Alzheimer's & Dementia: Translational Research & Clinical Interventions 2 (2016) 267-280

Featured Article

SAR110894, a potent histamine H3-receptor antagonist, displays disease-modifying activity in a transgenic mouse model of tauopathy

Philippe Delay-Goyet[a,*], Véronique Blanchard[a], Nathalie Schussler[a], Mati Lopez-Grancha[a], Jean Ménager[a], Véronique Mary[a], Eric Sultan[b], Armelle Buzy[c], Jean-Claude Guillemot[c], Jeanne Stemmelin[a], Philippe Bertrand[a], Thomas Rooney[a], Laurent Pradier[a], Pascal Barnéoud[a]

[a]*Neurodegeneration and Pain Unit, Sanofi R&D, Chilly-Mazarin, France*
[b]*Disposition, Safety and Animal Research, Sanofi R&D, Montpellier, France*
[c]*Translational Sciences Unit, Sanofi R&D, Chilly-Mazarin, France*

Abstract

Introduction: Tau hyperphosphorylation and neurofibrillary tangles are histopathologic hallmarks of tauopathies. Histamine H3-receptor antagonists have been proposed to reduce tau hyperphosphorylation in preclinical models.

Methods: We evaluated the ability of SAR110894, a selective histamine H3-receptor antagonist, to inhibit tau pathology and prevent cognitive deficits in a tau transgenic mouse model (THY-Tau22).

Results: SAR110894 treatment for 6 months (but not 2 weeks) in THY-Tau22 mice decreased both tau hyperphosphorylation at pSer396-pSer404 (AD2 signal) in the hippocampus and the number of AT8 (pSer199/202-Thr205) positive cells in the cortex and decreased the formation of neurofibrillary tangles in the cortex, hippocampus, and amygdala. Macrophage inflammatory protein 1-alpha messenger RNA expression was decreased in the hippocampus. SAR110894 also prevented episodic memory deficits, and this effect was still detected after treatment washout.

Discussion: Long-term SAR110894 treatment could have potential disease modifying activity in neurodegenerative tauopathies.

Keywords: Alzheimer's disease; Tau pathology; Cognition; Histamine H3-receptor; THY-Tau22; Transgenic

1. Background

The histamine H3-receptor (H3R) is expressed in many brain regions, including those involved in sleep/wake regulation and cognitive functions [1]. At the presynaptic level, inhibition of the H3R leads to increased release of several neurotransmitters, which induce activation of postsynaptic signaling pathways relevant to cognitions, making this receptor a potential drug target for regulating the enhancement of arousal or cognitive processes. Extensive preclinical data with H3R antagonists support their potential use for the treatment of human cognitive disorders, including Alzheimer's Disease (AD) [1]. The activation by H3R antagonists of postsynaptic signaling pathways also relevant to neuroprotection (such as the Akt/GSK3β pathway, which is involved in tau phosphorylation) was recently suggested [2,3].

The microtubule-associated tau protein is abundant in the nervous system, especially concentrated in neurons where it regulates the assembly and stabilization of the microtubule cytoskeleton [4]. In neurodegenerative tauopathies, such as AD, hyperphosphorylation of tau leads to its dissociation from microtubules, leading to the formation of neurofibrillary tangles (NFTs) composed of paired helical filaments and the progressive destabilization of the neuronal cytoskeleton. In AD, the amount and distribution

*Corresponding author. Tel.: +33 160496194; Fax: +33 160497442.
E-mail address: philippe.delay-goyet@sanofi.com

http://dx.doi.org/10.1016/j.trci.2016.10.002

of NFTs and/or hyperphosphorylated tau correlate with the severity of the disease [5,6].

A previous study has shown that a 2-week treatment with ABT-239, a H3R antagonist, decreases tau hyperphosphorylation (AT8 site) in ventral horn spinal motoneurons and hippocampal CA3 mossy fibers of tau × amyloid precursor protein mice [7]. This prompted us to investigate whether SAR110894, a different chemical class of H3R antagonist with higher affinity and selectivity than ABT-239 for H3R [8], had any effect on tau pathology in THY-Tau22 transgenic mice.

THY-Tau22 transgenic mice display age-dependent tau pathology in the brain with minor traces of human tau protein in the spinal cord. THY-Tau22 mice show hyperphosphorylation of tau on several AD-relevant tau epitopes, NFT-like inclusions (Gallyas and MC1-positive) with rare ghost tangles, and paired helical filament–like filaments, as well as mild astrogliosis starting from the age of 3 to 6 months. These mice also display cognitive deficits from the age of 6 months in the absence of any motor deficits [9].

SAR110894 is a potent and selective histamine receptor antagonist at human, rat, and mouse H3R and has shown efficacy in several animal models related to the cognitive deficits in AD. For example, SAR110894 prevented the occurrence of episodic memory deficits induced by scopolamine in rats or by the central infusion of the toxic amyloid fragment Aβ(25–35) in the object recognition test in mice [8]. In the present study, we evaluated the ability of SAR110894 to prevent cognitive deficits and inhibit tau pathology in THY-Tau22 mice after single, repeated, or chronic administration.

2. Methods

2.1. Animals and treatment

Male THY-Tau22 transgenic and nontransgenic littermates (wild-type [WT]) mice were used. In all experiments with repeated administration, mice were housed individually in an enriched environment. Housing was in a pathogen-free facility at a constant temperature of 22 ± 2°C and humidity (50 ± 10%) on a reverse light/dark cycle under a 12:12 light/dark cycle (light on at 7:00 AM) with ad libitum access to food and water.

SAR110894 was synthesized by Sanofi medicinal chemistry as a difumarate salt and was given orally to the animals in drinking water (0.002% and 0.02% for 2 weeks) or in the diet (0.0002%, 0.002%, and 0.02% for 6 months or 0.00067% and 0.002% for 4 or 6 months). Experiments and treatment groups are shown in Table 1. Body weights were measured weekly. All protocols were approved by the Ethical Committee for Animal Research of Sanofi-Aventis. Animal facilities and animal care and use programs were in accordance with French legislation, which implemented the European directive 86/609/EEC.

The animals were homogeneously distributed in the treatment groups according to their body weights and timing of test sessions at the different open fields used in the object recognition test.

The study investigators were not blinded to treatment assignment. Behavioral experiments were carried out by two different experimenters.

Further details are provided in Supplementary Material (see Supplementary Methods).

2.2. Shape and spatial memory assessment using the object recognition test

The test apparatus was based on that described by Ennaceur and Delacour [15] in rats and adapted for use in mice. The enclosure (59 × 59 × 30 cm) was made of polyvinyl chloride, with four black walls and a white floor and a camera located 150 cm above the enclosure. The observer was located in an adjacent room fitted with a video monitoring system. The task procedure consists of three sessions: habituation, acquisition, and recall, all carried out between 9:00 AM and 1:15 PM.

Further details are provided in Supplementary Material (see Supplementary Methods).

2.3. Brain tissue and blood sampling

At the completion of the experiments, blood samples were collected from awake animals from the mandibular vein and transferred into Sarstedt tubes containing lithium heparin. After centrifugation (1500–2000*g* for 10 minutes at 4°C) plasma samples were frozen in microtubes and stored at −80°C.

Mice were sacrificed without anesthesia, as anesthesia has been shown to increase tau hyperphosphorylation through hypothermia [16], and brains removed. Hippocampi, cingulate cortex, amygdala, and dentate gyrus or hemibrains were dissected from each mouse using a coronal acrylic slicer (Delta Microscopies) at 4°C and stored at −80°C until used for biochemical, RNA, or pharmacokinetics (PK) analyses. The second hemibrains (to be used in histopathologic studies) were immersion-postfixed for 7 days at 4°C with 4% formaldehyde (Carlo Erba/code 415691).

