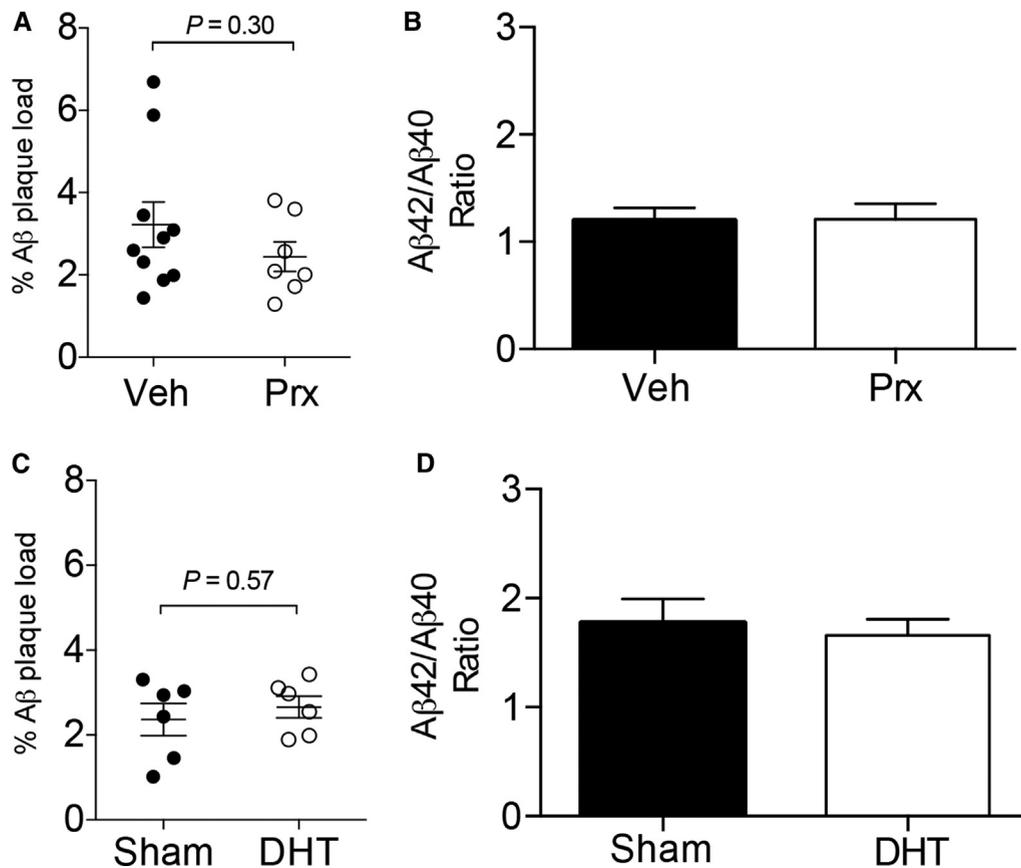


Fig. 5. No effect on A $\beta$  pathology in neocortex of 12-month-old APP/PS1 mice treated with paroxetine or subjected to a 5,7-DHT-induced depletion of 5-HT. (A, B) Dot diagram showing the % A $\beta$  plaque load in the neocortex of 6E10-stained sections of the left hemisphere (A), and bar diagram showing the A $\beta_{42}$ /A $\beta_{40}$  ratio in the right neocortex (B) of the same vehicle- and paroxetine-treated, 12-month-old APP/PS1 mice (Veh:  $n = 9$  (one outlier  $> 2$  standard deviations from the mean was excluded), Prx:  $n = 6$  (one sample not included in the ELISA for A $\beta_{40}$ ), Student  $t$  test,  $P = .95$ ). (C, D) Dot diagram showing the % A $\beta$  plaque load in 6E10-stained sections of the left hemisphere (C), and bar diagram showing the A $\beta_{42}$ /A $\beta_{40}$  ratio in the right neocortex (D) of the same 5,7-DHT- and sham-injected 12-month-old APP/PS1 mice. (Student  $t$  test,  $n = 6$ /group,  $P = .67$ ). Horizontal lines and bar height indicate the means and the error bars, the SEM. Abbreviations: A $\beta$ , amyloid- $\beta$ ; 5,7-DHT, 5,7-dihydroxytryptophan; APP, amyloid precursor protein; ELISA, enzyme-linked immunosorbent assay; Veh, vehicle; Prx, paroxetine; PS1, presenilin 1; 5-HT, 5-hydroxytryptamine; SEM, standard error of the mean.

