Molecular Psychiatry
https://doi.org/10.1038/s41380-020-0794-5

ARTICLE

Allosteric modulation of AMPA receptors counteracts Tau-related excitotoxic synaptic signaling and memory deficits in stress- and Aβ-evoked hippocampal pathology

Daniela Monteiro-Fernandes[1,2] · Joana Margarida Silva[1,2] · Carina Soares-Cunha[1,2] · Christina Dalla[3] · Nikolaos Kokras[3,4] · François Arnaud[5] · Rodolphe Billiras[5] · Viktoriya Zhuravleva[6] · Clarissa Waites[7,8] · Sylvie Bretin[5] · Nuno Sousa[1,2] · Ioannis Sotiropoulos[1,2]

Received: 1 December 2019 / Revised: 12 May 2020 / Accepted: 18 May 2020

Abstract

Despite considerable progress in the understanding of its neuropathology, Alzheimer's disease (AD) remains a complex disorder with no effective treatment that counteracts the memory deficits and the underlying synaptic malfunction triggered by the accumulation of amyloid beta (Aβ) and Tau protein. Mounting evidence supports a precipitating role for chronic environmental stress and glutamatergic excitotoxicity in AD, suggesting that targeting of glutamate receptor signaling may be a promising approach against both stress and AD pathologies. In light of the limited cognitive benefit of the direct antagonism of NMDA receptors in AD, we here focus on an alternative way to modify glutamatergic signaling through positive allosteric modulation of AMPA receptors, by the use of a PAM-AMPA compound. Using non-transgenic animal model of Aβ oligomer injection as well as the combined stress and Aβ i.c.v. infusion, we demonstrate that positive allosteric modulation of AMPA receptors by PAM-AMPA treatment reverted memory, but not mood, deficits. Furthermore, PAM-AMPA treatment reverted stress/Aβ-driven synaptic missorting of Tau and associated Fyn/GluN2B-driven excitotoxic synaptic signaling accompanied by recovery of neurotransmitter levels in the hippocampus. Our findings suggest that positive allosteric modulation of AMPA receptors restores synaptic integrity and cognitive performance in stress- and Aβ-evoked hippocampal pathology. As the prevalence of AD is increasing at an alarming rate, novel therapeutic targeting of glutamatergic signaling should be further explored against the early stages of AD synaptic malfunction with the goal of attenuating further synaptic damage before it becomes irreversible.

Introduction

Alzheimer's disease (AD), the most prevalent form of dementia, is characterized by progressive cognitive decline and neurodegeneration of brain regions pivotal for learning and memory such as the hippocampus [1]. Although the

Supplementary information The online version of this article (https://doi.org/10.1038/s41380-020-0794-5) contains supplementary material, which is available to authorized users.

✉ Ioannis Sotiropoulos
ioannis@med.uminho.pt

1 Life and Health Sciences Research Institute (ICVS), Medical School, University of Minho, Campus Gualtar, Minho, Portugal

2 ICVS/3B's-PT Government Associate Laboratory, Braga/Guimarães, Portugal

3 Department of Pharmacology, Medical School, National and Kapodistrian University of Athens, Athens, Greece

4 First Department of Psychiatry, Eginition Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece

5 Pôle Innovation Thérapeutique Neuropsychiatrie, Institut de Recherches Internationales Servier, Suresnes, France

6 Neurobiology and Behavior Graduate Program, Columbia University, New York, NY, USA

7 Department of Pathology and Cell Biology, Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University Medical Center, New York, NY, USA

8 Department of Neuroscience, Columbia University, New York, NY, USA

Published online: 28 May 2020

deposition of amyloid-β (Aβ) into plaques along with the inclusions of neurofibrillary tangles, composed of microtubule-associated Tau protein, are characteristic histopathological hallmarks of the AD brain [2], accumulating evidence suggests that synaptic malfunction and atrophy are better correlated with cognitive decline in patients [3, 4]. Human studies suggest that Aβ accumulation, synaptic dysfunction, and hippocampal volume loss occur years before the appearance of clinical symptoms of AD [5] with synaptic malfunction being an early pathological manifestation in AD [6]. Moreover, synaptic malfunction and atrophy are also found in conditions considered to be causally related to AD such as depression and lifetime stressful events [7, 8]. In line with clinical evidence that chronic stress may contribute to AD pathology [9–11], animal studies have shown that exposure to chronic stress induces Aβ overproduction and accumulation of pathological Tau, resulting in synaptic malfunction and cognitive decline [12–15].

A large body of evidence supports a role for imbalanced glutamatergic signaling in AD neuropathology [16–18]. For instance, glutamate receptors (e.g., AMPA receptors; AMPARs) are reported to be decreased in synapses of postmortem AD brain tissue [19] and Aβ is known to impair synaptic plasticity [20] by impacting on NMDA and AMPA receptors, thereby damaging mechanisms of synaptic plasticity that lead to memory deficits [21, 22]. Together with internalization of synaptic receptors, Aβ-driven altered calcium (Ca^{+2}) dynamics is also suggested to be involved in glutamate-related neuronal damage in AD; further, Ca^{+2} imbalances are implicated in destabilization of the cytoskeleton of dendritic spines, leading to spine collapse [23, 24]. Other studies indicate that the neuroexcitotoxic effects of Aβ depend on the accumulation of Tau protein and its synaptic missorting into synapses via the mediation of GluN2B receptors [25]. Providing support for the notion that stress-related diseases of the brain and AD pathology share common neurobiological substrates [9, 26], previous studies have implicated glutamate release and its receptors in stress-driven neurotoxicity [18, 27, 28]. Moreover, our recent studies have demonstrated an essential role of Tau and GluN2B signaling in synaptic malfunction caused by chronic stress through the mediation of glucocorticoids (GC) [29, 30]. In light of the limited benefit of direct NMDA antagonism in AD, we here focused on an alternative way to modify glutamatergic signaling, namely, through positive allosteric modulation of AMPARs. To this end, we investigated the efficacy of a PAM-AMPA compound against early stages of stress- and Aβ-evoked synaptic pathology in rats, using a multi-scale approach; besides evaluating memory and mood, we also performed neurochemical assays and molecular–cellular analyses of hippocampal tissue with an emphasis on synaptic proteins. The results of this work indicate that allosteric modulation of AMPARs in stress- and Aβ-treated animals effectively re-established synaptic integrity and attenuated downstream cognitive deficits, although mood impairments persisted.

Methods and materials

Animals and treatments

Male Wistar rats (Charles River Laboratories, France) were used in all studies. For modulation of AMPARs, we used the positive allosteric modulator of AMPAR, PAM-AMPA compound (S47445; Servier Laboratories, WO/2008/085506, France; see also Supplementary Information). For monitoring the short-term effect of AMPARs modulation against Aβ-driven pathology, we used 5 months old animals that were bilaterally injected in hippocampi with $Aβ_{1-40}$ (Eurogentec; 2.5 µg/µl, 4 µl) [31] (see also Fig. 1a–c and Supplementary Information). For monitoring the long-term effect of AMPARs modulation on the combined stress/Aβ-driven brain pathology, 12 months old animals were used, where chronic unpredictable stress protocol (4 weeks—see Supplementary Information) was combined with $Aβ_{1–40}$ i.c.v. infusion [32]. Animals that received combined stress/Aβ treatment also received i.p. injection of vehicle or PAM-AMPA compound (3 or 10 mg/Kg) for 4 weeks (see Fig. 2q–c and Supplementary Information). Similar PAM-AMPA administration to control (non-stressed/saline-infused) animals induced no significant effect on behavioral tests while absence of overall changes of hippocampal neurotransmitter levels were also found—for more information, see Supplementary Figs. 1 and 2.