2.4. Quantification of SAR110894 levels in blood and brain samples

For both plasma and brain tissue samples, after addition of the precipitant solution (acetonitrile), SAR110894 was quantified by liquid chromatography-mass spectrometry/mass spectrometry. Deuterium-labeled SAR110894 (D5-SAR110894) was used as an internal standard.

Further details are provided in Supplementary Material (see Supplementary Methods).

2.5. Histology and immunohistochemistry

Postfixed hemibrains were cryoprotected in 20% sucrose solution (for 48 hours at 4°C) before subsequent rapid freezing in isopentane. Serial sagittal cryostat tissue sections

Table 1
Doses and treatment duration used in the different experiments

Experiment (repeated administration)	Genotype	Age at beginning of treatment (mo)	Treatment	Number
2 wk in drinking water	THY-Tau22	5–6	Vehicle	9
AD2, AT8, Ser9-GSK3β signals (Western blot)			0.002%	9
			0.02%	9
6 mo in food	WT	2	Vehicle	16
Object recognition (during treatment)			0.0002%	15
AD2, AT8, pSer214-, pSer262-, pSer422-, and pThr181-tau			0.002%	15
signals (Western blot)			0.02%	15
AT8 immunostaining	THY-Tau22		Vehicle	17
NFTs (Gallyas silver staining)			0.0002%	17
MIP-1α, cystatin F, GFAP mRNA			0.002%	17
			0.02%	17
4 mo (+2 mo of treatment washout) or 6 mo in food	WT	2.5	Vehicle	15
Without or with treatment cessation			0.00067%	15
			0.002%	15
Pharmacokinetics	THY-Tau22		Vehicle	20
Object recognition test			0.00067%	20
Shape object recognition testing: 10 d after treatment cessation			0.002%	20
Spatial object recognition testing: 17 d after treatment cessation			0.002% + treatment cessation	20

Abbreviations: GFAP, glial fibrillary acidic protein; MIP-1α, macrophage inflammatory protein 1-alpha; mRNA, messenger RNA; NFTs, neurofibrillary tangles; PK, pharmacokinetics; WT, wild-type.

(20 μm thick) were collected onto Superfrost plus microscope glass slides (VWR, Radnor, PA) and then stored at −20°C or placed in phosphate-buffered saline–sodium azide 0.1% (S-2002, Sigma) containing wells to avoid any contamination and stored at 4°C.

Further details are provided in Supplementary Material (see Supplementary Methods).

2.6. Quantitative image analysis of Gallyas staining and AT8 immunostaining

Gallyas staining and AT8 immunostaining were quantitatively determined using microscopic virtual slide technology using an Olympus dotslide scanner system and a computer-based workstation (Explora Nova using Mercator software) [17-19].

Further details are provided in Supplementary Material (see Supplementary Methods).

2.7. Western blot analysis of tau phosphorylation in cortex and hippocampus homogenates

For immunoblot analysis, frozen mouse tissues (hippocampus or cortex) were homogenized using a Precellys 24 tissue homogenizer in 350 μL radioimmunoprecipitation assay buffer from Cell Signaling (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2 ethylene diamine tetraacetic acid, 1 mM ethylene glycol tetraacetic acid, 1% 4-nonylphenylpoly(ethylene glycol), 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, and 1 μg/mL leupeptin) to which protease inhibitors (Sigma cocktail 1% vol/vol) and phosphatase inhibitors (okadaic acid 1 μM and sodium fluoride 100 mM) were added. After centrifugation, 16,000*g* at 4°C for 10 minutes, the protein content in supernatants was determined with a BioRad DC Protein Assay kit, using bovine serum albumin as a standard. Equal amounts of protein (5 or 20 μg) were loaded on 15-well 4% to 12% Bis-Tris gels (NuPAGE; Invitrogen) and electrophoresis was performed at 200 V for 50 minutes in 3-(N-morpholino)propanesulfonic acid buffer, according to the manufacturer's instructions. Proteins were then transferred to polyvinylidene difluoride membranes (Invitrogen; Thermo Fisher Scientific Corporation, Waltham, MA) at 30 V for 2 hours in transfer buffer (Invitrogen) containing 20% methanol. After blocking in 5% nonfat dry milk in tris-buffered saline Tween 0.1%, blots were incubated overnight at 4°C with primary antibodies diluted in 5% bovine serum albumin in tris-buffered saline Tween 0.1%.

Further details are provided in Supplementary Material (see Supplementary Methods).

2.8. Messenger RNA extraction and quantitative real-time reverse transcription polymerase chain reaction analysis

A hemihippocampus from each mouse was placed in a Precellys CK14 tube including 50 ceramic beads (1.4 mm diameter) and 0.5 mL of Applied Biosystems' nucleic acid purification lysis solution (1×). The tissue was homogenized using a homogenizer Precellys 24 (Bertin Technology) during 2 × 10 seconds bursts. Total RNA was isolated using the 6100 PrepStation (Applied Biosystems), according to the manufacturer's instructions, including a DNase treatment (protocol isolation of total RNA from plant and animal tissue). To assess the quality and concentration of the total RNA, 1 μL was analyzed on a RNA LabChip (Agilent Technologies) using a 2100 Bioanalyzer (Agilent Technologies).

Further details are provided in Supplementary Material (see Supplementary Methods).

2.9. Statistical analysis

Results are expressed as the means ± standard error of the mean (SEM) or means ± standard deviation. Statistical analyses were performed using SAS system release 9.2 for HP-UX via Everstat 6.0 internal software. All data sets passed the Kolmogorov-Smirnov test for normality and Levene test for variance homogeneity.

Depending on the data obtained, each treated group was compared with a control group with a two-sided Dunnett test or a two-sided Wilcoxon test with Bonferroni-Holm multiplicity correction. For two-group comparison, the two-sided Student *t* test or two-sided Wilcoxon test was used.

3. Results

3.1. Plasma and brain exposure of SAR110894 in WT mice

The PK parameters for SAR110894 were measured in male WT mice after a 5-month repeated oral administration of SAR110894 (0.00067% or 0.002% in diet). SAR110894 concentrations were measured in the plasma and pons/