### 3.6. Short-term paroxetine treatment has no effect on A $\beta$ pathology

In contrast to the studies by Cirrito et al. [18] and Nelson et al. [20] in which mice were treated with SSRI for 4 to 5 months and brains were processed for A $\beta$  pathology at a younger age, we treated mice with paroxetine (10 mg/kg/day *per os*) or vehicle for 3 months and analyzed A $\beta$  pathology in 12-month-old male APP/PS1 mice (Table 1) by quantifying plaque load in the right neocortex, and by enzyme-linked immunosorbent assay for A $\beta_{42}$  and A $\beta_{40}$  in the left neocortex (Fig. 5A and 5B). There was no difference in plaque load between the paroxetine- and vehicle-treated APP/PS1 mice ( $P > .05$ , Student  $t$  test) (Fig. 5A). In addition, levels of A $\beta_{42}$  and A $\beta_{40}$  were unaffected by the treatment ( $P > .05$ , Student  $t$  test) (data not shown) as was the ratio of A $\beta_{42}$ /A $\beta_{40}$  ( $P > .05$ , Student  $t$  test) (Fig. 5B), a risk parameter for AD [34].

### 3.7. Chronic loss of 5-HT has no effect on A $\beta$ pathology or mouse behavior

We finally examined whether 5,7-DHT-induced serotonergic deafferentation of the forebrain of 9-month-old male APP/PS1 mice might impact A $\beta$  pathology at 12 months of age (Table 1). The serotonergic deafferentation was validated by demonstration of undetectable levels of 5-HT and loss of SERT-immunopositive fibers in the neocortex (data not shown). The plaque load was comparable in sham-injected and deafferented APP/PS1 mice ( $2.4 \pm 0.9\%$  vs.  $2.7 \pm 0.6\%$ ,  $P = .29$ , Student  $t$  test) (Fig. 5C). Similarly, the levels of soluble A $\beta_{42}$  and A $\beta_{40}$  in the neocortex from the same mice were unaffected by the treatment (data not shown,  $P > .05$ , Student  $t$  test), as was the ratio of A $\beta_{42}$ /A $\beta_{40}$  ( $P > .05$ , Student  $t$  test) (Fig. 5D). The behavioral testing at baseline in the open field test showed higher locomotor activity of APP/PS1 compared

with Wt mice ( $P < .01$ , Student  $t$  test. APP/PS1:  $n = 22$ ; Wt:  $n = 16$ ). However, no significant differences were observed in the open field and social interaction tests between sham-injected and deafferented APP/PS1 mice at 12 months (data not shown, Sham:  $n = 6$ ; 5,7-DHT:  $n = 6$ ). These results suggest that 3-month impairment of 5-HT signaling has no effect on neither A $\beta$  pathology nor behavior of 12-month-old APP/PS1 mice.

## 4. Discussion

### 4.1. Main findings

The main results of this study showed that chronic *per os* treatment with paroxetine of plaque-bearing 9-month-old APP/PS1 mice for up till 9 months was unable to mitigate A $\beta$  pathology. However, treatment with paroxetine increased mortality for transgenic mice exclusively and reduced locomotion in both transgenic and littermate control mice. In addition, neurotoxin-induced serotonergic deafferentation of 9-month-old APP/PS1 mice had no impact on A $\beta$  pathology in 12-month-old male APP/PS1 mice. These results were unexpected as previous studies have suggested that SSRIs mitigate A $\beta$  pathology [18–20,22]. Considering the therapeutic implications of the potential option to impact directly on A $\beta$  pathology in individuals developing AD, it is important to understand the background for these apparently contradictory results.

