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Treatment with the glutamate modulator riluzole prevents early life stress-induced cognitive deficits and impairments in synaptic plasticity in APPswe/PS1dE9 mice

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HIGHLIGHTS

- In APP/PS1 mice, early life stress impairs LTP and flexible spatial learning.
- Early life stress increases plaque load in APPswe/PS1dE9 mice.
- EAAT2 correlates positively with flexible spatial learning.
- Riluzole treatment prevented ELS changes in LTP, flexible spatial learning and plaque load.
- Thus, normalising glutamate signalling rescues ELS-induced deficits in AD mice.

ABSTRACT

Background: Environmental factors like stress affect age-related cognitive deficits and promote Alzheimer's disease (AD)-related pathology in mice. Excess glutamate has been proposed as a possible mediator underlying these effects in the hippocampus, a vulnerable brain region implicated in learning and memory.

Methods: Here, we examined a) whether stress applied during a sensitive developmental period early in life affects later synaptic plasticity, learning and memory and plaque load in the APPswe/PS1dE9 mouse model for Alzheimer's disease and b) whether these effects could be rescued using long-term treatment with the glutamate modulator riluzole.

Results: Our results demonstrate that ELS impairs synaptic plasticity in 6-month-old mice and increases plaque load in 12-month-old APPswe/PS1dE9 mice, while impairing flexible spatial learning in the Barnes maze at this age. Notably, spatial learning correlated well with hippocampal expression of the transporter EAAT2, which is important for extracellular glutamate uptake. The changes in LTP, plaque load and cognition after ELS were all prevented by riluzole treatment that started from post-weaning.

Conclusion: These results suggest that normalising glutamate signalling may be a viable therapeutic strategy for treating vulnerable individuals at risk of developing stress-aggravated AD, particularly in relation to adverse early life experiences.

1. Introduction

Alzheimer's disease (AD) is a common age-related neurodegenerative disorder characterised by progressive cognitive decline (Selkoe and Schenk, 2003) that is, in view of current human life expectancy (Jagust, 2013; Prince et al., 2013; Small et al., 2002), expected to increase in the future (Brookmeyer et al., 2007). While familial forms of AD are linked to rare genetic mutations (Querfurth and LaFerla, 2010; Scheltens et al., 2016), the cause of sporadic AD remains elusive. Various recent lines of evidence suggest that environmental factors play a role in AD risk (Baumgart et al., 2015; Herbert and Lucassen, 2016; Matthews et al., 2016; Xu et al., 2015). One of these environmental factors may be exposure to stress, particularly when experienced during the sensitive period of early life. For instance, individuals with a history of childhood adversity have a higher probability to develop later diseases (Ferraro et al., 2016; Schafer and Ferraro, 2012), and a higher prevalence and severity of mild cognitive impairment at an older age (Kang et al., 2017; Wang et al., 2016). Likewise, evidence from rodent studies indicates...
that early life stress (ELS) triggers age-related cognitive decline (Oitzl et al., 2000; Solas et al., 2010; Vallée et al., 1999). Such ELS-induced accelerations of cognitive decline are often accompanied by (neuro) biological changes of aging, such as a reduced telomere length (Price et al., 2013), reductions in adult hippocampal neurogenesis (Bath et al., 2016; Lucassen et al., 2015; Naninck et al., 2015), and enhanced neuro-inflammatory profiles (Hoeijmakers et al., 2016; Johnson and Kaffman, 2018). In line with the hypothesis that ELS may affect the course of AD related changes, ELS has been shown to worsen cognitive decline in various genetic mouse models for AD both following pre- (Siersksma et al., 2013) and postnatal stress (Hui et al., 2017; Lesuis et al., 2018). Yet how early life adversity aggravates aging and AD is unknown.