Behavior testing

In the open field (OF) test, animals were allowed to freely move for 5 min while, in the elevated plus maze (EPM) test, animals explored the apparatus for 5 min. In the forced swim test (FST), animals were placed in a transparent cylinder filled with water for 5 min. For the Y maze test, animals were allowed to explore the two arms of the apparatus (15 min) while, 3 h later, animals were allowed to explore all three arms of the apparatus (5 min); the ratio between the distance spent in the novel arm and the total distance traveled in the maze was used. In the novel place and object recognition tests (NPR and NOR, respectively), animals were allowed to freely explore two identical objects placed in the test arena (10 min). For monitoring short-term memory, one object was placed in a novel position and animals explored the arena for 10 min. For long-term memory assessment, one object was replaced by a new one (novel object). The discrimination index was calculated based on the formula: (a) for short-term memory [time of exploration

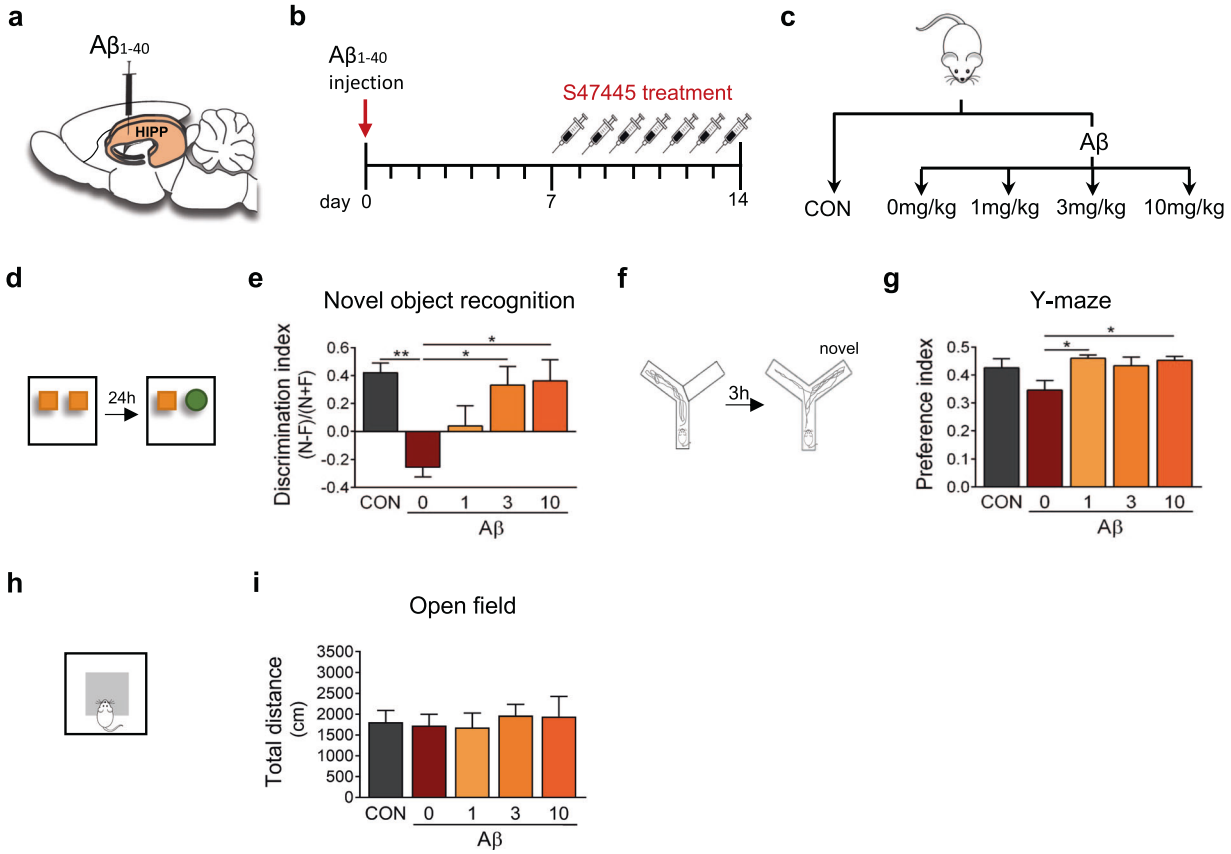


Fig. 1 Allosteric modulation of AMPA receptors by PAM-AMPA compound blocked the cognitive deficits induced by Aβ injection in the hippocampus. a, **b** Schematic representation of the Aβ animal model where Aβ oligomers were bilaterally injected in the dorsal rat hippocampus (**a**); animals received daily intraperitoneal (i.p.) injection of the allosteric modulator of AMPA receptors, the PAM-AMPA compound, for 7 consecutive days. **c** Experimental design of the experimental set where Aβ-injected animals received 0, 1, 3, or 10 mg/kg of PAM-AMPA; control animals received saline hippocampal injection and vehicle i.p. injections for seven days. **d**–**e** In novel object recognition (NOR) test, Aβ-injected animals presented a decreased discrimination index when compared with the control (CON) group indicating deficits of long-term recognition memory. In contrast to 1 mg/Kg dose, 3 mg/kg and 10 mg/kg of PAM-AMPA ameliorated this reduction of the discrimination index. **f**–**g** In Y-maze test, the Aβ + 1 mg and the Aβ + 10 mg groups exhibited increased preference index in comparison with the Aβ-injected animals while the Aβ + 3 mg group exhibited a tendency for increase. **h**–**j** No significant difference of total distance traveled in the open field (OF) arena was found among all groups. All numeric data are represented as mean ± SEM, $*p < 0.05$, $**p < 0.01$.

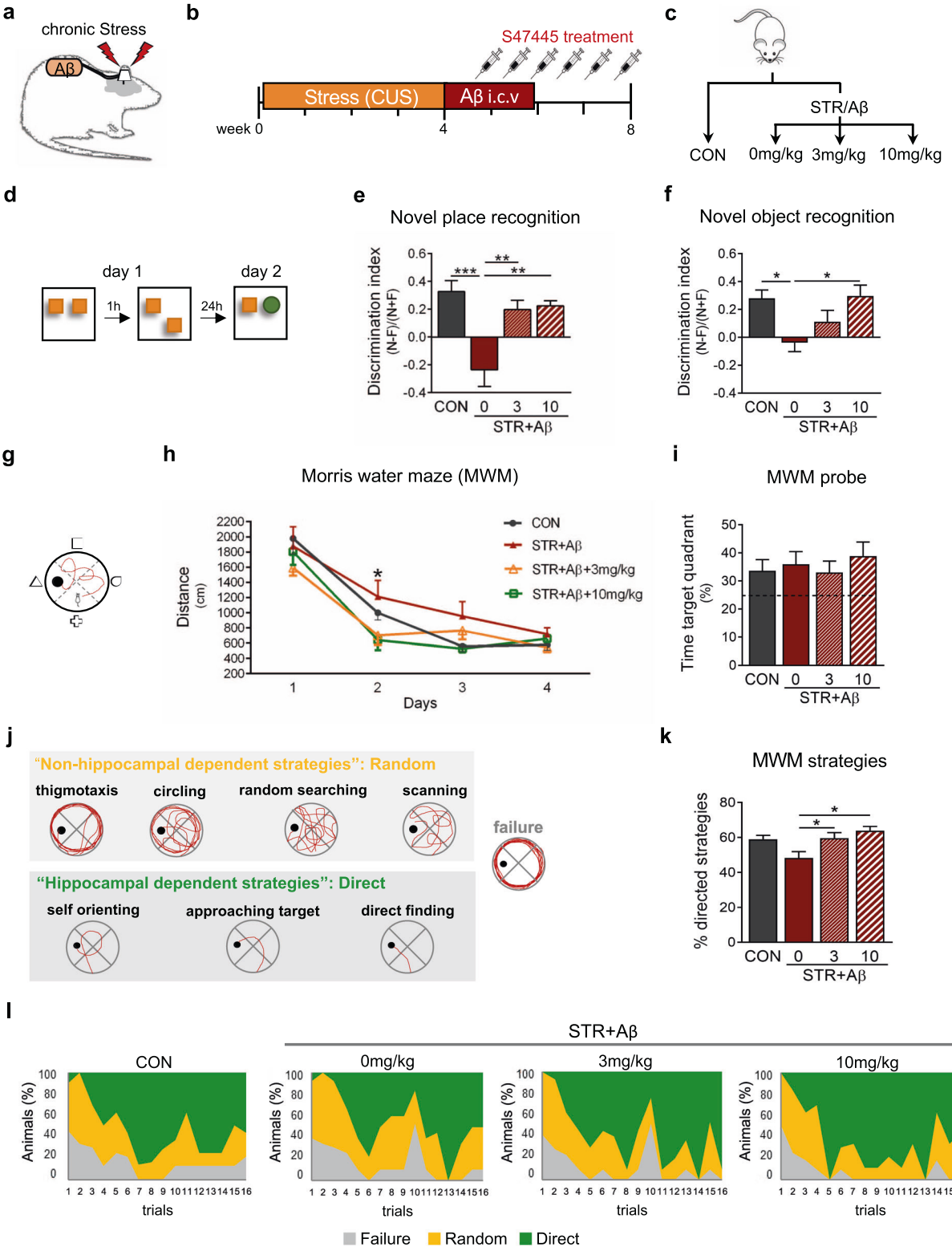
of the displaced object—time of exploration of familial object]/total time exploration; (b) for long-term memory [time of exploration of the novel object—time of exploration of familial object]/total time exploration. In the morris water maze (MWM) test, animals were tested 4 consecutive days (four trials per day) and the swum distance to reach the platform was used. In addition, the swimming pattern followed by each animal during the trials was classified as previously described [33] and categorized into non-direct and direct (hippocampus-dependent) strategies; the percentage of direct strategies was calculated by the number of trials that the animals chose a direct strategy to encounter the platform [33]—see also Supplementary Information.