### 4.2. What is already known

Previous studies have reported reduced A $\beta$  pathology after shorter periods of SSRI treatment in younger APP/PS1 and 3  $\times$  TgAD mice [18–20]. In Cirrito et al. [18], citalopram treatment of APP/PS1 mice from 3 to 7 months of age, resulted in a lower plaque load in neocortex of APP/PS1 mice. In the study by Sheline et al. [19], 4 weeks of citalopram treatment of 6-month-old APP/PS1 mice impaired the formation and growth of cortical plaques at 7 months [18]. Another study using 17-month-old APP/PS1 mice suggested that 5-week treatment with fluoxetine (10 mg/kg/day *i.p.*) was sufficient to mitigate plaque load; however, as acknowledged by the authors, the plaque load was not quantified [22]. In contrast, a study from our own group showed no or minor effect on the A $\beta$  levels in neocortex and hippocampus in APP/PS1 mice treated from 3 to 9 months of age with escitalopram in a dose of 5 mg/kg/day *per os*, leading to  $\approx 80\%$  occupancy of SERT [31]. This suggests that the differences in results cannot simply be explained by differences in the time of treatment start, but that other factors, such as sex, genetic background, and type of SSRI should be taken into consideration. Studies suggest that the R-enantiomer present in citalopram but not in escitalopram may reduce levels of A $\beta$  by SERT-independent mechanisms, possibly by increasing the  $\gamma$ -secretase activity, increasing the non-amyloidogenic processing of APP, and reducing the production of A $\beta$  [35,36].

### 4.3. Strengths and limitations

The fact that the APP/PS1 and Wt mice were all littermates, of the same sex, and housed identically during their entire life span affords credibility to our findings of (1) a lack of effect of chronic paroxetine treatment on established A $\beta$  pathology and (2) premature death by paroxetine treatment in APP/PS1 mice. APP/PS1 mice are known to exhibit seizure activity [37,38], which also occurs frequently in patients with AD [39]. Recently, fluoxetine administered in a dose of 15 mg/kg/day *i.p.* for 55 days to 19-week-old male APP/PS1 mice was found to increase seizure activity and mortality [40]. Although, the lowest paroxetine dose used in our study was 5 mg/kg/day, which is higher than the 0.3 mg/kg/day considered therapeutic in humans [27], it would be important to know if paroxetine in a dose of 0.3 mg/kg/day and therapeutic concentrations of e.g. citalopram increase seizure activity and mortality of AD transgenic mice. Importantly, we did not observe any mortality in the APP/PS1 mice treated with escitalopram (5 mg/kg/day *per os*) from 3 to 9 months of age [31]. It is also possible that chronic SSRI treatment, especially when administered *i.p.*, may enhance stress and reduce survival in APP/PS1 mice. Under stressful conditions, SSRI treatment exaggerates, rather than ameliorates depressive-like symptoms in C57BL/6 mice [41]. In the present study, APP/PS1 and littermate Wt mice were treated with a clinically relevant paroxetine dose starting at an age when A $\beta$  pathology is well established, but still increasing [28], similar to the progression of A $\beta$  pathology in patients with AD [42]. Despite these similarities, the present results have been obtained in a mouse model and cannot be directly extrapolated to AD, which is a complex, polygenic neurodegenerative disease. Finally, it is increasingly recognized that A $\beta$  pathology and associated molecular pathways represent only one out of several pathogenic mechanisms in AD, including tau pathology [43,44], brain inflammation [45], and cholinergic deficits [46], that can be modulated by serotonergic signaling in AD [47,48].

### 4.4. What does this study add and how to move forward?

In contrast to previous short-term studies using the same mouse model, this extended study demonstrated that chronic, long-term treatment with paroxetine did not ameliorate already established A $\beta$  pathology in old APP/PS1 transgenic mice and selectively increased mortality in the APP/PS1 transgenic mice, but not in controls. Future studies, examining the effect of chronic treatment with newer generation SSRIs in APP/PS1 and tau transgenic mice, should be performed to determine whether the observed lack of beneficial effects and the serious adverse effect is specific for paroxetine. Knowing the answer to these questions is important for adjusting or reconsidering the wide-spread use of SSRIs in individuals with mild cognitive impairment and AD.

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Authors' contribution: M.Se., M.Si., L.Ø.O., A.M., J.B.G., O.W., and B.F. designed the experiments, were responsible for carrying out the experiments, data analysis, and wrote the manuscript. For the behavioral experiments E.B. and K.L.L. helped with design and data analysis. A.M.K. assisted with surgery, and C.U.L. with histology in the same experiment. A.A.B. contributed to carrying out the experiments, and A.M. performed the SERT occupancy studies and assisted with statistical analysis. All authors approved the manuscript.

## Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.trci.2018.04.005>.