Studies in transgenic animal models for AD have implicated glutamatergic N-methyl-D-aspartate (NMDA) receptors in AD and reveal that glutamatergic synapses are particularly affected (Haass and Selkoe, 2007; Kamenetz et al., 2003; Kessels et al., 2013; Rowan et al., 2003; Townsend et al., 2006; Turner et al., 2005; Walsh et al., 2002). Whereas synaptic NMDA activity is critical for long-term potentiation (LTP) and memory formation, excessive extra-synaptic NMDA activation has been associated with the induction of long-term depression and even excitotoxicity (Hardingham, 2006; Hardingham and Bading, 2010; Rusakov and Kullmann, 1998). Glutamate uptake by the excitatory amino acid transporter 2 (EAAT2, also known as GLT-1 or Slc1a2) is the primary mechanism via which extracellular glutamate regulates physiological glutamatergic neurotransmission in the brain (Furuta et al., 1997; Huang and Bergles, 2004; Tzingounis and Wadiche, 2007). Interestingly, the expression of gluta
tamate transporters, including EAAT2, is decreased after early life stress (Odeon et al., 2015), in aging (Brothers et al., 2013; Potier et al., 2010) as well as in AD (Jacob et al., 2007; Masliah et al., 1996) and has been associated with neurodegeneration (Masliah et al., 1996).

Since (early life) stress can disturb glutamatergic signalling and function, the effects of ELS and AD may thus converge at glutamatergic transmission (O'Connor et al., 2013; Musazzi et al., 2011). In the present study we therefore tested in APPswe/PS1dE9 mice whether ELS affects mechanisms which are critical for the uptake of glutamate from synapses (i.e. EAAT2), synaptic plasticity, and whether these effects can be modulated by the glutamate modulator riluzole. This drug alters glutamatergic neurotransmission by decreasing presynaptic glutamate release, and by facilitating glial glutamate uptake via increased EAAT2 expression (Azbill et al., 2006; Frizzo et al., 2004; Fumagalli et al., 2008; Pereira et al., 2017; Pittenger et al., 2008). Riluzole increases synaptic connectivity, strengthens neural connectivity (Larkum and Nevian, 2008), and enhances LTD (De Roo et al., 2008). Moreover, riluzole prevents age-related cognitive decline in rodents (Pereira et al., 2014) and AD related changes in gene expression (Pereira et al., 2017). Our present results show not only that ELS affects synaptic plasticity and spatial memory in APPswe/PS1dE9 mice, in close correlation with EAAT2 expression in the hippocampus, but also that these deficits in LTP and spatial memory in 12-month-old AD mice were completely prevented by prolonged riluzole treatment.

2. Materials and methods

2.1. Mice and breeding

All experimental procedures were conducted under Dutch national law and European Union directives on animal experiments (2010/63/EU), and were approved by the animal welfare committee of the University of Amsterdam. Wild type (WT) and APPswe/PS1dE9 male littermates (Jankowsky et al., 2001) of 6 and 12 (± 1) months of age were used. To obtain mice, two 10 week old C57BL/6J virgin WT females (Harlan B.V., Venray, The Netherlands) and one heterozygous male APPswe/PS1dE9 mouse were housed together for one week to allow mating. Pregnant females were housed individually in a standard cage covered with a filter top and monitored daily for the birth of pups (Arp et al., 2016; Lesuis et al., 2018, 2016; Rice et al., 2008). When a litter was born before 10.00 a.m., the previous day was considered the day of birth (postnatal day 0; PND 0), after which the early life stress paradigm was initiated from PND 2-9. At PND 21, mice were weaned and ear biopsies were collected for identification and genotyping. Mice were housed with 2-6 same sex littermates per cage. All experimental mice were left undisturbed (except for cage cleaning once a week) until the start of the experimental procedures at 6 and 12 months of age. Number of mice used: 6 months old: 56 mice; 12 months old: 57 mice.