Mass spectrometry and HPLC analyses

The cerebrospinal fluid (CSF) was analyzed by mass spectrometry analysis (LC-MS/MS). The signal intensity obtained for each peptide was normalized to the BSA signal obtained in each CSF sample—see also Supplementary Information. Dorsal hippocampal levels of monoamines (NA, DA, and 5-HT) and their metabolites [HVA (homovanillic acid), DOPAC (3,4-dihydroxyphenylacetic acid), 5-HIAA (5-hydroxyindoleacetic acid)] were measured using high-performance liquid chromatography (HPLC) with electrochemical detection as previously described [29]—see also Supplementary Information.

Subcellular fractionation and western blot analysis

Hippocampal tissue was fractionated using a protocol for preparation of the cytosolic and postsynaptic density (PSD)-enriched synaptossomal fraction as previously described [29]. For western blot analysis, samples were analyzed as previously described [29]—see also Supplementary Information.



Statistical analysis

Statistical analysis and graphic representation were performed by GraphPad Prisma 6.0 (GraphPad Software, La Jolla, USA) using One-way ANOVA and appropriate posthoc tests for pair comparisons. For MWM analysis, repeated-measurement ANOVA was performed. Values were accepted as significant if $p < 0.05$ and all the results were expressed as mean ± SEM.

◀ **Fig. 2 Prolong modulation of AMPA receptors attenuated the hippocampal-dependent memory impairment of stress/Aβ-exposed animals. a–c** Representation of the combined stress/Aβ animal model used (**a**) where the animals were exposed to chronic unpredictable stress protocol for 4 weeks followed by Aβ i.c.v. infusion by mini-osmotic pump for the following two weeks. Intraperitoneal injections of PAM-AMPA compound were daily performed during the next 4 weeks (**b**). Experimental groups included stress/Aβ-exposed animals treated with 0, 3, 10 mg/kg of PAM-AMPA for 4 weeks while control, non-stressed animals (CON) received saline i.c.v. infusion for 4 weeks. **d–f** In novel place recognition (NPR) task, the stress/Aβ group presented a decrease in the discrimination index when compared with the CON group while both stress/Aβ + 3 mg and stress/Aβ + 10 mg exhibited higher discrimination index indicating that both PAM-AMPA doses attenuated the stress/Aβ-driven deficits of short-term recognition memory. In novel object recognition (NOR) task, stress/Aβ group exhibited a decreased discrimination index in comparison with the control group suggesting impairment of long-term recognition memory. This decrease of discrimination index was mitigated in stress/Aβ + 10 mg, but not stress/Aβ + 3 mg, group indicating that only the high dose reverted long-term memory deficits. In Morris water maze (MWM) test, stress/Aβ + 3 mg and stress/Aβ + 10 mg groups presented a reduced distance swum to reach the target platform in the second day of the task in comparison with the stress/Aβ group (**g**); note that in the MWM probe test, no significant difference was found among groups while all the animal groups swum more than 25% in the target quadrant (**i**). **j** Schematic representation of the division of the spatial navigation strategies that animals swum during the MWM learning phase into three categories: (1) failure, where animals never find the platform, (2) random strategies, where the animals exhibit non-hippocampal-dependent, random searching or circling strategies, and (3) direct strategies, where the animals employ an hippocampal-dependent strategy, very spatially directed swimming path towards the escaping platform. **k** Stress/Aβ + 3 mg and stress/Aβ + 10 mg groups employed significantly higher percentage of directed, hippocampus-dependent strategies than stress/Aβ group. **l** Stacked area charts displaying the percentage of failures (gray), random (yellow), and direct (green) strategies across all trials of the learning period of the MWM test for all groups where stress/Aβ + 10 mg animals followed higher percentage of direct strategies accompanied by reduced random strategies and failures. All numeric data presented as mean ± SEM, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Results

Short-term allosteric modulation of AMPARs blocks memory deficits driven by Aβ hippocampal injection

AD pathogenesis is widely believed to be driven by the overproduction of the amyloid-beta peptide (Aβ), known to cause synaptic malfunction and related memory deficits [22]. As glutamatergic signaling imbalance is suggested to be implicated in the Aβ-driven neuronal and cognitive deficits [16, 17], we first evaluated the efficacy of a positive modulation of the AMPARs by a PAM-AMPA compound against Aβ-driven memory deficits using animals bilaterally injected with Aβ oligomers in their hippocampus (Fig. 1a); this model is suggested to mimic early phase of Aβ-driven AD neuropathology [31]. We tested three different dosages of the compound (1, 3, and 10 mg/Kg), which was delivered intraperitoneally for 7 days (Fig. 1b, c). To assess the impact of short-term treatment of PAM-AMPA on memory performance, we used the novel object recognition (NOR) test, monitoring the time that animals spent exploring the novel and the familiar objects (Fig. 1d). As shown in Fig. 1e, Aβ-injected animals presented a clear reduction in the discrimination index ($p = 0.006$) compared with control (vehicle-treated; CON) animals, indicating deficits of recognition memory [One-way ANOVA; $F_{[4,24]} = 4.550$, $p = 0.007$]. Furthermore, Aβ-injected animals that received 1 mg/Kg of PAM-AMPA compound (Aβ + 1 mg) exhibited no differences in the discrimination index compared with the Aβ-injected animals, suggesting that this dose did not alter cognitive performance. However, animals that received 3 or 10 mg/Kg of PAM-AMPA compound (Aβ + 3 mg and Aβ + 10 mg, respectively) showed a significantly higher discrimination index ($p_{3\ mg/Kg} = 0.048$, $p_{10\ mg/Kg} = 0.035$) compared with Aβ-injected animals, indicating that these doses of PAM-AMPA compound reverted the Aβ-driven impairment of the recognition memory.

Next, we used the Y-maze test to evaluate short-term memory based on the distance that animals traveled in the different arms of the apparatus (Fig. 1f). One-way ANOVA analysis revealed significant differences of the preference index for the novel arm [$F_{[4,22]} = 3.039$, $p = 0.04$]. Specifically, Aβ + 1 mg group exhibited an increased preference index in comparison with the Aβ-injected group ($p = 0.042$), indicating cognition-improving effect of 1 mg/Kg dose of PAM-AMPA compound. While Aβ + 3 mg group presented a tendency for an increased preference index compared with Aβ-injected group, the Aβ + 10 mg group exhibited a significant elevation of its preference index ($p = 0.04$), suggestive of improvement of short-term memory (Fig. 1g). Importantly, no differences in the total distance traveled in the OF apparatus were detected among groups [One-way ANOVA; $F_{[4,22]} = 0.153$, $p = 0.959$], indicating that the tested doses of PAM-AMPA compound exerted no effects in locomotor activity of the animals (Fig. 1h, i). Altogether, the above behavioral findings suggest that short-term administration of PAM-AMPA compound attenuated cognitive impairments induced by hippocampal injection of Aβ oligomers.

Prolong modulation of AMPARs attenuates cognitive, but not mood, deficits of stressed/Aβ-treated animals

Clinical and experimental studies suggest lifetime stressful events and depression as precipitating factors of AD, while emerging evidence suggests chronic stress as the linking parameter between mood and cognitive deficits [9]. Thus, we next monitored the potentially beneficial impact of allosteric modulation of AMPARs against mood and

memory deficits present in a previously described animal model that combines exposure to chronic stress and Aβ i.c.v infusion [32]. In this experiment, prolonged treatment (30 days) with the two higher doses of PAM-AMPA compound (3 and 10 mg/Kg) was performed (Fig. 2a–c). For assessing short- and long-term recognition memory, we used the novel place and object recognition (NPR and NOR, respectively) tasks (Fig. 2d). In the NPR task, the group of animals exposed to chronic stress in combination with Aβ i.c.v infusion (stress/Aβ) displayed a significant reduction of discrimination index ($p = 0.0003$) when compared with CON animals (Fig. 1e) [One-way ANOVA; $F_{[3,30]} = 9.053$, $p = 0.0002$]. Moreover, stress/Aβ animals that received either 3 or 10 mg/Kg of PAM-AMPA compound (stress/Aβ + 3 mg and stress/Aβ + 10 mg, respectively) exhibited an increased discrimination index in comparison with the stress/Aβ group ($p_{3\ mg/Kg} = 0.003$ and $p_{10\ mg/Kg} = 0.002$), suggesting that both doses of PAM-AMPA reverted the stress/Aβ-driven cognitive deficits. In the NOR test (Fig. 2f), a similar pattern was found with the stress/Aβ group exhibiting a marked reduction of discrimination index ($p = 0.024$) when compared with CON animals [One-way ANOVA; $F_{[3,30]} = 4.538$, $p = 0.009$]. However, in contrast to stress/Aβ + 3 mg, the group treated with the higher dosage of PAM-AMPA compound (stress/Aβ + 10 mg) presented an increase of the discrimination index when compared with the stress/Aβ animals ($p = 0.016$).