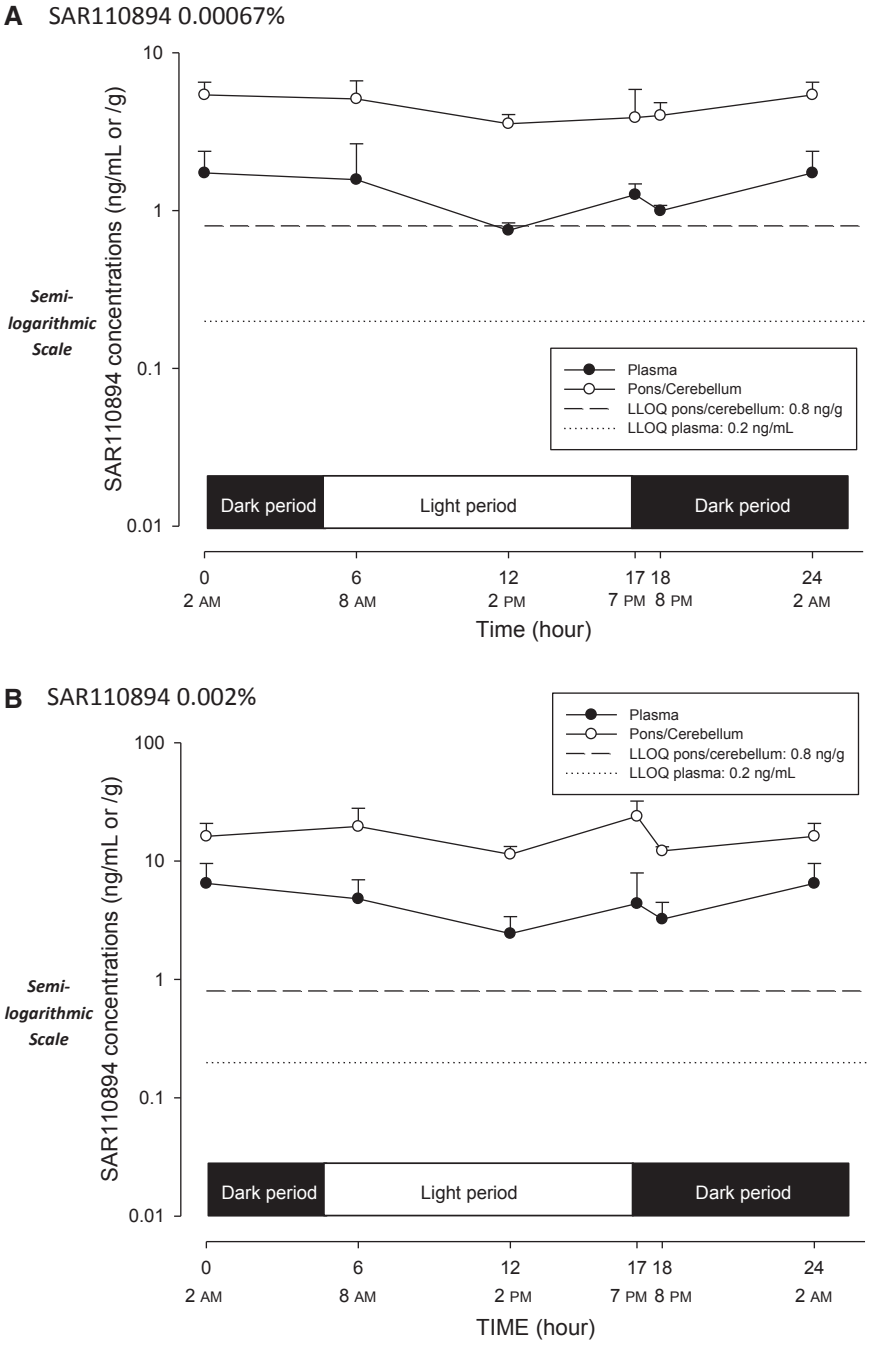

Fig. 1. Time course of total SAR110894 concentrations in plasma and pons/cerebellum after a 5-month repeated oral administration of 0.00067% (A) and 0.002% (B) SAR110894 in diet to male wild-type mice. Results are expressed as the mean +standard deviation. n = 3 per group. LLOQ, lower limit of quantitation.

Table 2
Pharmacokinetic parameters of SAR110894 after a 5-month repeated oral (in diet) administration of SAR110894 to male wild-type mice

Dose (% in diet)	Tissue	C_{max} ng/mL or ng/g (nM)	t_{max} (h)	C_{min} ng/mL or ng h/g (nM)	t_{min} (h)	$AUC_{(0-24\ h)}$ ng h/mL or ng/g	C_{max}/C_{min} ratio	Brain/plasma $AUC_{(0-24\ h)}$ ratio	0.002%/0.00067% $AUC_{(0-24\ h)}$ ratio
0.00067	Plasma	1.73 (6.63)	2:00 AM	1.26 (4.83)	7:00 PM	31	1.4	—	—
	Pons/cerebellum	5.41 (20.7)	2:00 AM	3.55 (13.6)	2:00 PM	110	1.5	3.5	—
0.002	Plasma	6.48 (24.8)	2:00 AM	2.44 (9.35)	2:00 PM	110	2.7	—	3.5
	Pons/cerebellum	23.9 (91.6)	7:00 PM	11.4 (43.7)	2:00 PM	390	2.1	3.5	3.5

Abbreviation: AUC, area under curve.

cerebellum on the last day of treatment and showed a moderate interanimal variability. The concentrations in both plasma and brain were roughly constant over the period of sampling collection (i.e., 24 hours; Fig. 1A and B) with C_{max}/C_{min} ratios ranging from 1.4 to 2.7 (Table 2). Both the C_{max} and the area under curve $(AUC)_{(0-24\ h)}$ in plasma and brain increased in a dose proportional manner (C_{max} from 6.63 to 24.8 nM and from 20.7 to 91.6 nM, respectively; $AUC_{(0-24\ h)}$ from 31 to 110 ng h/mL and from 110 to 390 ng h/g, respectively; Table 2). The SAR110894 "brain/plasma" exposure ratio was 3.5 at both doses demonstrating good brain penetration of the compound.

Taking into account the exposure in the plasma after a single oral administration (data not shown), 0.002% SAR110894 in the diet was calculated to be bioequivalent to a 1 mg/kg single oral dose. This dose has been shown to be active in a variety of animal models [8].

3.2. Chronic treatment with SAR110894 reduced cognitive deficits in THY-Tau22 mice

THY-Tau22 mice have been reported to have impaired spatial learning, social recognition memory, and appetitive learning (but not to have any impairment in passive avoidance, fear learning, or extinction learning), with the first signs of cognitive impairment arising by the age of 7 to 8 months [20]. We used the object recognition test because we observed cognitive impairment in shape memory as soon as 5.5 months (internal data not published) and both the shape and spatial test designs were applied because they are dependent on cortical [21–27] and hippocampal [28,29] functions, respectively. Fig. 2 shows that 6- to 7-month-old THY-Tau22 mice displayed an impairment of shape (panels A and C) and spatial (panels B and D) episodic memory compared with age-matched WT mice in the object recognition test.

SAR110894 administered during 4 or 6 months starting at the age of 2 months prevented cognitive deficits in THY-Tau22 mice (Fig. 2). SAR110894 (0.002% and 0.02%) reduced the deficits in both shape episodic memory (recognition index [means ± SEM]: 64 ± 2%, $P = .0010$ and 61 ± 3%, $P = .0104$, respectively; Fig. 2A) and spatial episodic memory (recognition index [means ± SEM]: 58 ± 3%, $P = .0153$ and 60 ± 2%, $P = .0002$, respectively; Fig. 2B). PK parameters of SAR110894 at the three doses tested were measured (Supplementary Table 2).

In an independent confirmatory study, 4 months of treatment with a lower dose of SAR110894 (0.00067%) reduced the deficit in spatial episodic memory (Fig. 2D) but had no effect on the deficit in shape episodic memory (Fig. 2C).

The procognitive effect of SAR110894 (0.002%) on shape episodic memory (Fig. 2C) and spatial episodic memory (Fig. 2D) still persisted after 1 or 2 weeks of treatment washout, respectively. After 1 week of washout, the concentrations of SAR110894 were less than the level of quantification in both the plasma and pons/cerebellum (Supplementary Table 3).