2.2. Early life stress

At postnatal day (PND) 2, litters were culled to 6 pups per litter, and dams and their litters were randomly assigned to the early life stress (ELS) or control condition until PND 9, after which all mice were treated equally, as described before (Arp et al., 2016; Lesuis et al., 2018, 2016; Naninck et al., 2015; Rice et al., 2008). Briefly, control dams were provided with a standard amount of sawdust bedding and nesting material (one square piece of cotton nesting material (5 × 5 cm; TecniLab-BMI, Someren, the Netherlands)). ELS dams were provided with a strongly reduced amount of sawdust bedding and half the nesting material (1/2 piece of nesting material), and a fine-gauge stainless steel mesh was placed 1 cm above the cage floor.

2.3. Riluzole treatment

Riluzole (Selleckchem, The Netherlands) was added to the drinking water from weaning (PND 28) onwards, and provide fresh every 3-4 days. Bottles were shielded from light to prevent light exposure. A dosage of 4.0 mg/kg per day per animal (adapted from (Pereira et al., 2017)) was dissolved in tap water and stirred until the water was completely transparent.

2.4. Field potential recordings

Field potential recordings were conducted in 6-month-old male animals. At PND 180 ± 14 mice were sacrificed between 9 and 10 a.m. through quick decapitation. Immediately after decapitation, the brain was rapidly removed, and collected in ice-cold oxygenated (95% O2/5% CO2) solution containing (in mM): Cholinechloride (120), glucose (10), NaHCO3 (25), MgSO4 (6), KCl (3.5), NaH2PO4 (1.25), CaCl2 (0.5). Coronal slices (350 μm) were cut using a microtome (Leica VT1000S). For recovery, slices were incubated for 20 min in warm (32°C) oxygenated standard artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl (120), KCl (3.5), MgSO4 (1.3), NaH2PO4 (1.25), CaCl2 (2.5), glucose (10), NaHCO3 (25), after which the sections were maintained at room temperature (22°C). Sections containing the dorsal hippocampal CA1 area (bregma −2.0 mm to −3.2 mm) were placed in a recording chamber with a constant flow of oxygenated aCSF. Field excitatory synaptic potentials (fEPSPs) were recorded as described previously (Bagot et al., 2009; Pu et al., 2007; Wiegert et al., 2006). fEPSPs were evoked using a stainless steel bipolar stimulation electrode (60 μm diameter, insulated except for the tip) positioned on the Schaffer collaterals and recorded through a glass electrode (2.5-MΩ impedance, filled with aCSF) positioned in the CA1 stratum radiatum. A stimulus-response curve was generated by gradually increasing the stimulus intensity to define a level that generated the half-maximal response that was used for the remainder of the experiment. Once the input-output curve for each recording was established, baseline synaptic transmission was monitored by stimulating at 0.033 Hz for 10 min. When recordings were stable, afferent fibres were stimulated at 10 Hz for 90 s (Mayford et al., 1996; Wiegert et al., 2006). We used this paradigm since it elicits synaptic plasticity at the threshold for LTD and LTD, and is therefore well-suited to examine subtle and potentially bi-directional changes in synaptic plasticity (Derks et al., 2016; Mayford et al., 1996;
Wiegert et al., 2006). Next, the degree of potentiation was determined by recording fEPSPs every 30 s for 1 h. Synaptic transmission was measured by determining the slope of the fEPSP. The average baseline value was normalised to 100% and all values of the experiment were normalised to this baseline average.

### 2.5. Barnes maze

Mice (12 months) were transferred to a reversed light/dark cycle (lights on 8 p.m., lights off 8 a.m.) one month before behavioural testing commenced and were single-housed in the behaviour room for one more week before testing. Three days prior to testing, mice were handled for 5 min per day. Testing was conducted during the dark, active phase of the mice between 12 and 6 p.m. During testing, recording was done with a video camera connected to a computer with Ethovision software version 14 (Noldus, The Netherlands). Twelve-month-old APPswe/PS1dE9 and WT male mice were tested for spatial memory in the spatial Barnes maze task. A classic set up was used (110 cm diameter, 12 exit holes) in which mice were trained for one (day 1 and 2) or two (day 3 and 4) sessions a day (adapted from Lesuis et al., 2018). During training, mice were placed in the centre of the maze twice (inter-trial interval of 30 min) and were allowed to navigate to the exit hole leading to the home cage (acquisition learning). Behavioural flexibility was tested by relocating the exit hole to another location on the maze (180°) for two sessions per day on two consecutive days. Cages containing used bedding material were placed at equal distances under the maze to avoid guidance by odour cues, the board was rotated after each trial, and the maze was cleaned with 25% EtOH to dissipate odour cues. The location of the exit hole was always fixed relative to the distal extra-maze cues in the room. The distance the mice travelled until the exit hole was reached was analysed.