## RESEARCH IN CONTEXT

1. Systematic review: Chronic treatment with selective serotonin reuptake inhibitors (SSRIs) has been suggested to mitigate amyloid- $\beta$  ( $A\beta$ ) pathology in mouse models of Alzheimer's disease and in patients with Alzheimer's disease, in addition to having an antidepressant mechanism of action.
2. Interpretation: We tested the hypothesis that chronic treatment with paroxetine, a selective serotonin reuptake inhibitor, would mitigate  $A\beta$  pathology in plaque-bearing double-transgenic APP<sub>swc</sub>/PS1 $\Delta E9$  mutants. In addition, we addressed whether serotonin depletion influences  $A\beta$  pathology. Contrary to our hypothesis, chronic, long-term therapy with paroxetine did not mitigate  $A\beta$  pathology. However, the therapy increased mortality for transgenic mice exclusively. Depletion of serotonin did not exacerbate  $A\beta$  pathology.
3. Future directions: Future studies, examining the effect of chronic treatment with newer generation selective serotonin reuptake inhibitors in APP/PS1 and tau transgenics, should be performed to determine whether the observed lack of beneficial effects and the serious adverse effect are specific for paroxetine.

## References

- [1] Hendricksen M, Thomas AJ, Ferrier IN, Ince P, O'Brien JT. Neuropathological study of the dorsal raphe nuclei in late-life depression and Alzheimer's disease with and without depression. *Am J Psych* 2004;161:1096–102.
- [2] Sierksma AS, van den Hove DL, Steinbusch HW, Prickaerts J. Major depression, cognitive dysfunction and Alzheimer's disease: Is there a link? *Eur J Pharmacol* 2010;626:72–82.
- [3] Ramirez MJ, Lai MK, Tordera RM, Francis PT, Bowen DM. Serotonergic therapies for cognitive symptoms in Alzheimer's disease: Rationale and current status. *Drugs* 2014;74:729–36.
- [4] Arai H, Kosaka K, Iizuka R. Changes of biogenic amines and their metabolites in postmortem brains from patients with Alzheimer-type dementia. *J Neurochem* 1984;43:388–93.
- [5] Palmer AM, Wilcock GK, Esiri MM, Francis PT, Bowen DM. Monoaminergic innervation of the frontal and temporal lobes in Alzheimer's disease. *Brain Res* 1987;401:231–8.
- [6] Geerlings MI, den Heijer T, Koudstaal PJ, Hofman A. History of depression, depressive symptoms, and medial temporal lobe atrophy and the risk of Alzheimer disease. *Neurol* 2008;70:1258–64.
- [7] Harrington KD, Gould E, Lim YY, Ames D, Pietrzak RH, Rembach A, et al., AIBL Research Group. Amyloid burden and incident depressive symptoms in cognitively normal older adults. *Int J Geriatr Psych* 2017;32:455–63.
- [8] Ye Q, Bai F, Zhang Z. Shared genetic risk factors for late-life depression and Alzheimer's disease. *J Alz Dis* 2016;52:1–15.
- [9] Kessing LV, Forman JL, Andersen PK. Do continued antidepressants protect against dementia in patients with severe depressive disorder? *Int Clin Psychopharmacol* 2011;26:316–22.
- [10] Rosenberg PB, Mielke MM, Han D, Leoutsakos JS, Lyketsos CG, Rabins PV, et al. The association of psychotropic medication use with the cognitive, functional, and neuropsychiatric trajectory of Alzheimer's disease. *Int J Geriatr Psych* 2012;27:1248–57.
- [11] Jones HE, Joshi A, Shenkin S, Mead GE. The effect of treatment with selective serotonin reuptake inhibitors in comparison to placebo in the progression of dementia: a systematic review and meta-analysis. *Age Ageing* 2016;45:448–56.
- [12] Moranos J, Nwankwo C, Patten SB, Mousseau DD. The association of antidepressant drug usage with cognitive impairment of dementia, including Alzheimer disease: a systematic review and meta-analysis. *Depress Anxiety* 2017;34:217–26.
- [13] Bartels C, Wagner M, Wolfsgruber S, Ehrenreich H, Scheider A, for the Alzheimer's Disease Neuroimaging Initiative. Impact of SSRI therapy on risk of conversion from mild cognitive impairment to Alzheimer's dementia in individual with previous depression. *Am. J. Psychiatry* 2018;175:232–41.
- [14] Madsen K, Neumann WJ, Holst K, Marner L, Haahr MT, Lehel S, et al. Cerebral serotonin 4 receptors and amyloid- $\beta$  in early Alzheimer's disease. *J Alzheimers Dis* 2011;26:457–66.
- [15] Garcia-Alloza M, Hirst WD, Chen CP, Lasheras B, Francis PT, Ramirez MJ. Differential involvement of 5-HT<sub>1B/ID</sub> and 5-HT<sub>6</sub> receptors in cognitive and non-cognitive symptoms in Alzheimer's disease. *Neuropsychopharmacol* 2004;29:410–6.
- [16] Hasselbalch SG, Madsen K, Svarer C, Pinborg LH, Holm S, Paulson OB, et al. Reduced 5-HT<sub>2A</sub> receptor binding in patients with mild cognitive impairment. *Neurobiol Aging* 2008;29:1830–8.
- [17] Guiard BP, Di Giovanni G. Central serotonin-2A (5-HT<sub>2A</sub>) receptor dysfunction in depression and epilepsy: the missing link? *Front Pharmacol* 2015;6:46.
- [18] Cirrito JR, Disabato BM, Restivo JL, Verges DK, Goebel WD, Sathyan A, et al. Serotonin signaling is associated with lower amyloid- $\beta$  levels and plaques in transgenic mice and humans. *Proc Natl Acad Sci U.S.A* 2011;108:14968–73.