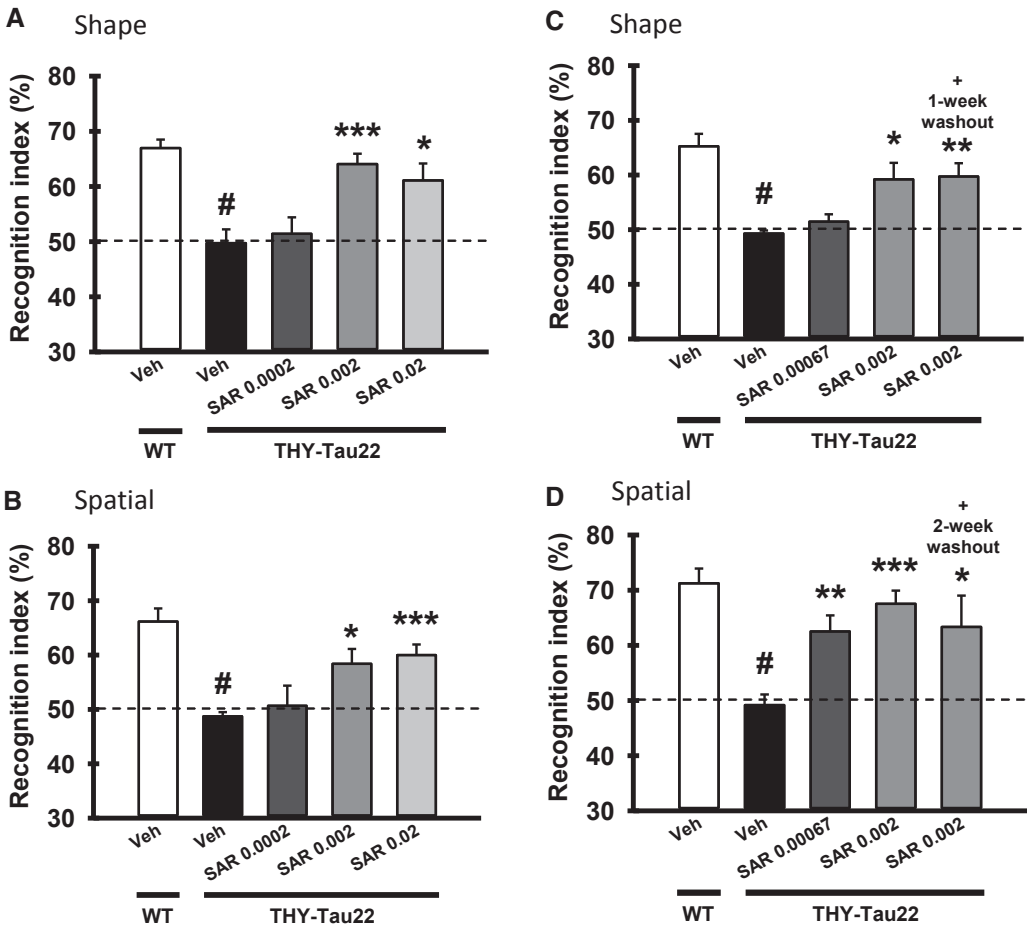

Fig. 2. Chronic 4-month oral treatment with SAR110894 starting at the age of 2 months prevented shape (A, C) and spatial (B, D) object recognition deficits in 6- to 6.5-month-old (A, B) (first experiment) or 6.5- to 7-month-old (C, D) (second experiment) THY-Tau22 mice. The procognitive effect of SAR110894 on shape (C) and spatial (D) memory still persisted after 1 or 2 weeks of treatment washout, respectively. Results are expressed as the mean recognition index (%) + standard error of the mean. $^{\#}P < .001$ versus WT vehicle (Student *t* test (A) and Wilcoxon test (B–D)), $^{*}P < .05$, $^{**}P < .01$, $^{***}P \leq .001$ versus THY-Tau22 vehicle (Dunnett test (A) or Wilcoxon test with Bonferroni-Holm correction (B–D)), n = 10 to 13 mice per group (A, B) or 7 to 11 mice per group (C, D). WT, wild-type; Veh, vehicle. Doses of SAR110894 (SAR) are expressed in percents. Dotted line corresponds to chance level.

3.3. Effect of subacute and chronic treatment with SAR110894 on tau pathology in THY-Tau22 mice

3.3.1. Tau phosphorylation

Acute administration of SAR110894 (3 and 10 mg/kg orally) had no effect on tau hyperphosphorylation (AD2 or AT8 epitopes) in the cingulate cortex of 3-month-old THY-Tau22 mice (data not shown). PK parameters of SAR110894 (10 mg/kg orally) were measured (Supplementary Table 1).

Moreover, a subacute 2-week treatment with SAR110894 (0.002% and 0.02% in drinking water, bioequivalent to single oral doses of 1 and 10 mg/kg) also had no effect on tau hyperphosphorylation (AD2 or AT8) in the hippocampus of 5- to 6-month-old THY-Tau22 mice (Fig. 3A and B) or on the level of Ser9-GSK3β (inactive form of GSK3β; Fig. 3C).

To determine if longer treatment with SAR110894 was needed to observe an effect on tau pathology, THY-Tau22 mice were treated with SAR110894 for 6 months starting at the age of 2 months (the same animals were used for cognition readouts, see Section 3.2). Using this chronic treatment protocol, SAR110894 (0.002%) significantly decreased (−37%, $P = .0291$) tau phosphorylation at residues 396/404 (AD2 signal) measured by immunoblot in the hippocampus (Fig. 4A) and showed a trend to decrease (−57%, $P = .2766$) the AD2 signal in the cortex (Fig. 4B).

In a second independent Western blot evaluation in cortex homogenates comparing "vehicle group" and the dose of "0.002%," the inhibition of AD2 signal in the cortex on treatment was confirmed and significant (−37%, $P = .0191$; Fig. 4F). SAR110894 (0.002%) also significantly decreased tau phosphorylation at pSer214 (−35%, $P = .0052$) in the cortex but had no effect on tau phosphorylation at pThr181 (AT270 signal; −16%, $P = .4830$) and pSer422 (−20%, $P = .3019$). Total tau (Tau5 signal) was unchanged (−17%, $P = .0950$).

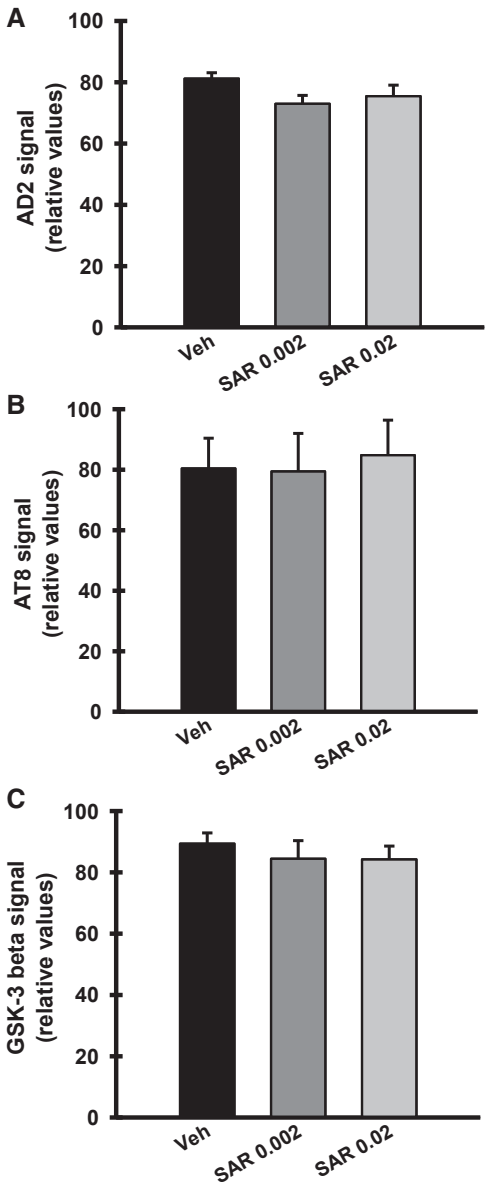

Fig. 3. Two-week oral treatment of 5- to 6-month-old THY-Tau22 mice with SAR110894 had no effect on tau hyperphosphorylation or Ser-9 GSK3β phosphorylation in the hippocampus. (A) Tau hyperphosphorylation on AD2 epitope, (B) tau hyperphosphorylation on AT8 epitope, and (C) Ser-9 phosphorylation. Western blot quantifications are expressed as relative values and represented as the means +standard error of the mean (Dunnett tests (A, C) or Wilcoxon tests with Bonferroni-Holm correction (B)), n = 9 mice per group. Veh, vehicle. Doses of SAR110894 (SAR) are expressed in percents.

SAR110894 also had no statistically significant effect on tau phosphorylation at residues Ser199/202-Thr205 (AT8 signal) in the hippocampus (−22%, $P = .5560$; Fig. 4D) or in the cortex (−29%, $P = .8054$; Fig. 4E).

Akt, pAkt(Ser473), pAkt(Thr308), ERK1, pERK1(Thr202/Tyr204), ERK2, pERK2(Thr185/Tyr187), and demethylated PP2A levels were not changed by SAR110894 (0.002%) treatment (Western blot evaluation, see Supplementary Table 4).