### 2.6. Tissue preparation

One week after behavioural testing, mice were sacrificed by quick decapitation, between 8.00 and 9.00 p.m. (beginning of the inactive phase). The brains were removed, and the left hemisphere was immersion-fixed in 4% paraformaldehyde in phosphate buffer (0.1 M PB, pH 7.4) for 48 h and then stored in 0.01% sodium-azide in 0.1 M PB at 4°C until further processing. Paraformaldehyde-fixed tissue was over-night cryoprotected in 30% sucrose/0.1 M PB. Frozen hemispheres were cut in 40 μm thick coronal sections in six parallel series using a sliding microtome and stored in antifreeze solution (30% Ethylene glycol, 20% Glycerol, 50% 0.05 M PBS) at −20°C until immunohistochemical staining.

### 2.7. DAB immunohistochemistry

Immunocytochemistry was used to visualise amyloid plaques. Prior to staining, sections were mounted on glass (Superfrost Plus slides, Menzel, Braunschweig, Germany) and antigen retrieval was performed by heating the sections in 0.1 M citrate buffer (pH 6) in a microwave (Samsung M6235) to a temperature of ±95°C for 15 min. Sections were incubated with 0.3% H2O2 for 15 min to block endogenous per-oxidase activity, and were next incubated for 30 min in blocking buffer (1% BSA, 0.3% Triton X-100 in 0.05 M TBS). Primary antibody 6E10 (1:1500, BioLegend) was incubated for 2 h at room temperature and overnight at 4°C. Sections were incubated with biotinylated secondary antibody (1:200, sheep anti-mouse, GE Healthcare) for 2 h at room temperature followed by a 90 min incubation with avidin-biotin complex (ABC kit, Elite Vectastain Brunswchwig Chemie, Amsterdam, 1:800). Subsequent chromogen development was performed with diamino-benzidine (DAB; 20 mg/100 mL 0.05 M Tris, 0.01% H2O2).

### 2.8. Fluorescent immunohistochemistry

A random subset of brains (N = 4-5 mice/group) was used for EAAT2 immunohistochemistry. All stainings were performed on parallel series from the same brains within an age group. Sections were incubated with blocking mix containing goat anti-mouse Fab fragments (1:200) in 0.1 M PBS. Primary mouse anti-EAAT2 (1:250, Cell Signalling) was incubated for 1 h at RT followed by incubation at 4°C overnight. Sections were incubated in the secondary antibodies (1:200 sheep anti-mouse) for 2 h, and mounted and coverslipped with Vectashield.

### 2.9. Imaging and quantification

Quantification was performed on coronal sections of the left hemisphere on 8–10 sections per animal of matched anatomical levels along the rostro-caudal axis (Lesuis et al., 2017). Using a Nikon DS-Ri2 microscope, representative images of 20x magnification were systematically captured. For images from DAB staining, ImageJ software was used to binarise the pictures to 8-bit black-and-white pictures, and a fixed intensity threshold was applied defining the DAB staining. Measurements were performed for the percentage area covered by DAB staining (Christensen et al., 2009; Marlatt et al., 2013). EAAT2 fluorescence was measured using ImageJ in 50 μm intervals from the cellular layer in the CA1 of the hippocampus (Pereira et al., 2017). All images were quantified by an experimenter blinded to the experimental procedures and animals.