In addition, we also monitored spatial reference memory using the morris water maze (MWM) test (Fig. 2g). Repeated-measures ANOVA revealed an overall difference in the distance swum to reach the escaping platform across testing days [$F_{(3,116)} = 3.956$, $p = 0.010$] (Fig. 2h). Further analysis showed that stress/Aβ + 3 mg and stress/Aβ + 10 mg groups exhibited a reduced distance swum to reach the hidden platform in the second day of the MWM test ($p_{3\ mg/Kg} = 0.036$ and $p_{10\ mg/Kg} = 0.023$) when compared with stress/Aβ group, indicating improvement of spatial reference memory upon PAM-AMPA treatment. No differences in time swum in the target quadrant were found among groups during the probe test (Fig. 2i). In addition, spatial navigation swimming strategies in the MWM test were analyzed as previously described [33], with particular focus on the hippocampus-dependent direct strategies which reflect spatially directed swimming path towards the escaping platform (Fig. 2j). We found that both stress/Aβ + 3mg and stress/Aβ + 10 mg groups adopted more directed (hippocampal-dependent) strategies when compared with the stress/Aβ animals ($p_{3\ mg/Kg} = 0.020$ and $p_{10\ mg/Kg} = 0.007$), another index of improved hippocampal function evoked by the PAM-AMPA [One-way ANOVA $F_{[3,30]} = 4.755$, $p = 0.007$] (Fig. 1k). Similar PAM-AMPA-induced improvement was also found in stacked area charts, where the stress/Aβ + 10mg and stress/Aβ + 3mg groups employed higher direct strategies accompanied by reduced random strategies and failures when compared with stress/Aβ animals (Fig. 1l).

OF test showed no overall differences among groups in the total distance traveled suggesting an absence of significant effect of PAM-AMPA on locomotor activity (Fig. 3a, b). Anxiety levels were assessed by the elevated plus maze (EPM) test (Fig. 3c). As shown in Fig. 3d, e, stress/Aβ group exhibited decreased time and entries in the open arms of the EPM apparatus in comparison with CON group ($p = 0.023$ and $p = 0.04$, respectively), suggesting increased anxiety levels [for time $F_{[3,31]} = 3.430$, $p = 0.028$; for entries $F_{[3,31]} = 3.768$, $p = 0.020$]. Interestingly, stress/Aβ + 3 mg and stress/Aβ + 10 mg groups exhibited similar time and number of entries in open arms to stress/Aβ group, suggesting that PAM-AMPA had no anxiolytic properties in this model. Finally, we evaluated the learned helplessness parameter of the depressive symptomatology using the forced swim test (FST) (Fig. 3f). As shown in Fig. 3g, h, stress/Aβ group presented a significant increase in immobility time when compared with CON group ($p = 0.001$), indicating depressive-like behavior [$F_{[3,30]} = 9.103$, $p = 0.0002$]. Furthermore, both stress/Aβ + 3 mg and stress/Aβ + 10 mg groups presented an increase in time spent immobile during the test when compared with CON animals ($p_{3\ mg/Kg} = 0.0002$ and $p_{10\ mg/Kg} = 0.012$), indicating that PAM-AMPA had no impact in depressive-like behavior found in stress/Aβ group. Overall, the above behavioral data provide the first in vivo evidence of the cognition-enhancing properties of long-term PAM-AMPA treatment against the combined stress/Aβ-driven deficits. However, PAM-AMPA had no anxiolytic or anti-depressive action in these animals.

Sustained modulation of AMPARs reverts the reduction in monoamine levels induced by stress/Aβ exposure

Hippocampal synaptic malfunction and reductions in monoamine levels have been implicated in both stress and AD pathologies [9, 29]. Thus, we next monitored the levels of hippocampal neurotransmitters and their metabolites by HPLC-ED analysis. As shown in Fig. 4, stress/Aβ group presented a significant decrease in the levels of noradrenaline (NA) [$F_{[3,27]} = 3.120$, $p = 0.042$] when compared with CON group ($p = 0.021$) (Fig. 4b). Moreover, in contrast to stress/Aβ + 3 mg, the stress/Aβ + 10 mg group exhibited a significant enhancement in the levels of NA when compared with the stress/Aβ animals ($p = 0.029$). In addition, the stress/Aβ animals exhibited decreased levels of serotonin (5HT) when compared with the control ones ($p = 0.021$) [$F_{[3,27]} = 4.990$, $p = 0.007$] (Fig. 4c) that was accompanied

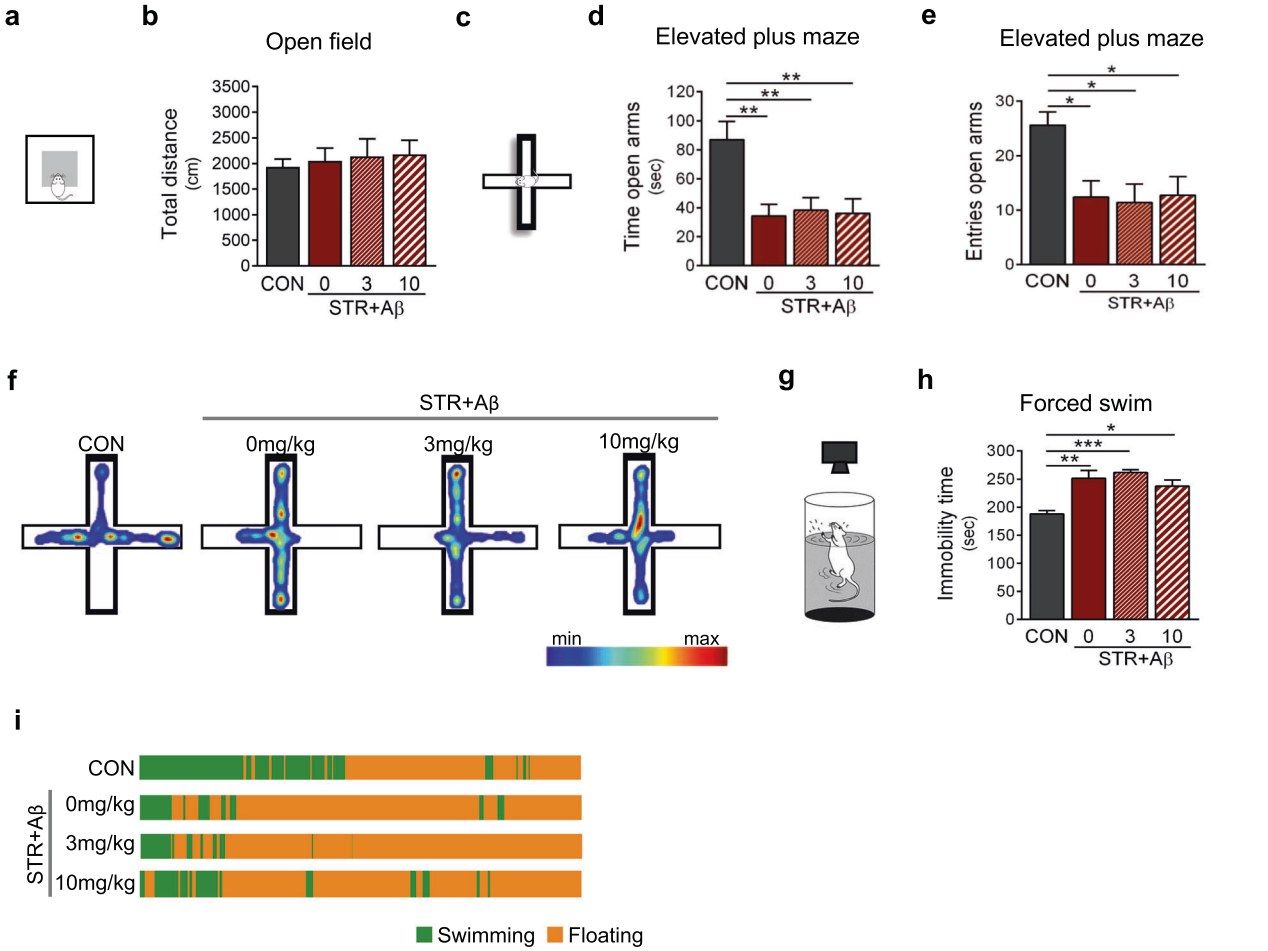
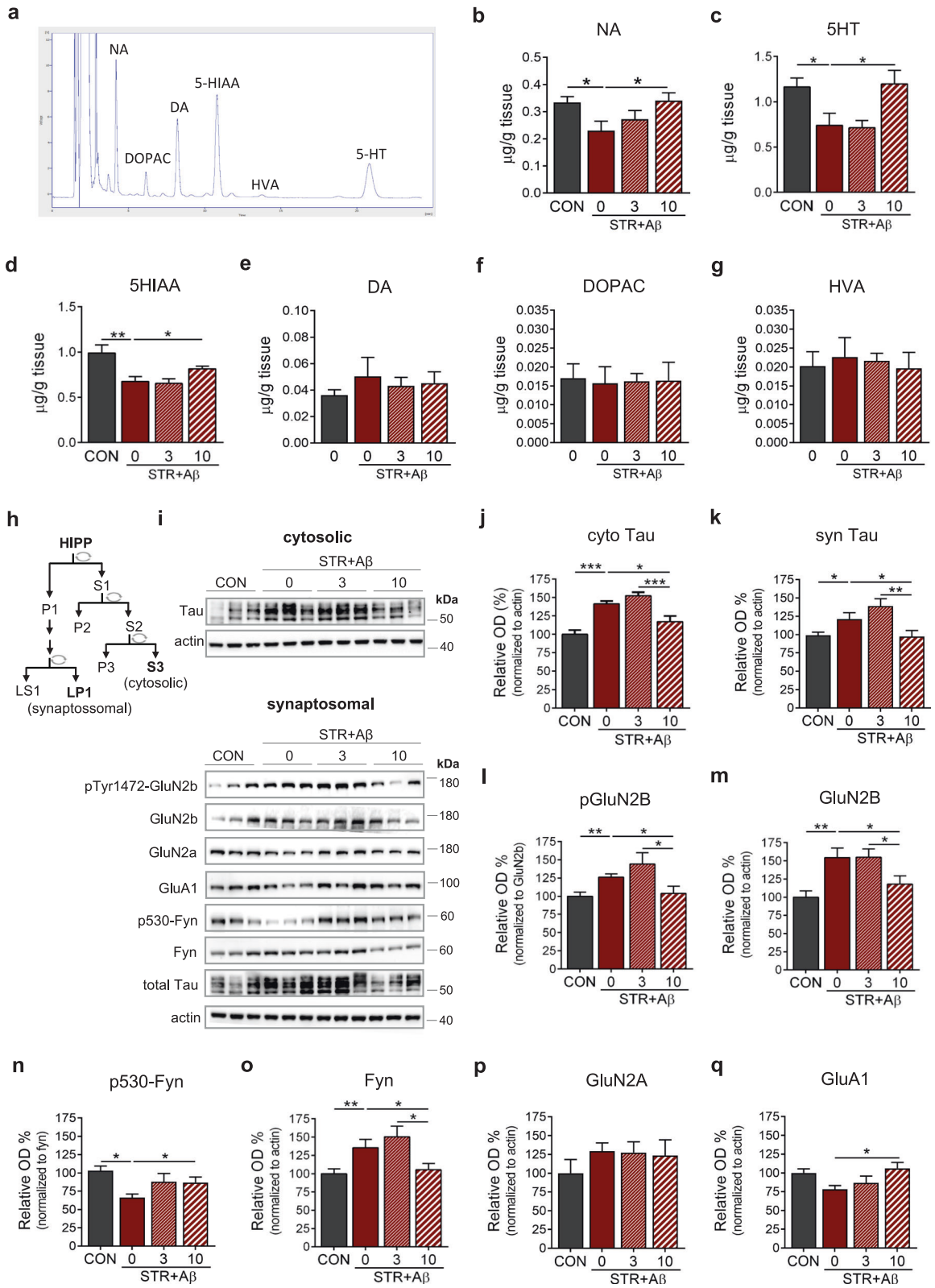