- [19] Sheline YI, West T, Yarasheski K, Swarm R, Jasielec MS, Fisher JR, et al. An antidepressant decreases CSF A $\beta$  production in healthy individuals and in transgenic AD mice. *Sci Transl Med* 2014;6:236re4.
- [20] Nelson RL, Guo Z, Halagappa VM, Pearson M, Gray AJ, Matsuoka Y, et al. Prophylactic treatment with paroxetine ameliorates behavioral deficits and retards the development of amyloid and tau pathologies in 3xTgAD mice. *Exp Neurol* 2007;205:166–76.
- [21] Jankowsky JL, Slunt HH, Ratovitski T, Jenkins NA, Copeland NG, Borchelt DR. Co-expression of multiple transgenes in mouse CNS: A comparison of strategies. *Biomol Eng* 2001;17:157–65.
- [22] Ma J, Gao Y, Jiang L, Chao FL, Huang W, Zhou CN, et al. Fluoxetine attenuates the impairment of spatial learning ability and prevents neuron loss in middle-aged APP<sup>swe</sup>/PSEN1<sup>dE9</sup> double transgenic Alzheimer's disease mice. *Oncotarget* 2017;8:27676–92.
- [23] Liu Y, Yoo MJ, Savonenko A, Stirling W, Price DL, Borchelt DR, et al. Amyloid pathology is associated with progressive monoaminergic neurodegeneration in a transgenic mouse model of Alzheimer's disease. *J Neurosci* 2008;28:13805–14.
- [24] Von Linstow CU, Severino M, Metaxas A, Waider J, Babcock AA, Lesch KP, et al. Effect of aging and Alzheimer's disease-like pathology on brain monoamines in mice. *Neurochem Int* 2017;108:238–45.
- [25] Tesseur I, Pimenova AA, Lo AC, Ciesielska M, Lichtenthaler SF, De Maeyer JH, et al. Chronic 5-HT<sub>4</sub> receptor activation decreases A $\beta$  production and deposition in hAPP/PS1 mice. *Neurobiol Aging* 2013;34:1779–17789.
- [26] Fisher JR, Wallace CE, Tripoli DL, Sheline YI, Cirrito JR Redundant G(s)-coupled serotonin receptors regulate amyloid-beta metabolism in vivo. *Mol Neurodegrad* 2016;11:45.
- [27] Sanchez C, Reines EH, Montgomery SA. A comparative review of escitalopram, paroxetine, and sertraline: Are they all alike? *Int Clin Psychopharmacol* 2014;29:185–96.
- [28] Babcock AA, Ilkjaer L, Clausen BH, Villadsen B, Dissing-Olesen L, Bendixen AT, et al. Cytokine-producing microglia have an altered beta-amyloid load in aged APP/PS1 Tg mice. *Brain Behav Immun* 2015;48:86–101.
- [29] Olesen LØ, Bouzinova EV, Severino M, Sivasaravanaparan M, Hasselstrøm JB, Finsen B, et al. Behavioural phenotyping of APP<sup>swe</sup>PS1<sup>dE9</sup> mice: age-related changes and effect of long-term paroxetine treatment. *PLoSOne* 2016;11:e0165144.
- [30] Olesen LØ, Sivasaravanaparan M, Severino M, Babcock AA, Bouzinova EV, West MJ, et al. Neuron number and neurogenesis in the dentate gyrus of aged APP<sup>swe</sup>/PS1<sup>dE9</sup> transgenic mice: effect of long-term treatment with paroxetine. *Neurobiol Dis* 2017;104:50–60.
- [31] von Linstow CU, Waider J, Grebing M, Metaxas A, Lesch KP, Finsen B. Serotonin augmentation therapy by escitalopram has minimal effects on amyloid- $\beta$  levels in early-stage Alzheimer's-like disease in mice. *Alz Res Ther* 2017;9:74.