### 2.10. Statistical analysis

Data were analysed using SPSS 22.0 (IBM software). Data are expressed as mean ± standard error of the mean (S.E.M.). Data were considered statistically significant when p < 0.05. Outliers were determined using a Grubb’s test, which identifies a maximum of one value to be excluded from the analysis. Repeated measures ANOVA was performed to assess Barnes maze learning curves over the different trials, and to assess synaptic plasticity. Greenhouse-Geisser correction was applied when the assumption of sphericity was violated. To enhance the readability of the graphs, the repeated measures data for the LTP and Barnes maze have been split up in separate graphs (Fig. 1A and B and Fig. 2A–D), although statistical analysis was performed on all data combined. To compare between groups accounting for the main and interaction effects of genotype (WT vs. APPswe/PS1dE9), condition (Ctrl vs. ELS), and treatment (water vs. Riluzole), a 2 × 2 × 2 ANOVA was performed, with planned contrasts as post hoc tests to correct for the relevant comparisons conducted. Pearson’s correlation test was conducted to determine correlations.

### 3. Results

#### 3.1. Early life stress model

APPswe/PS1dE9 and WT littermates were housed with limited nesting and bedding materials from PND 2 to 9 in order to induce ELS. In line with previous reports (Lesuis et al., 2018; Naninck et al., 2015) this procedure reduced body weight gain (Ctrl: 3.6 ± 0.11 g; ELS: 2.5 ± 0.08 g; t(55) = 8.06, p = 0.001), indicative of effective stress exposure. Since effects of ELS are particularly sex-specific (Loi et al., 2017; Naninck et al., 2015), all experiments were further conducted with male mice. From PND 28 onwards, half of the mice received riluzole supplementation to their drinking solution. Water consumption was measured at 3 different time points throughout the experiment (Table 1). No differences in consumption of water with or without riluzole were observed (see Table 1).
PS1dE9 water vs. riluzole: \( p < 0.001 \); ELS-APPswe/PS1dE9 water vs. (1,63) = 22.62, \( p < 0.001 \); post hoc difference between: Ctrl-APPswe/PS1dE9 and ELS-APPswe/PS1dE9 mice (genotype*treatment: \( F(1,63)=62.41, p < 0.001 \)), most strongly in APPswe/PS1dE9 mice (F(1,63) = 15.34, \( p < 0.001 \)). Post hoc testing revealed a significant effect of riluzole treatment in ELS-WT mice (\( p = 0.01 \)), Ctrl-APPswe/PS1dE9 mice (\( p < 0.001 \)), and ELS-APPswe/PS1dE9 mice (\( p < 0.001 \)).

### 3.3. Barnes maze training

We next investigated whether ELS-induced changes in synaptic plasticity also affect spatial memory performance in WT and APPswe/PS1dE9 mice (Lesuis et al., 2018), and whether such effects could be prevented by riluzole in 12-month-old mice. For acquisition learning, there was a mild but significant effect of treatment, in which riluzole resulted in a shorter distance to locate the exit hole (F(1,58) = 6.91, \( p = 0.01 \)) (Fig. 2A and B). However, neither genotype nor condition affected performance on acquisition learning (genotype effect: \( F(1,58)=0.27, p = 0.61 \); condition effect: \( F(1,58)=1.31, p = 0.26 \)). No effects were observed when examining the last trial of acquisition learning, indicating that after 6 training sessions, all groups learned to find the location of the exit hole to a similar degree (Fig. 2C).