The effect of a 6-month treatment with SAR110894 on tau phosphorylation was further evaluated by immunohistochemistry, a technique more sensitive than Western blot analysis when the antigen is heterogeneously distributed. As illustrated in Fig. 5, the pathological process of abnormal tau hyperphosphorylation in neurons was reduced after SAR1100894 treatment. Within the cortex, the number of AT8-positive neuronal cell bodies was significantly decreased by 47% ($P = .0010$) and 32% ($P = .0250$) after treatment with SAR110894 (0.002% and 0.02%, respectively; Fig. 5C). In addition, a marked decrease in the intensity of AT8 immunostaining was observed within the neuronal cell bodies and fibers in the cortex of mice treated with SAR110894 (0.002%; data not shown). No significant decrease in AT8-positive neuronal cell number was detected in other brain regions (i.e., the amygdala and both CA1 and dentate gyrus hippocampal subareas; data not shown).

3.3.2. Neurofibrillary tangles

The effect of SAR110894 on tau pathology was further evaluated by measuring NFTs using the Gallyas silver impregnation staining. Abnormal tau protein aggregation was reduced after 6 months of SAR110894 treatment in THY-Tau22 transgenic mice, as shown in Fig. 6. The number of NFTs in the cortex was significantly decreased by −48% ($P = .0001$) and −41% ($P = .0019$), at SAR110894 0.002% and 0.02%, respectively (Fig. 7A). SAR110894 also significantly decreased the number of NFTs in the hippocampus (−25%, [$P < .0001$] and −22% [$P = .0006$]) at the doses of 0.002% and 0.02%, respectively (Fig. 7B) and in the amygdala (−31% [$P = .0065$]) at 0.02% (Fig. 7C).

3.4. SAR110894 treatment decreased hippocampal neuroinflammation in THY-Tau22 mice

A previous study has shown that, in addition to developing tau pathology, THY-Tau22 mice display a mild astrogliosis in the hippocampus [9]. Increased expression of glial fibrillary acidic protein (GFAP; marker of astrogliosis) was indeed observed in the hippocampus in our study, together with an increased expression of macrophage inflammatory protein 1-alpha (MIP-1α; a marker of microgliosis) and cystatin F (a marker of microgliosis expressed during demyelination [30]). After 6 months of treatment, SAR110894 significantly decreased the expression of MIP-1α by −35% ($P = .0087$) and −36% ($P = .0184$) at the doses of 0.0002 and 0.002%, respectively (Fig. 8A). SAR110894 had no effect on the expression of GFAP or cystatin F in the hippocampus (Fig. 8B and C, respectively).

4. Discussion

In the present study, we examined the effect of SAR110894, a selective and potent H3R antagonist [8], on cognitive deficits, tau hyperphosphorylation, and the

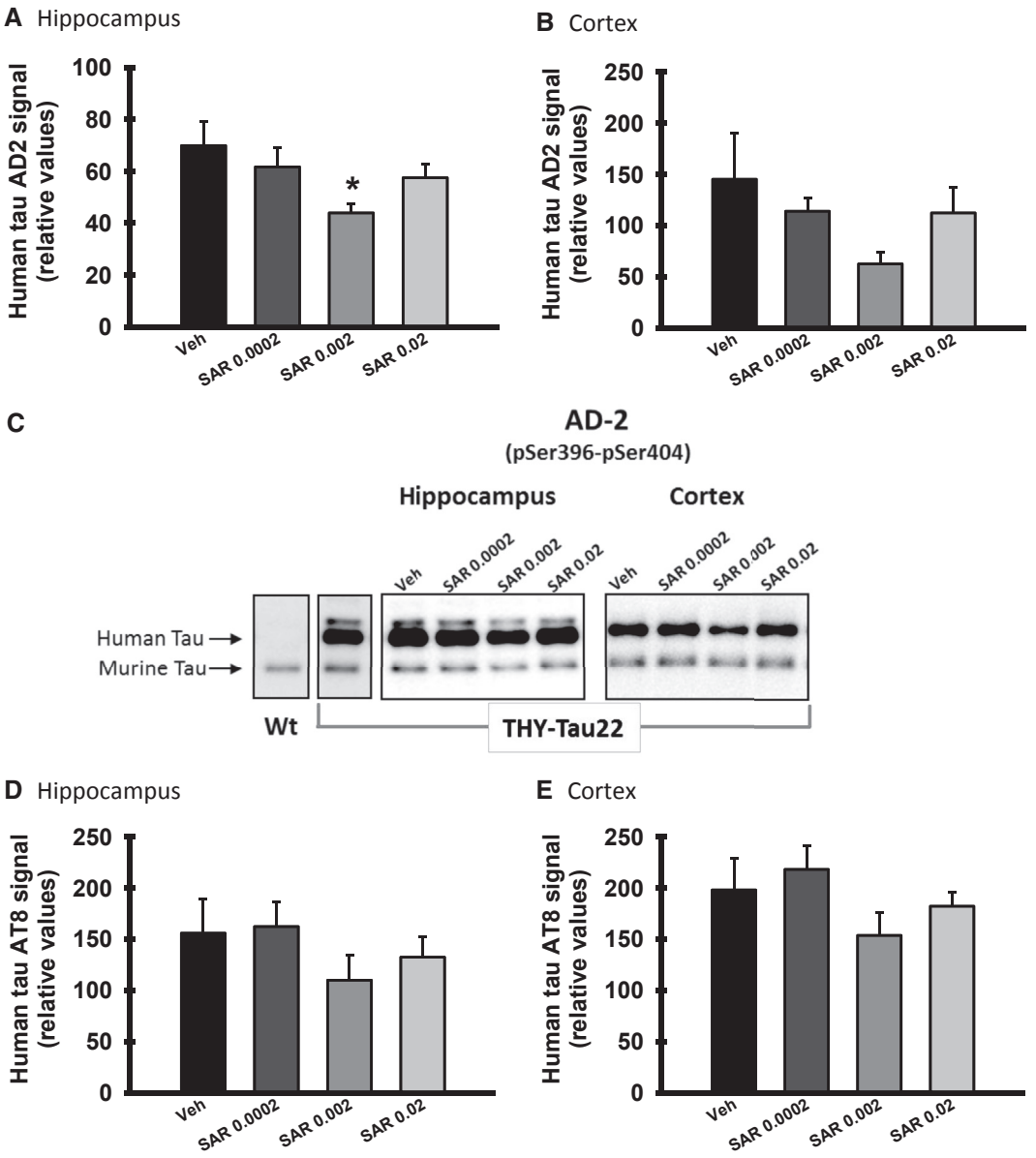

Fig. 4. Chronic oral treatment with SAR110894 during 6 months starting at 2 months in THY-Tau22 mice decreased tau hyperphosphorylation on AD2 (pSer396-pSer404) (A, B, C, F) and Tau pSer214 (F) but not on AT8 (D–F), AT270 (Thr181) (F), or Tau pSer422 (F) epitopes in the hippocampus (A, C, D) and cortex (B, C, E, F). (C) Representative Western blots with AD2 antibody in wild-type and THY-Tau22 mice showing murine endogenous tau (around 50 kDa) and human transgenic tau 4R1N (around 60 kDa). (F) Quantitation and representative Western blots with all phosphorylation-dependent antibodies tested at the dose of SAR 0.002% (AD2, AT8, Tau pSer214, AT270, and Tau pSer422) or phosphorylation-independent (Tau5). Western blot quantifications are expressed as the relative means + standard error of the mean (A, B, D, E) or expressed as individual data and relative means ± standard error of the mean (F). *$P < .05$, **$P < .01$ versus THY-Tau22 Vehicle (Dunnett tests (A), Wilcoxon tests with Bonferroni-Holm correction (B, D, E, F [AT8 only]) or Student t test (F [AD2, Tau pSer214, AT270, Tau pSer422, and Tau5])), n = 8 to 9 mice per group (A, B, D, E) or 7 to 9 mice per group (F). Veh, vehicle. Doses of SAR110894 (SAR) are expressed in percents.