Fig. 3 Modulation of AMPA receptors did not improve the anxious and depressive-like behavior of stress/Aβ-treated animals. a, **b** No differences in total distance traveled in the open field arena among all experimental groups suggesting absence of any effect on locomotion of the animals. **c–e** Representative graphs of animal presence in open and closed arms of elevated plus maze (EPM) apparatus (**c**). Stress/Aβ group exhibited a significantly reduction in time (**d**) and number of entries (**e**) in the open arms of the EPM apparatus indicating anxious behavior. No difference was found between stress/Aβ + 10 mg or stress/Aβ + 3 mg when compared with stress/Aβ group indicating that AMPAR modulation exhibited no anxiolytic action in stress/Aβ animals. In forced swim test (**f**), representative heatmap of animal swimming and immobility behavior among groups in forced swim test (**h**). Stress/Aβ, stress/Aβ + 3 mg, and stress/Aβ + 10 mg presented increased immobility time when compared with the control groups indicating that treatment with PAM-AMPA did not exhibit antidepressant action (**g**). All numeric data presented as mean ± SEM, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

by reduced levels of the 5HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) ($p = 0.005$) [$F_{[3,27]} = 6.64$, $p = 0.001$] (Fig. 4d). Again, the dose of 10 mg/Kg of PAM-AMPA in stress/Aβ animals increased levels of 5HT and 5HIAA when compared with the stress/Aβ animals ($p < 0.05$ in both). (Fig. 4c, d). Interestingly, no differences among groups were found in levels of dopamine (DA), DOPAC, or HVA (Fig. 4e–g). Altogether, these findings suggest that the higher dose (10 mg/Kg) of PAM-AMPA compound attenuated the reduced levels of NA and 5-HT neurotransmitters in hippocampus of stress/Aβ animals. In addition, given its direct interaction with the extracellular space in the brain, the CSF may reflect the associated pathological alterations of the brain. Thus, mass spectrometry analysis of the CSF of the animals showed that different proteins related to synaptic structure and integrity exhibited a tendency for increase (but not significant) in stress/Aβ animals treated by the higher dose (10 mg/Kg) of PAM-AMPA compound (see Supplementary Table 1).

Prolonged PAM-AMPA treatment reverts synaptic missorting of Tau and GluN2B-related excitotoxic signaling

Synaptic dysfunction in AD is suggested to involve Aβ-driven imbalanced glutamatergic signaling related to GluN2B and thought to be an early pathological manifestation in AD, preceding synapse and neuronal loss [25, 34]. Interestingly, missorting of soluble Tau to the synapse is suggested to mediate this Aβ-driven synaptic malfunction



[24, 25]. WB analysis revealed that stress/Aβ group presented a significant increase in cytosolic levels of Tau in the hippocampus compared with CON ($p < 0.0001$) indicating accumulation of Tau [One-way ANOVA: $F_{[3,40]} = 16.020$, $p < 0.0001$] (Fig. 4i, j). In contrast to stress/Aβ + 3 mg animals, stress/Aβ + 10 mg animals presented a significant reduction in the Tau levels both in comparison with the stress/Aβ ($p = 0.021$) and the stress/Aβ + 3 mg

◀ **Fig. 4 Prolong modulation of AMPA receptors attenuated stress/Aβ-driven Tau-related excitotoxic synaptic signaling and neurotransmitter reduction. a–g** HPLC analysis of hippocampal neurotransmitter levels (**a**) showed that stress/Aβ group exhibited a decrease of noradrenaline (NA) levels that was reverted in stress/Aβ + 10 mg group (**b**). Similar effects were detected in serotonin (5HT) and its metabolite, 5HIAA, where stress/Aβ-driven decreases were attenuated in stress/Aβ + 10 mg animals (**c**, **d**). No differences were found in the levels of DA (**e**), DOPAC (**f**), or HVA (**g**) among experimental groups**. h–i** Protocol followed for obtaining PSD-enriched synaptossomal and cytosolic fractions and representative blots of molecular analysis of hippocampus. **j–k** Stress/Aβ group exhibited elevated Tau levels in both cytosolic and synaptossomal fraction, suggesting its accumulation and synaptic missorting of Tau. In contrast to stress/Aβ + 3 mg group, stress/Aβ + 10 mg animals presented reduced Tau levels in both fractions. **l–m** Further neurosynaptossomal analysis revealed an increase in the levels of GluN2B and pTyr1472-GluN2b in stress/Aβ animals, which was reverted in stress/Aβ + 10 mg group. **n–o** A decrease in the levels of p530-Fyn (inactive Fyn) was detected in stress/Aβ group that was accompanied by elevated levels of Fyn. Treatment with the 10 mg dose of PAM-AMPA (stress/Aβ + 10 mg group) attenuated these changes. **p**, **q** Whereas no significant changes of GluN2A subunit levels were detected, exposure to stress/Aβ induced a decrease in the GluA1 subunit levels that were mitigated by the 10 mg PAM-AMPA treatment. All numeric data presented as mean ± SEM, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

($p = 0.0004$) groups. Similarly, a stress/Aβ-driven increase of Tau was also found in the synaptossomal fraction of the stress/Aβ group, suggesting synaptic missorting and accumulation of Tau (Fig. 4i, k). Furthermore, the stress/Aβ + 10 mg group presented a significant decrease in Tau levels, suggesting that the higher dose of S47455 reverted cytosolic accumulation and synaptic missorting of Tau found in the stress/Aβ animals.