When the exit hole was relocated to a new location, riluzole again improved performance, resulting in a shorter distance travelled to the exit hole (F(1,58) = 24.90, \( p < 0.001 \)) (Fig. 2D and E). In addition, APPswe/PS1dE9 mice took a longer distance to find the exit hole (F(1,58) = 9.97, \( p = 0.003 \)). Analysis of the last trial, as an indication of how well mice had learned to locate the exit hole, revealed an effect of treatment, genotype and condition, as well as a condition x treatment interaction effect (treatment: \( F(1,58)=39.03, p < 0.001 \); genotype: \( F(1,58)=5.95, p = 0.018 \); condition: \( F(1,58)=8.56, p = 0.005 \); condition x treatment: \( F(1,58)=7.68, p = 0.003 \)) (Fig. 2F). Post hoc testing revealed that in APPswe/PS1dE9 mice, ELS resulted in a longer distance to the exit hole than Ctrl animals. Riluzole treatment also resulted in a shorter travelling distance to the exit hole in both groups.

### 3.4. EAAT2 expression

Immunocytochemical labelling revealed that EAAT2 was reduced in the distal portion of the CA1 area with age (F(1,34) = 81.38, \( p = 0.001 \)) (Fig. 3A). We further found that EAAT2 expression in aged riluzole-treated animals was enhanced when compared to untreated young and aged mice (treatment effect: \( F(1,34)=250.22, p = 0.001 \)). Moreover, in water-treated animals, the APPswe/PS1dE9 genotype reduced EAAT2 expression at all ages (F(1,34) = 5.6, \( p = 0.025 \)). We found an interaction effect between condition x treatment (F(1,34) = 14.42, \( p = 0.001 \)) and genotype x treatment (F(1,34) = 8.76, \( p = 0.006 \), reflecting the enhanced EAAT2 expression following riluzole treatment in aged ELS and APPswe/PS1dE9 mice.

Importantly, EAAT2 expression correlated significantly with cognitive performance of the last learning trial of the Barnes maze in aged mice (\( r = -0.75, n = 32, p = 0.001 \)) (Fig. 3B), which suggests a potential mechanism by which riluzole may rescue cognitive performance.

### 3.5. Hippocampal plaque load

Finally, we investigated plaque load, an important pathological hallmark of AD, and we found a significant interaction effect between condition and treatment in the hippocampal CA1 area (F(1,37) = 7.52, \( p = 0.009 \)). ELS-APPswe/PS1dE9 mice treated with water displayed an increased plaque load, which was absent in APPswe/PS1dE9 animals treated with riluzole treatment (\( p < 0.05 \)) (Fig. 3C). Plaque load did not correlate with cognitive decline (\( r = 0.09, n = 32, p = 0.59 \)) (Fig. 3D).
4. Discussion

Previous studies have reported that early life stress can alter flexible spatial learning, synaptic plasticity and amyloid levels in 12-month-old APPswe/PS1dE9 mice (Lesuis et al., 2018). In the current study, we investigated whether riluzole, a glutamate modulator (Brothers et al., 2013; Pittenger et al., 2008), can rescue these effects. We found that ELS-induced impairments in synaptic plasticity, flexible spatial learning and plaque load in APPswe/PS1dE9 mice can be rescued by prolonged riluzole treatment from post-weaning onward, likely by regulating EAAT2 expression.

Fig. 2. Chronic riluzole-treated aged APPswe/PS1dE9 mice were protected against ELS-induced deficits in Barnes maze performance. (A,B) The distance travelled before the mice located the exit hole was comparable between all groups (water-treated mice: full line; riluzole-treated mice: dashed line). (C) The distance travelled during the last trial of acquisition learning was also comparable between all groups. (D) When the exit hole was relocated to a novel location, in WT mice, long-lasting riluzole treatment (dashed line) resulted in a slight improvement in the distance travelled to the exit hole, compared to water-treated mice (full line). (E) Water-treated APPswe/PS1dE9 mice took longer to locate the exit hole compared to WT mice, especially when exposed to ELS. The distance travelled was improved in all groups after chronic riluzole treatment. (F) The distance travelled to the exit hole when the exit hole was relocated to a new location was reduced by long-term riluzole treatment in all groups, except for Ctrl-WT mice. Ctrl-WT-water: N = 7; ELS-WT-water: N = 9; Ctrl-APPswe/PS1dE9-water: N = 9; ELS-APPswe/PS1dE9-water: N = 9; Ctrl-WT-riluzole: N = 7; ELS-WT-riluzole: N = 8; Ctrl-APPswe/PS1dE9-riluzole: N = 8; ELS-APPswe/PS1dE9-riluzole: N = 9. *: p < 0.05.