formation of NFTs in THY-Tau22 mice, a tau transgenic mouse model. SAR110894 has been tested using different routes of administration (gavage, drinking water, and incorporation in food) to fully evaluate its pharmacological activity using different experimental protocols. THY-Tau22 mice selectively overexpress human 4-repeat tau in the brain and display age-dependent tau pathology [9]. These mice also exhibit behavioral and memory deficits in a broad variety of experimental paradigms. For example, THY-Tau22 mice display spatial reference memory deficits in a Y-maze test at the age of 8 to 13 months [10,13,31] and in the Morris Water maze at the age of 7 to 12 months [11,12,14,20,31]. They also show impaired social recognition memory and appetitive learning deficits at the age of 9 to 10 months [20]. Our data provide the first demonstration that THY-Tau22 mice also have deficits in shape and spatial episodic

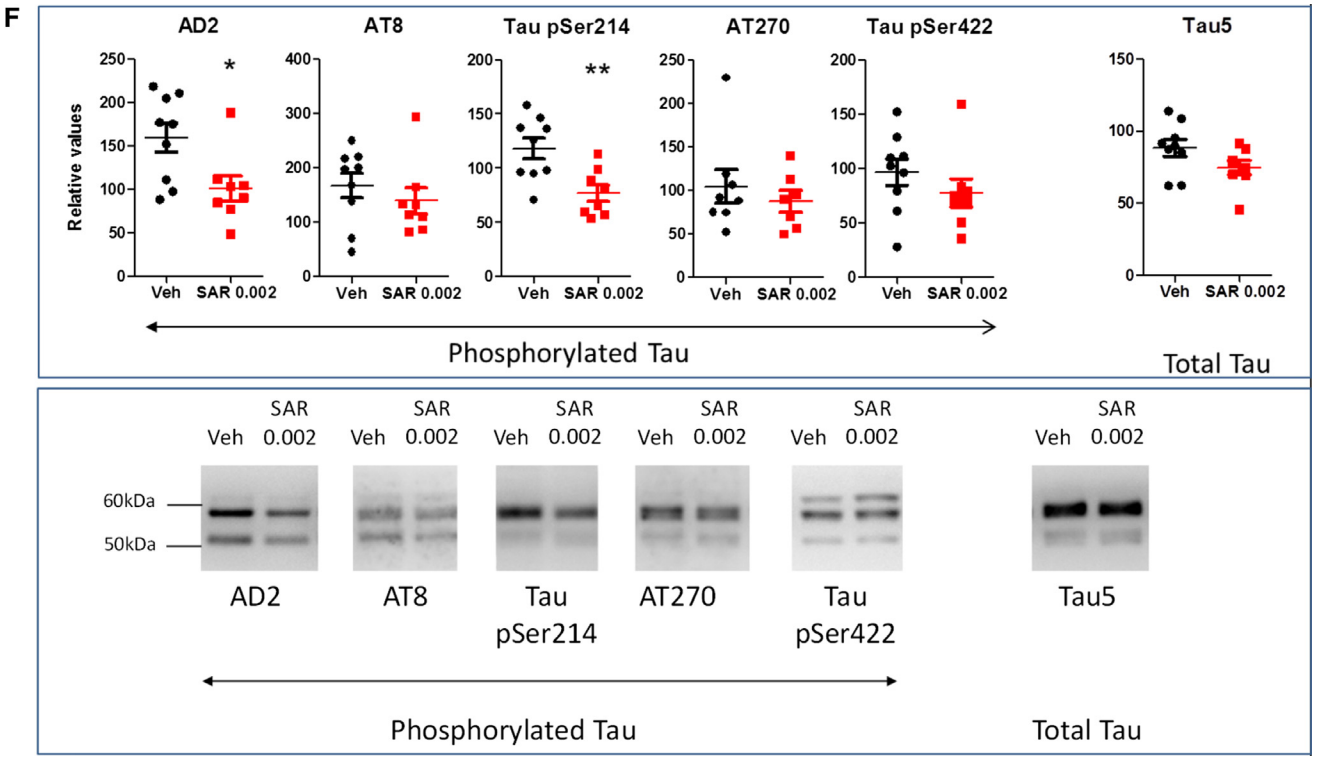

Fig. 4. (*continued*).

memory in the object recognition test from the age of 6 months.

Previous studies have indicated that the age-dependent cognitive impairment in THY-Tau22 mice is closely correlated with the development of tau pathology in the hippocampus [9,31,32] and impaired brain-derived neurotrophic factor-dependent hippocampal synaptic transmission [11]. Moreover, the potentiation of spatial learning deficits induced by early and progressive obesity in THY-Tau22 mice is accompanied by a worsening of hippocampal tau pathology [12]. Finally, pharmacological intervention with chronic caffeine intake induced a decrease in tau hyperphosphorylation and proinflammatory and oxidative stress makers in the hippocampus of THY-Tau22 mice that was correlated with a reduction in cognitive impairment [14].

We previously showed that acute treatment with SAR110894 has procognitive activity in several animal models displaying cognitive deficits relevant to those found in patients with AD [8]. For example, in the object recognition test SAR110894 prevented the occurrence of scopolamine-induced memory deficits in rats and decreased the memory deficits induced by infusion of Aβ(25–35) in mice. SAR110894 also decreased memory deficits observed in the same behavioral test in APPxPS1 transgenic mice (data not shown).

The acute procognitive activity of SAR110894 in THY-Tau22 mice is consistent with a symptomatic effect related to the increased release of several neurotransmitters, such as histamine and acetylcholine, induced by inhibition of the H3R, that have been implicated in the regulation of arousal and cognitive processes [1] because in our study acute treatment with SAR110894 had no effect on tau hyperphosphorylation. In contrast, the improvement in cognitive function after chronic treatment with SAR110894, which was maintained after treatment washout, was correlated with a significant decrease in tau hyperphosphorylation, a reduction in the number of NFTs, and the decreased expression of MIP-1α (a marker of microgliosis and recently proposed to play a role in synaptic plasticity mechanisms involved in learning and memory functions [33]), suggesting a potential disease modifying effect.

Our finding that chronic, but not acute, treatment with SAR110894 decreases tau pathology in THY-Tau22 mice is different from previously published data obtained with another H3R antagonist, ABT-239, which has been shown to decrease tau hyperphosphorylation (AT8-positive cells) in the spinal cord and hippocampus of tau × amyloid precursor protein AD-transgenic mice after 2 weeks of treatment [7]. The reason for these different findings is unclear, but could be because of the different animal models used or to differences in the techniques used to measure tau hyperphosphorylation (i.e., immunohistochemistry vs. Western blot). These technical differences could also account for the different effect of SAR110894 and ABT239 on the activity of GSK3β because ABT-239 increased the levels of pSer9-GSK3β (inactive form of GSK3β) in the cortex of CD1 mice and reversed the reduction in hippocampal