Synaptic missorting of Tau has been suggested to play a role in synaptic toxicity by enhancing the postsynaptic targeting of the kinase Fyn, which subsequently leads to phosphorylation and stabilization of GluN2B subunit in the synapse, resulting in the over-activation of NMDA receptors, and related excitotoxic signaling [35]. As shown in Fig. 4l, m, stress/Aβ group presented increase levels of pTyr1472-GluN2b in the synaptic fraction ($p = 0.001$), [$F_{[3,43]} = 4.363$, $p = 0.008$], which was accompanied by increased levels of GluN2B ($p = 0.004$). Interestingly, both increases were attenuated in stress/Aβ + 10 mg animals (for both cases, $p < 0.05$), while the same was not true for stress/Aβ + 3 mg animals. Next, we also measured the levels of total Fyn and p530-Fyn (inactivated form) (Fig. 4n, o), which were found to be increased ($p = 0.008$) and decreased ($p = 0.012$), respectively, in the stress/Aβ group [$F_{(3,43} = 3.543$, $p = 0.022$]. Treatment with 10 mg dose of PAM-AMPA (stress/Aβ + 10 mg group) reverted these changes (for both cases, $p < 0.05$). No changes in the levels of GluN2A were detected among experimental groups. However, levels of GluA1 subunit of AMPARs were reduced in stress/Aβ animals ($p < 0{,}05$), a finding that is in line with previous studies that show internalization of AMPARs by Aβ [21] [$F_{[3,41]} = 3.414$, $p = 0.026$]. Interestingly, stress/Aβ + 10 mg group exhibited a significant increase in GluA1 levels when compared with the stress/Aβ animals ($p = 0.032$), indicating that PAM-AMPA treatment counteracted stress/Aβ-driven GluA1 decrease. Overall, the above findings suggest that prolonged treatment with the PAM-AMPA compound at the dose of 10 mg/Kg attenuated Tau accumulation and excitotoxic synaptic signaling that may underlie the cognitive improvement detected in stress/Aβ-exposed animals.

Discussion

Based on its increasing incidence and high societal impact, dementia, and AD in particular, was recently declared by the World Health Organization as a priority health problem, highlighting the urgent need for better understanding of disease etiopathogenesis and identification of novel therapeutic targets [36]. Clinical studies suggest that stressful life experiences and prolonged exposure to stress are likely etiological factors in AD [10]. Consistent with this, animal studies have shown that exposure to chronic stress or high levels of GC, the main stress hormones, trigger APP misprocessing and Aβ overproduction [13, 32] followed by generation of different forms of pathological Tau that culminate in memory deficits [12, 14, 15, 37, 38]. Moreover, emerging evidence suggests that common neurobiological substrates may be involved in synaptic malfunction and atrophy in both stress-related and AD pathologies [9, 37], with Tau protein, its synaptic missorting and related glutamatergic excitotoxic signaling having a central role [29, 30]. There is growing consensus that previous stressful experiences may leave a trace of vulnerability that, together with other risk factors (e.g., age, Aβ), act cumulatively to precipitate AD pathology [26]. Thus, the usage of chronic stress in the modeling of etiopathogenic conditions that precipitate AD could be considered as an alternative approach to study the early events of sporadic AD, the type of AD that counts for 95–99% of all cases. Indeed, exclusive reliance on transgenic models during early-phase development of AD therapeutics may explain the failure of test drugs in subsequent clinical trials [39].

As synaptic malfunction increasingly appears to be the earliest pathological manifestation in AD, preceding synaptic and/or neuronal loss [6, 34], modulation of synaptic plasticity [39, 40] [e.g., behavioral-driven gamma oscillations [40], environmental enrichment [41]] seems to be a promising approach against AD neuropathology and cognitive decline. Whereas dysregulation of glutamatergic transmission may underpin Aβ-induced synaptic malfunction [22, 28], partial antagonists of the glutamate NMDA receptor (e.g., memantine) have proven to have only

transient effects on memory improvement in AD patients [42]. Thus, more attention has recently focused on other glutamate receptors, especially AMPARs because of their crucial role in fast excitatory synaptic transmission and long-term potentiation (LTP); the latter represents a form of synaptic plasticity involved in memory formation [17]. Notably, AMPAR levels are downregulated in the AD brain [21]. With respect to therapeutics, it is interesting that positive allosteric modulators of AMPAR function have been reported to slow AMPAR desensitization and/or deactivation [43, 44], thus enhancing synaptic plasticity [44, 45] and memory [46]. The PAM-AMPA compound employed in the present work was previously shown to be highly selective; it has no affinity for orthosteric binding sites and, while it lacks inherent agonistic potency at AMPAR, its activity requires agonist-activated AMPAR [47, 48]. Further, this PAM-AMPA compound enhances glutamate-evoked responses in vitro independently of other glutamate ionotropic (NMDA and kainate) receptors and is non-neurotoxic in in vitro and in vivo settings [47]. However, the effect of this PAM-AMPA compound against AD-related synaptic malfunction was not previously monitored.

In the present systematic exploration of this PAM-AMPA compound as a potential AD therapeutic agent, we found that short-term (7 days) allosteric modulation of AMPAR ameliorates cognitive deficits that become manifest following Aβ injection into the rat hippocampus. Furthermore, chronic (4 weeks) treatment with the drug was also found to improve short- and long-term memory in animals exposed to a double insult of chronic stress and Aβ infusion. Interestingly, in light of the known reciprocal regulatory relationships between cognition and emotion [49], as well clinical reports of increased levels of anxiety and depression in AD patients [50], the PAM-AMPA compound did not dampen the anxiety- and depressive-like behavior in stress/Aβ-treated animals; this observation suggests that AMPAR signaling does not play a central role in the alleviation of affective symptomatology [51]. This most likely reflects the engagement of distinct circuits and/or signaling mechanisms between mood and cognitive deficits in AD. Accordingly, although approved antidepressant treatments improve emotional and behavioral symptoms, they exhibit a limited benefit on memory deficit in AD patients [52] highlighting the need to devise combinatorial therapies that can help manage co-existing cognitive and emotional deficits in AD subjects.

Previous reports suggest that the accumulation of the soluble Tau, rather than the tangle-associated aggregated form, is the main toxic element of synaptic malfunction in AD [34, 53]. As before, we here found that combined exposure to stress and Aβ lead to accumulation of soluble Tau in both cytosolic and synaptic compartments of the hippocampus, an effect mitigated by the PAM-AMPA compound. This was accompanied by a reduction in synaptic levels of Fyn, a kinase translocated to postsynaptic sites by Tau where it contributes to glutamate-induced synaptotoxicity [25, 28, 34]. This result was strengthened by our observation that treatment with the PAM-AMPA compound reduced synaptic levels of the NMDAR subunit GluN2B as well as of pY1472-GluN2B; the latter is crucial for stabilizing NMDAR at the synaptic membrane, thus increasing vulnerability to excitotoxicity [54]. Another important finding in the present study was that the PAM-AMPA compound led to upregulated GluA1-containing AMPAR in stress/Aβ-treated animals, thus potentially promoting synaptic strength and memory formation. In this context, it is pertinent to recall that AMPAR levels are reduced in AD brains and that the removal of AMPARs from synapses appears to be one of the earliest events associated with elevated Aβ [55, 56]. Interestingly, it is recently described that Aβ preferentially damage the synaptic insertion of GluA1, but not GluA2, in the LTP mechanism of synaptic plasticity [57, 58]. Also, GluA3 subunit of AMPAR is shown to be important for the synaptotoxic action of Aβ as deletion of GluA3 block the Aβ-induced deficits on synaptic plasticity mechanisms [57–59]. Future studies should further characterize the beneficial action of this PAM-AMPA compound on different AMPAR subunits as different AMPAR subunits may respond differentially to Aβ [57, 58] while this PAM-AMPA compound exhibits a greater amount of potentiation on GluA1flop [47].

In addition to disrupted postsynaptic function, presynaptic dysregulation may also contribute to the pathogenesis of AD, with recent work indicating that Tau accumulation in the presynaptic compartment may be an underpinning mechanism [60] by impairing synaptic vesicle dynamics and therefore, neurotransmission [61, 62]. Supporting this idea, results presented here show that allosteric modulation of AMPAR in stress/Aβ-treated animals restores hippocampal levels of NA and serotonin (5-HT) to those found in control hippocampi. Both monoamines are strongly implicated in cognitive functions [63, 64] and are required for hippocampus-dependent memory [65], and further, hypoactivity of the 5-HTergic system is associated with accelerated cognitive decline and dementia severity [66]. Altogether, the above data support a recovery of synaptic integrity driven by AMPARs modulation, a finding that is in line with previous studies which have shown that this PAM-AMPA compound displayed neurotropic and connectivity-enhancing properties reverting neuronal atrophy of hippocampus in old mice [67, 68].

As pointed by human and animal studies, Aβ accumulation, synaptic dysfunction and hippocampal volume loss occur years before the appearance of AD clinical symptoms [5] with synaptic malfunction being an early pathological

manifestation in AD brain [6]. Thus, the absence of cognitive benefit in many clinical studies targeting Aβ synaptic malfunction in later (e.g., moderate or severe) stages of AD could be attributed to the fact that neurodegeneration, neuronal death and brain lesion(s) are too extensive to be ameliorated [69]. For instance, a recent clinical study using the same PAM-AMPA compound of the current study in a mixed cohort of mild and moderate AD patients did not show beneficial effects on memory [70]. Thus, new clinical studies have started to treat patients when their memory loss is mild or absent in order to block further synaptic damage before it becomes irreversible [39].