Table 1
Consumption of water with and without riluzole at different time points throughout the experiment.

<table>
<thead>
<tr>
<th></th>
<th>PND 35</th>
<th>6 months</th>
<th>11 months</th>
</tr>
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<tbody>
<tr>
<td>Water</td>
<td>4.1 ± 1.0 (20)</td>
<td>4.7 ± 1.0 (20)</td>
<td>5.2 ± 1.0 (20)</td>
</tr>
<tr>
<td>Water + Rizulole</td>
<td>4.2 ± 1.0 (16)</td>
<td>4.7 ± 1.0 (16)</td>
<td>5.5 ± 0.9 (16)</td>
</tr>
<tr>
<td>Ns</td>
<td>Ns</td>
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Water consumption is expressed as average ml/mouse/day. Data expressed as mean ± S.E.M (number of mice).

4. Discussion

Previous studies have reported that early life stress can alter flexible spatial learning, synaptic plasticity and amyloid levels in 12-month-old APPswe/PS1dE9 mice (Lesuis et al., 2018). In the current study, we investigated whether riluzole, a glutamate modulator (Brothers et al., 2013; Pittenger et al., 2008), can rescue these effects. We found that ELS-induced impairments in synaptic plasticity, flexible spatial learning and plaque load in APPswe/PS1dE9 mice can be rescued by prolonged riluzole treatment from post-weaning onward, likely by regulating EAAT2 expression.

Our current model for ELS has previously been shown to induce (age-related) impairments in spatial learning, memory processes (reviewed by (Walker et al., 2017; Yam et al., 2017)) and synaptic plasticity (Brunson et al., 2005). In addition, it has been shown that ELS aggravates AD-related neuropathology, including increased soluble Aβ levels, increased plaque load, and impaired cognitive performance (Hoiejmakers et al., 2016; Lesuis et al., 2018, 2016). In agreement, we found that ELS impaired synaptic plasticity in WT mice. In addition, LTP was impaired in APPswe/PS1dE9 mice which is in line with earlier studies showing impairments in synaptic plasticity in (transgenic) mouse models of AD (Jacobsen et al., 2006; Rowan et al., 2003). Moreover, ELS exposure in APPswe/PS1dE9 mice further decreased synaptic plasticity, and even resulted in LTD-like changes. We then investigated whether alterations in glutamatergic signalling might attenuate these effects by long-term treatment with the glutamate modulator riluzole, administered immediately after weaning. While riluzole...
did not affect LTP in Ctrl-reared wild type mice, it increased LTP in all other experimental groups, suggesting that the impairments resulting from both ELS and an APPsw/PS1dE9 background are indeed mediated by disturbances in glutamatergic signalling. Interestingly, riluzole treatment was most effective in APPsw/PS1dE9 mice. This effect was most pronounced in the first 10 min after stimulation, which could point to a different recovery of the presynaptic glutamate release between WT and APPsw/PS1dE9 mice after the 90 s of high frequency stimulation (which may have resulted in a depletion of synaptic vesicles). These effects of riluzole may be related to one of the many pathways associated to synaptic plasticity that are differentially regulated by AD (Pereira et al., 2017) and the exact nature of this interaction requires further investigation. Clearly, riluzole was able to prevent ELS and APPsw/PS1dE9-induced alterations in synaptic plasticity in 6-month-old mice.