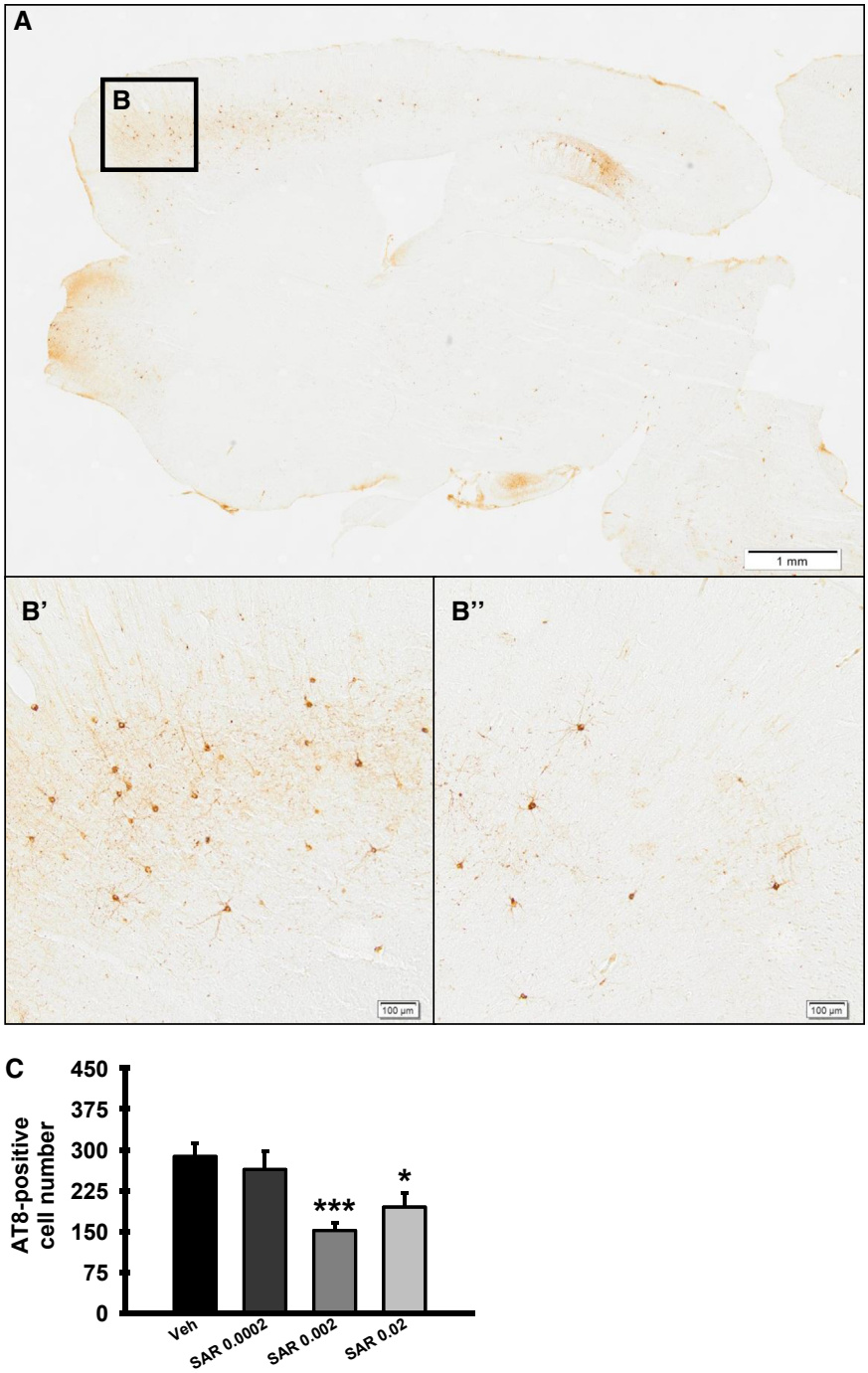

Fig. 5. Chronic oral treatment with SAR110894 during 6 months starting at 2 months decreased the number of AT8-positive cells in the cortex of 8-month-old THY-Tau22 mice. (A) Illustrative images of tau pathology detected by AT8 immunostaining in a representative vehicle-treated animal. Large image in (A) corresponds to sagittal section within medial part of the hemibrain. Delimited subarea (B) over image (A) indicates cortical subarea. B′ and B″ inserts (with higher magnification view): SAR110894 decreases tau hyperphosphorylation in the cortical area of an SAR110894 0.002%-treated animal (B″) versus a vehicle-treated animal (B′). Scale bar = 1 mm (A) or 100 µm (B′, B″). (C) Quantification of decrease in number of AT8-positive cells. AT8 immunostaining is expressed as the mean positive cell bodies count + standard error of the mean. $*P < .05$, $***P \leq .001$, versus THY-Tau22 vehicle (Dunnett tests on log-transformed data). n = 9 mice per group. Veh, vehicle. Doses of SAR110894 (SAR) are expressed in percents.

pSer9-GSK3β levels in Tg2576 (APP) AD-transgenic mice, whereas SAR110894 had no effect on pSer9-GSK3β levels in THY-Tau22 mice.

The fact that SAR110894 has no effect on the level of GSK3β activation and several other kinases implicated in tau phosphorylation, nor it has similar effects on tau phosphorylation from one brain area to another and it does not show clear dose dependencies, suggests that the inhibition of tau pathology after chronic treatment with SAR110894 is not mediated by a direct inhibition of a tau kinase. The