- [32] Lambertsen KL, Gramsbergen JB, Sivasaravanaparan M, Ditzel N, Sevelsted-Møller LM, Oliván-Viguera A, et al. Genetic KCa3.1-Deficiency produces locomotor hyperactivity and alterations in cerebral monoamine levels. *PLoS One* 2012;7:e47744.
- [33] West MJ, Østergaard K, Andreassen O, Finsen B. Counting in situ hybridized neurons with modern unbiased stereological methods. *J Comp Neurol* 1996;370:11–22.
- [34] Hampel H, Shen Y, Walsh DM, Aisen P, Shaw LM, Zetterberg H, et al. Biological markers of amyloid  $\beta$ -related mechanisms in Alzheimer's disease. *Exp Neurol* 2010;223:334–46.
- [35] Payton S, Cahill CM, Randall JD, Gullans SR, Rogers JT. Drug discovery targeted to the Alzheimer's APP mRNA 5'-untranslated region: the action of paroxetine and dimercaptopropanol. *J Mol Neurosci* 2003;20:267–75.
- [36] Morse LJ, Payton SM, Cuny GD, Rogers JT. FDA-preapproved drugs targeted to the translational regulation and processing of the amyloid precursor protein. *J Mol Neurosci* 2004;24:129–36.
- [37] Minkevičienė R, Rheims S, Dobszay MB, Zilberter M, Hartikainen J, Fülöp L, et al. Amyloid beta-induced neuronal hyperexcitability triggers progressive epilepsy. *J Neurosci* 2009;29:3453–62.
- [38] Ziyatdinova S, Gurevicius K, Kutchiashvili N, Bolkvadze T, Nissinen J, Tanila H, et al. Spontaneous epileptiform discharges in a mouse model of Alzheimer's disease are suppressed by antiepileptic drugs that block sodium channels. *Epilepsy Res* 2011;94:75–85.
- [39] Born HA. Seizures in Alzheimer's disease. *Neurosci* 2015;286:251–63.
- [40] Sierksma ASR, de Nijs L, Hoogland G, Vanmierlo T, van Leeuwen FW, Rutten BP, et al. Fluoxetine treatment induces seizure behavior and premature death in APP<sup>swe</sup>/PS1<sup>dE9</sup> mice. *J Alz Dis* 2016;51:677–82.
- [41] Alboni S, van Dijk M, Poggini S, Milior G, Perrotta M, Drenth T, et al. Fluoxetine effects on molecular, cellular and behavioral endophenotypes of depression are driven by the living environment. *Mol Psych* 2017;22:552–61.
- [42] Jack CR, Wiste HJ, Lesnick GT, Weigand SD, Knopman DS, Vemuri P, et al. Brain  $\beta$ -amyloid load approaches a plateau. *Neuro* 2013;80:890–6.
- [43] Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med* 2016;8:595–608.
- [44] Li C, Götz J. Tau-based therapies in neurodegeneration: Opportunities and challenges. *Nat Rev Drug Disc* 2017;16:863–83.
- [45] Heneka MT, Carson MJ, Khoury JE, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol* 2015;14:389–405.
- [46] Ramos-Rodriguez JJ, Pacheco-Herrero M, Thyssen D, Murillo-Carretero MI, Berrococo E, Spires-Jones TL, et al. Rapid  $\beta$ -amyloid deposition and cognitive impairment after cholinergic denervation in APP/PS1 mice. *J Neuropathol Exp Neurol* 2013;72:272–85.
- [47] Smith GS, Kramer E, Ma Y, Hermann CR, Dhawan V, Chaly T, et al. Cholinergic modulation of the cerebral metabolic response to citalopram in Alzheimer's disease. *Brain* 2009;132:392–401.
- [48] Frozza RL, Lourance MV, De Felice FG. Challenges for Alzheimer's disease therapy: Insights from novel mechanisms beyond memory defects. *Front Neurosci* 2018;12:37.