LTP is an important cellular model for learning and memory (Kessels and Malinow, 2009; Malinow and Malenka, 2002), and functional brain abnormalities have been observed in humans decades before the development of other symptoms (Reiman et al., 2004; Sterling et al., 2009). We therefore tested whether ELS affected learning and memory in APPsw/PS1dE9 mice. Previously, we have reported that 12-month-old ELS-APPsw/PS1dE9 mice are impaired in flexible spatial learning, indicating that EAAT2 is indeed relevant for memory formation. Increased immunoreactivity for EAAT2 in the CA1 area of the hippocampus in all groups, irrespective of their genetic background or early life experience. Interestingly, EAAT2 expression correlated significantly with flexible spatial learning, indicating that EAAT2 is indeed relevant for memory formation. Increased EAAT2 levels in the CA1 area of the hippocampus in all groups, was strongest in the ELS-APPsw/PS1dE9 mice. This was again normalised by chronic riluzole treatment. N = 8-9/group. (D) No correlation was observed between plaque load and distance travelled in the last trial of the Barnes maze.

In line with this, we observed that EAAT2 immunoreactivity was significantly reduced with aging, while both ELS and an APPsw/PS1dE9 background further lowered EAAT2, which was strongest in APPsw/PS1dE9 mice exposed to ELS. Riluzole treatment strongly increased EAAT2 levels in the CA1 area of the hippocampus in all groups, irrespective of their genetic background or early life experience. Interestingly, EAAT2 expression correlated significantly with flexible spatial learning, indicating that EAAT2 is indeed relevant for memory formation. Increased immunoreactivity for EAAT2 was observed in the same region as where we observed decreases in synaptic plasticity in ELS-APPsw/PS1dE9 mice. In addition, others have previously observed increased spine clustering in the same area in riluzole-treated rats, which also correlated with cognitive performance (Pereira et al., 2014), suggesting a potential mechanism by which riluzole can increase cognitive performance. However, in addition to regulating glutamate levels, the drug has additional pharmacological effects such as inhibiting Na+ channels (Bellingham, 2011). A possible contribution of these mechanisms to the present results cannot be ruled out.

Synaptic dysfunction is an important mechanism implicated in AD-related cognitive deficits (Selkoe, 2002; DeKosky and Scheff, 1990) and presenting as one of the first symptoms of AD (Sterling et al., 2009; Reiman et al., 2004). Amyloid-β (Aβ), one of the hallmarks of AD neuropathology, is closely related to glutamatergic dysregulation, since...
Aβ oligomers disrupt glutamate uptake, reduce synaptic transmission, facilitate LTD and inhibit LTP (Li et al., 2009; Cheng et al., 2009). This is thought to occur through an excessive activation of extra-synaptic NMDA receptors (Li et al., 2011, 2009), and a decrease in the expression of synaptic NMDA receptors (Snyder et al., 2005). In parallel, neuronal activity, regulated by glutamatergic signalling increases the release of Aβ (Kamenetz et al., 2003), possibly resulting in vicious cycle of neurotoxicity. In the current study, we find that plaque load was increased following ELS, an effect that was rescued by riluzole treatment. Likewise, we have previously shown that in APPsw/PS1dE9 mice, soluble Aβ40 and Aβ42 levels are increased following ELS (Lesuis et al., 2018), although plaque load was not affected in this study. EAAT2 overexpression has previously been shown to decrease pathological markers in an AD mouse model (Takahashi et al., 2015), again supporting the hypothesis that improved regulation of glutamatergic signalling via enhanced EAAT2 uptake could potentially mitigate Aβ toxicity and worsen cognitive performance. This may suggest that normalising glutamate levels prevents Aβ pathology.

5. Conclusions
The present results indicate that riluzole rescues deficits in flexible spatial learning in 12-month-old ELS-exposed APPsw/PS1dE9 mice. The effects of riluzole are possibly mediated by alterations in synaptic plasticity that emerge already from a young age onwards (at least 6 months) since LTP deficits were completely rescued by riluzole supplementation. Future studies are required to investigate in more detail the critical time windows in which riluzole can prevent the ELS-imposed impairments. Ultimately, reducing glutamatergic signalling could represent a future therapeutic strategy for treating vulnerable individuals at risk of developing stress-aggravated AD, particularly in relation to adverse early life experiences.

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