Further support for the causal relationship between imbalanced AMPAR-mediated glutamatergic signaling and synaptic pathology in AD is provided by a recent study which shows that deficits in AMPA signaling induces early synaptic dysfunction in a transgenic model of AD [56]. Interestingly, treatment with an anti-Aβ serum restored AMPA signaling and synaptic plasticity, reinforcing the idea that existing therapies, if administered early enough, can prevent or slow the progression of AD pathology [56]. The results of the present work are consistent with that notion and specifically suggest that therapeutic targeting of glutamatergic signaling deserves further exploration in the quest to delay the onset and incidence of sporadic AD.

Acknowledgements We thank Drs Luisa Pinto and Patricia Patricio (BnML & ICVS, School of Medicine, University of Minho, Portugal) for their administrative support. In addition, we would like to thank Dr Osborne F.X. Almeida (Max Planck Institute of Psychiatry, Germany) for his critical and constructive comments and feedback on the paper. This work was funded by the Institut de Recherches Internationales Servier (France). Carina Soares-Cunha is holder of a post-doctoral fellowship from the Programa de Atividades Conjuntas (PAC), through MEDPERSYST project (POCI-01-0145-FEDER-016428), supported by the Portugal 2020 Programme Fund (FEDER) while these studies were also supported by the European Regional Development Fund COMPETE (FCOMP-01-0124-FEDER-037298) as well as the project NORTE-01-0145-FEDER-000013, supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development.

Compliance with ethical standards

Conflict of interest This study was partly financially supported by the Institut de Recherches Internationales Servier (France) which has no influence on the experimental performance, tissue collection, and analysis as well as data analysis. SB and FA and RB are employees of Institut de Recherches Internationales Servier.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Goedert M, Spillantini MG. A century of Alzheimer's disease. Science. 2006;314:777–81.
2. Duyckaerts C, Delatour B, Potier MC. Classification and basic pathology of Alzheimer disease. Acta Neuropathol. 2009;118:5–36.
3. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med. 2011;1:1–23.
4. Koffie RM, Meyer-Luehmann M, Hashimoto T, Adams KW, Mielke ML, Garcia-Alloza M, et al. Oligomeric amyloid beta associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. Proc Natl Acad Sci USA. 2009;106:4012–7.
5. Sperling R, Mormino E, Johnson K. The evolution of preclinical Alzheimer's disease: Implications for prevention trials. Neuron. 2014;84:608–22.
6. Scheff SW, Price DA, Schmitt FA, Mufson EJ. Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. Neurobiol Aging. 2006;27:1372–84.
7. Kang HJ, Voleti B, Hajszan T, Rajkowska G, Stockmeier CA, Licznerski P, et al. Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. Nat Med. 2012;18:1413–7.
8. Popoli M, Yan Z, McEwen B, Sanacora G. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nat Rev Neurosci. 2011;13:22–37.
9. Sotiropoulos I, Cerqueira JJ, Catania C, Takashima A, Sousa N, Almeida OFX. Stress and glucocorticoid footprints in the brain—the path from depression to Alzheimer's disease. Neurosci Biobehav Rev. 2008;32:1161–73.
10. Elgh E, Lindqvist Astot A, Fagerlund M, Eriksson S, Olsson T, Näsman B. Cognitive dysfunction, hippocampal atrophy and glucocorticoid feedback in Alzheimer's disease. Biol Psychiatry. 2006;59:155–61.
11. Simard M, Hudon C, van Reekum R. Psychological distress and risk for dementia. Curr Psychiatry Rep. 2009;11:41–7.
12. Green KN, Billings LM, Roozendaal B, McGaugh JL, LaFerla FM. Glucocorticoids increase amyloid-β and Tau pathology in a mouse model of Alzheimer's Disease. J Neurosci. 2006;26:9047–56.
13. Jeong YH, Park CH, Yoo J, Shin KY, Ahn S-M, Kim H-S, et al. Chronic stress accelerates learning and memory impairments and increases amyloid deposition in APPV717I-CT100 transgenic mice, an Alzheimer's disease model. FASEB J. 2006;20:729–31.
14. Sotiropoulos I, Catania C, Pinto LG, Silva R, Pollerberg GE, Takashima A, et al. Stress acts cumulatively to precipitate Alzheimer's disease-like Tau pathology and cognitive deficits. J Neurosci. 2011;31:7840–7.
15. Silva JM, Rodrigues S, Sampaio-Marques B, Gomes P, Neves-Carvalho A, Dioli C, et al. Dysregulation of autophagy and stress granule-related proteins in stress-driven Tau pathology. Cell Death Differ. 2019;26:1411–27.
16. Rudy CC, Hunsberger HC, Weitzner DS, Reed MN. The role of the tripartite glutamatergic synapse in the pathophysiology of Alzheimer's disease. Aging Dis. 2015;6:131–48.
17. Henley JM, Wilkinson KA. AMPA receptor trafficking and the mechanisms underlying synaptic plasticity and cognitive aging. Dialogues Clin Neurosci. 2013;15:11–27.
18. Lesuis SL, Lucassen PJ, Krugers HJ. Early life stress impairs fear memory and synaptic plasticity; a potential role for GluN2B. Neuropharmacol. 2019;149:195–203.
19. Jacob CP, Koutsilieri E, Bartl J, Neuen-Jacob E, Arzberger T, Zander N, et al. Alterations in expression of glutamatergic transporters and receptors in sporadic Alzheimer's disease. JAD. 2007;11:97–116.
20. Chapman PF, White GL, Jones MW, Cooper-Blacketer D, Marshall VJ, Irizarry M, et al. Impaired synaptic plasticity and

learning in aged amyloid precursor protein transgenic mice. Nat Neurosci. 1999;2:271–6.
21. Hsieh H, Boehm J, Sato C, Iwatsubo T, Tomita T, Sisodia S, et al. AMPAR removal underlies abeta-induced synaptic depression and dendritic spine loss. Neuron. 2006;52:831–43.
22. Shankar GM, Bloodgood BL, Townsend M, Walsh DM, Selkoe DJ, Sabatini BL. Natural oligomers of the Alzheimer amyloid-protein induce reversible synapse loss by modulating an NMDA-Type glutamate receptor-dependent signaling pathway. J Neurosci. 2007;27:2866–75.
23. Wu H-Y, Hudry E, Hashimoto T, Kuchibhotla K, Rozkalne A, Fan Z, et al. Amyloid beta induces the morphological neurodegenerative triad of spine loss, dendritic simplification, and neuritic dystrophies through calcineurin activation. J Neurosci. 2010;30:2636–49.
24. Zempel H, Thies E, Mandelkow E, Mandelkow E-M. A oligomers cause localized Ca2+ elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. J Neurosci. 2010;30:11938–50.
25. Ittner LM, Ke YD, Delerue F, Bi M, Gladbach A, van Eersel J, et al. Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. Cell. 2010;142:387–97.
26. Sotiropoulos I, Sousa N. Tau as the converging protein between chronic stress and Alzheimer's disease synaptic pathology. Neurodegener Dis. 2016;16:22–5.
27. Yang C-H, Huang C-C, Hsu K-S. Behavioral stress enhances hippocampal CA1 long-term depression through the blockade of the glutamate uptake. J Neurosci. 2005;25:4288–93.
28. Lesuis SL, Kaplick PM, Lucassen PJ, Krugers HJ. Treatment with the glutamate modulator riluzole prevents early life stress-induced cognitive deficits and impairments in synaptic plasticity in APPswe/PS1dE9 mice. Neuropharmacol. 2019;150:175–83.
29. Lopes S, Vaz-Silva J, Pinto V, Dalla C, Kokras N, Bedenk B, et al. Tau protein is essential for stress-induced brain pathology. Proc Nat Acad Sci USA. 2016;113:E3755–63.
30. Pinheiro S, Silva J, Mota C, Vaz-Silva J, Veloso A, Pinto V, et al. Tau mislocation in glucocorticoid-triggered hippocampal pathology. Mol Neurobiol. 2016;53:4745–53.
31. Prediger RDS, Franco JL, Pandolfo P, Medeiros R, Duarte FS, Di Giunta G, et al. Differential susceptibility following β-amyloid peptide-(1-40) administration in C57BL/6 and Swiss albino mice: evidence for a dissociation between cognitive deficits and the glutathione system response. Behav. Brain Res. 2007;177:205–13.
32. Catania C, Sotiropoulos I, Silva R, Onofri C, Breen KC, Sousa N, et al. The amyloidogenic potential and behavioral correlates of stress. Mol Psychiatry. 2009;14:95–105.
33. Graziano A, Petrosini L, Bartoletti A. Automatic recognition of explorative strategies in the Morris water maze. J Neurosci Methods. 2003;130:33–44.
34. Hoover BR, Reed MN, Su J, Penrod RD, Kotilinek LA, Grant MK, et al. Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. Neuron. 2010;68:1067–81.
35. Salter MW, Kalia LV. Src kinases: a hub for NMDA receptor regulation. Nat Rev Neurosci. 2004;5:317–28.
36. Cummings J, Lee G, Ritter A, Zhong K. Alzheimer's disease drug development pipeline: 2018. Alzheimers Dement (N. Y). 2018;4:195–214.
37. Vaz-Silva J, Gomes P, Jin Q, Zhu M, Zhuravleva V, Quintremil S, et al. Endolysosomal degradation of Tau and its role in glucocorticoid-driven hippocampal malfunction. EMBO J. 2018;37:e99084.
38. Sotiropoulos I, Silva JMGM, Kimura T, Rodrigues AJ, Costa PS, Almeida OFX, et al. Female hippocampus vulnerability to environmental stress, a precipitating factor in tau aggregation pathology. J Alzheimers Dis. 2015;43:763–74.
39. Panza F, Lozupone M, Logroscino G, Imbimbo BP. A critical appraisal of amyloid-β-targeting therapies for Alzheimer disease. Nat Rev Neurol. 2019;15:73–88.
40. Iaccarino HF, Singer AC, Martorell AJ, Rudenko A, Gao F, Gillingham TZ, et al. Gamma frequency entrainment attenuates amyloid load and modifies microglia. Nature. 2016;540:230–5.
41. Griñán-Ferré C, Izquierdo V, Otero E, Puigoriol-Illamola D, Corpas R, Sanfeliu C, et al. Environmental enrichment improves cognitive deficits, AD hallmarks and epigenetic alterations presented in 5xFAD mouse model. Front Cell Neurosci Front Cell Neurosci. 2018;12:224.
42. Weiner MW, Sadowsky C, Saxton J, Hofbauer RK, Graham SM, Yun S, et al. Magnetic resonance imaging and neuropsychological results from a trial of memantine in Alzheimer' s disease. Alzheimer's Dement. 2011;7:425–35.
43. Kumar J, Mayer ML. Functional insights from glutamate receptor ion channel structures. Annu Rev Physiol. 2013;75:313–37.
44. Lynch G, Gall CM. Ampakines and the threefold path to cognitive enhancement. Trends Neurosci. 2006;29:554–62.
45. Black MD. Therapeutic potential of positive AMPA modulators and their relationship to AMPA receptor subunits. A review of preclinical data. Psychopharmacol. 2005;4:154–63.
46. Bernard K, Danober L, Thomas J, Mu C, Cordi A, Desos P, et al. S 18986: A positive allosteric modulator of AMPA-type glutamate receptors pharmacological profile of a novel cognitive enhancer. CNS Neurosc Ther. 2010;16:193–212.
47. Bretin S, Louis C, Seguin L, Wagner S, Thomas J-Y, Challal S, et al. Pharmacological characterisation of S 47445, a novel positive allosteric modulator of AMPA receptors. PLoS One. 2017;12:e0184429.
48. Giralt A, Gómez-Climent MÁ, Alcalá R, Bretin S, Bertrand D, María Delgado-García J, et al. The AMPA receptor positive allosteric modulator S 47445 rescues in vivo CA3-CA1 long-term potentiation and structural synaptic changes in old mice. Neuropharmacology. 2017;123:395–409.
49. Dolan RJ. Emotion, cognition, and behavior. Science. 2002;298:1191–4.
50. Grossberg GT, Desai AK. Management of Alzheimer's Disease. J Gerontol A Biol Sci Med Sci. 2003;58:M331–53.
51. Refojo D, Schweizer M, Kuehne C, Ehrenberg S, Thoeringer C, Vogl AM, et al. Glutamatergic and dopaminergic neurons mediate anxiogenic and anxiolytic effects of CRHR1. Science. 2011;333:1903–7.
52. Mossello E, Boncinelli M, Caleri V, Cavallini MC, Palermo E, Di Bari M, et al. Is antidepressant treatment associated with reduced cognitive decline in Alzheimer's Disease? Dement Geriatr Cogn Disord. 2008;25:372–9.
53. Jaworski T, Lechat B, Demedts D, Gielis L, Devijver H, Borghgraef P, et al. Dendritic degeneration, neurovascular defects, and inflammation precede neuronal loss in a mouse model for tau-mediated neurodegeneration. Am J Pathol. 2011;179:2001–15.
54. Decker H, Jürgensen S, Adrover MF, Brito-Moreira J, Bomfim TR, Klein WL, et al. N-methyl-D-aspartate receptors are required for synaptic targeting of Alzheimer's toxic amyloid-β peptide oligomers. J Neurochem. 2010;115:1520–9.
55. Miyamoto T, Kim D, Knox JA, Johnson E, Mucke L. Increasing the receptor tyrosine kinase EphB2 prevents amyloid-β-induced depletion of cell surface glutamate receptors by a mechanism that requires the PDZ-binding motif of EphB2 and neuronal activity. J Biol Chem. 2016;291:1719–34. 22
56. Baglietto-Vargas D, Prieto GA, Limon A, Forner S, Rodriguez-Ortiz CJ, Ikemura K, et al. Impaired AMPA signaling and cytoskeletal alterations induce early synaptic dysfunction in a mouse model of Alzheimer's disease. Aging Cell. 2018;17:e12791.

57. Tanaka H, Sakaguchi D, Hirano T. Amyloid-β oligomers suppress subunit-specific glutamate receptor increase during LTP. Alzheimer's Dement. 2019;5:797–808.
58. Reinders NR, Pao Y, Renner MC, Silva-Matos CM, da, Lodder TR, Malinow R, et al. Amyloid-β effects on synapses and memory require AMPA receptor subunit GluA3. PNAS. 2016;113:E6526–34.
59. Berchtold NC, Sabbagh MN, Beach TG, Kim RC, Cribbs DH, Cotman CW. Brain gene expression patterns differentiate mild cognitive impairment from normal aged and Alzheimer disease. Neurobiol Aging. 2014;35:1961–72.
60. Tai H-C, Serrano-Pozo A, Hashimoto T, Frosch MP, Spires-Jones TL, Hyman BT. The synaptic accumulation of hyperphosphorylated tau oligomers in Alzheimer disease is associated with dysfunction of the ubiquitin-proteasome system. Am J Pathol. 2012;181:1426–35.
61. Zhou L, McInnes J, Wierda K, Holt M, Herrmann AG, Jackson RJ, et al. Tau association with synaptic vesicles causes presynaptic dysfunction. Nat Commun. 2017;8:1–13.
62. McInnes J, Wierda K, Snellinx A, Bounti L, Wang Y-C, Stancu I-C, et al. Synaptogyrin-3 mediates presynaptic dysfunction induced by Tau. Neuron. 2018;97:823–835.e8.
63. Borodovitsyna O, Flamini M, Chandler D. Noradrenergic modulation of cognition in health and disease. Neural Plast. 2017; 6031478. https://doi.org/10.1155/2017/6031478.
64. Švob Štrac D, Pivac N, Mück-Šeler D. The serotonergic system and cognitive function. Transl Neurosci. 2016;7:35–49.
65. Zhang L, Ouyang M, Ganellin CR, Thomas SA. The slow after-hyperpolarization: a target of β1-adrenergic signaling in hippocampus-dependent memory retrieval. J Neurosci. 2013;33:5006–16.
66. Lai MKP, Tsang SWY, Francis PT, Keene J, Hope T, Esiri MM, et al. Postmortem serotoninergic correlates of cognitive decline in Alzheimer's disease. Neuroreport. 2002;13:1175–8.
67. Calabrese F, Savino E, Mocaer E, Bretin S, Racagni G, Riva MA. Upregulation of neurotrophins by S 47445, a novel positive allosteric modulator of AMPA receptors in aged rats. Pharm Res. 2017;121:59–69.
68. Giralt A, Gómez-climent MÁ, Alcalá R, Bretin S, Delgado-garcía JM, Pérez-navarro E, et al. The AMPA receptor positive allosteric modulator S 47445 rescues in vivo CA3-CA1 long-term potentiation and structural synaptic changes in old mice. Neuropharmacolgy. 2017;123:395–409.
69. Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, et al. Long-term effects of abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. Lancet. 2008;372:216–23.
70. Bernard K, Gouttefangeas S, Bretin S, Galtier S, Robert P, Holthoff-Detto V, et al. A 24-week double-blind placebo-controlled study of the efficacy and safety of the AMPA modulator S47445 in patients with mild to moderate Alzheimer's disease and depressive symptoms. Alzheimers Dement (N. Y). 2019;5:231–40.