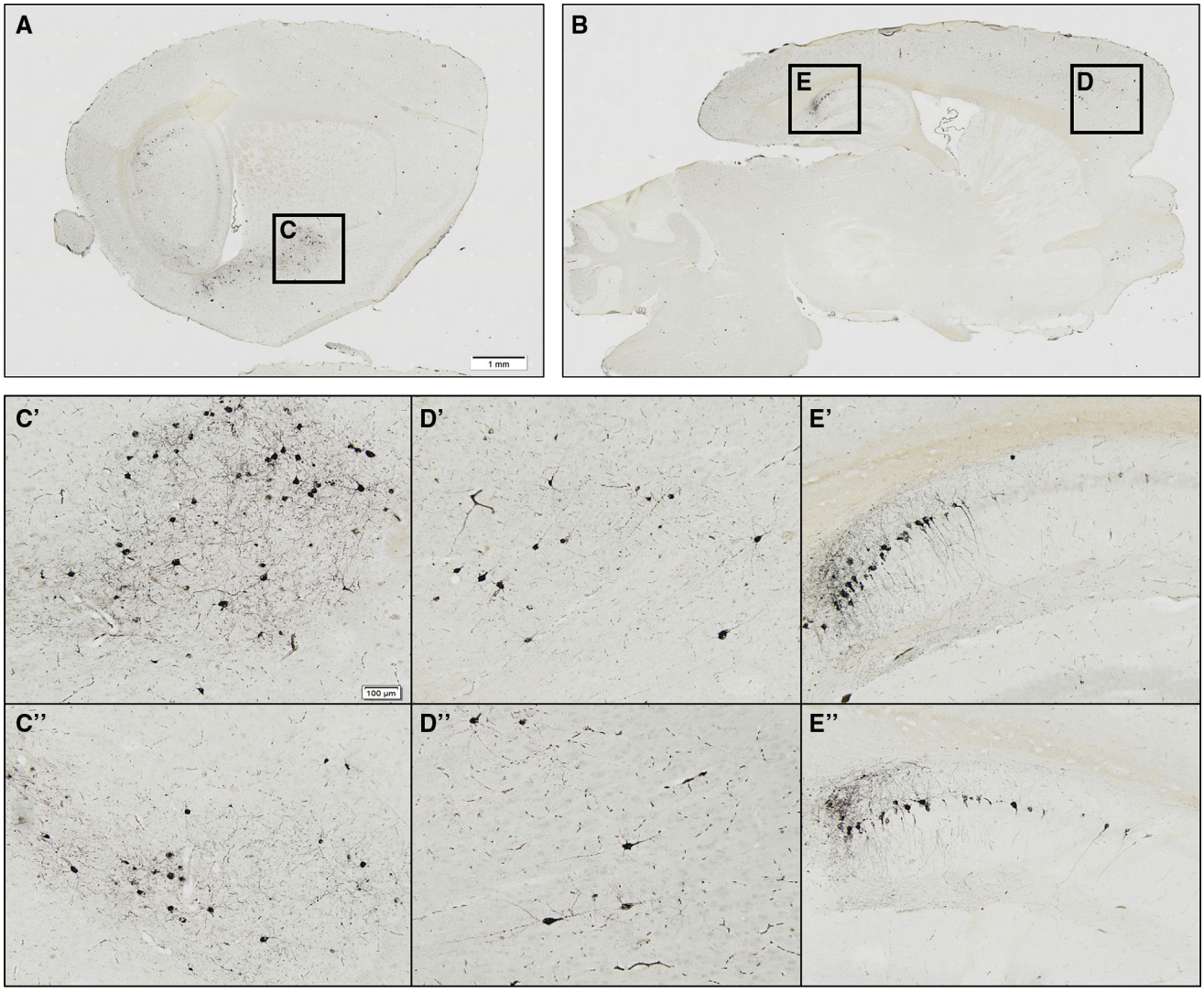

Fig. 6. Chronic oral treatment with SAR110894 during 6 months starting at 2 months decreases the number of neurofibrillary tangles in the cortex. Illustration of tau pathology detected by Gallyas staining in vehicle-treated animals (A, B) corresponds to sagittal sections in the lateral and medial parts of the hemibrain, respectively. Subarea images in (A) and (B) indicate three brain areas with marked tau pathology at the age of 8 months: amygdala (C), cortex (D), and hippocampus (E). (C′–E″) Different inserts (with higher magnification) demonstrate decrease in neurofibrillary tangles induced by SAR110894 in the amygdala (C′ and C″), cortex (D′ and D″), and hippocampus (E′ and E″) of an SAR110894 0.002%-treated animal (C″, D″, E″) versus a vehicle-treated animal (C′, D′, E′). Scale bar = 1 mm (A, B) or 100 µm (C′–E″).

inhibition of tau pathology by SAR110894 could therefore be mediated by an inhibition of a Gαi/o-coupled H3R-regulated intracellular kinases (e.g., ERK1/2, PI3K/Akt, GSK3β, and PKA), which are known to induce tau phosphorylation [2,3]. Moreover, SAR110894 decreases the hyperphosphorylation of tau epitopes that are known to be phosphorylated by H3R-regulated kinases: pSer-214, phosphorylated by PKA; pSer396-pSer404, phosphorylated by GSK3β and ERK1/2. However, at the dose of 0.002%, we did not detect any significant change of the expression and phosphorylation state of different kinases (Akt and Erk1/2) measured by Western blot in cortex. It is also unlikely that inhibition of phosphorylation at any single tau epitope can explain the significant decrease in the number of NFTs observed with SAR110894. In addition, an indirect effect could be mediated by a long-term activation of postsynaptic receptors by evoked release of neurotransmitters, such as histamine, dopamine, and acetylcholine. By its effect on arousal and increased perception of environmental stimuli, SAR110894 could also mimic the effect reported by complex environment experience on the reduction in levels of hyperphosphorylated tau in APPswe/PS1ΔE9 transgenic mice, another model of AD [34].

Over the past years, numerous H3R antagonists have been developed in the clinic for cognitive disorders, including AD, but none reached the late development stage [35–39]. In short, duration trials (4–24 weeks) aimed at demonstrating a symptomatic effect on cognition in patients with mild or

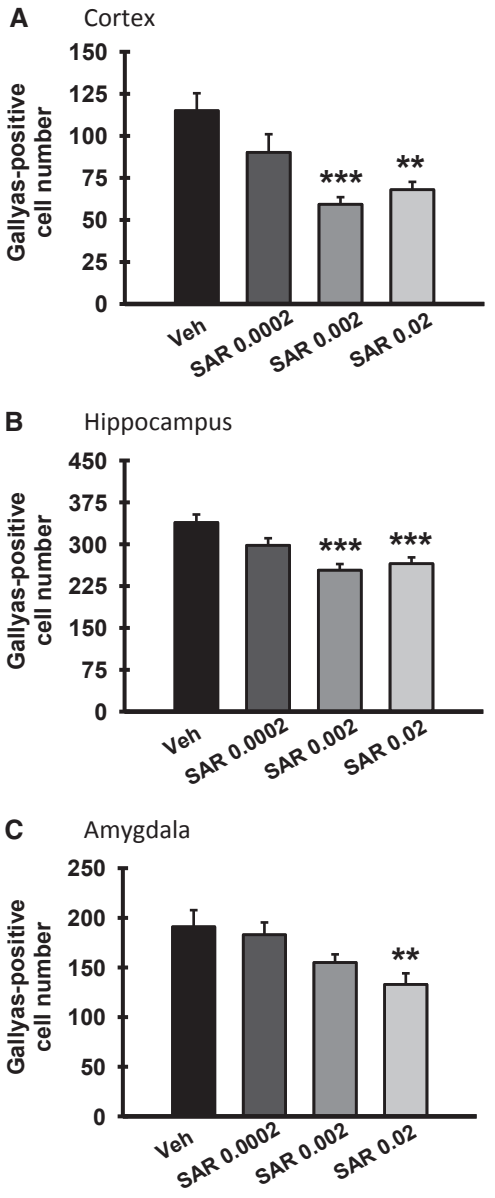

Fig. 7. Quantification of decrease in Gallyas staining in the cortex (A), the hippocampus (B), and the amygdala (C) of 8-month-old THY-Tau22 mice after chronic oral treatment with SAR110894 during 6 months starting at 2 months. Results are expressed as the mean positive cell count + standard error of the mean. $^{**}P < .01$, $^{***}P < .001$ versus THY-Tau22 vehicle (Dunnett tests [on log-transformed data in panel A]). n = 9 mice per group. Veh, vehicle. Doses of SAR110894 (SAR) are expressed in percents.

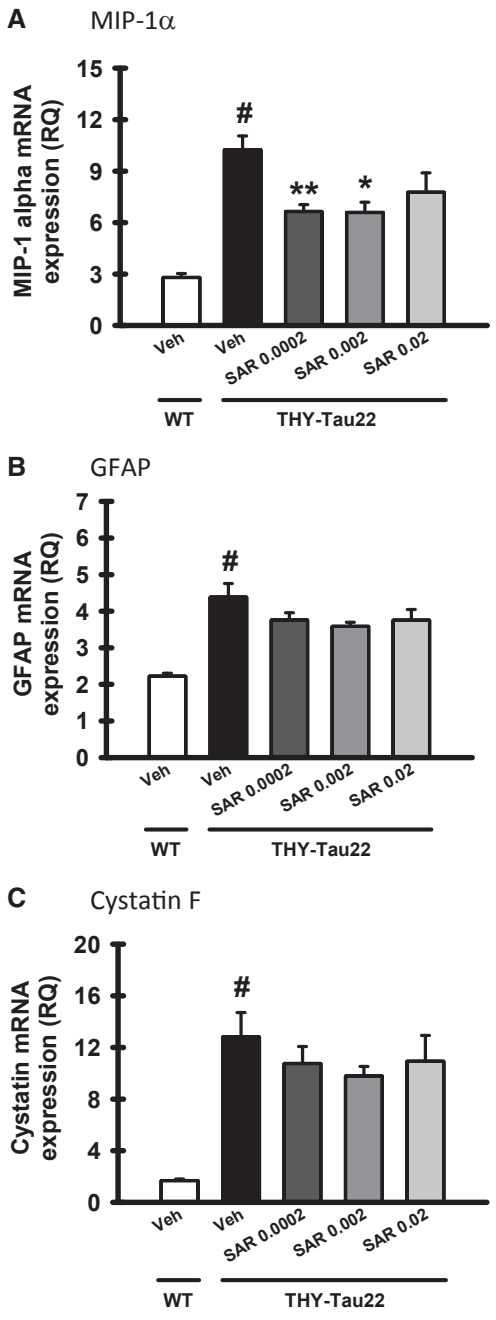

Fig. 8. Chronic oral treatment with SAR110894 during 6 months starting at 2 months reduced the expression of MIP-1α (A), a marker of microgliosis in the hippocampus of 8-month-old THY-Tau22 mice. GFAP (B) and cystatin (C) levels were not modified by the treatment. qPCR quantifications are expressed as the means +standard error of the mean. $^{\#}P < .01$ versus WT vehicle (Wilcoxon), $^{*}P < .05$, $^{**}P < .01$ versus THY-Tau22 vehicle (Wilcoxon tests with Bonferroni-Holm correction). n = 7 to 8 mice per group. MIP-1α, macrophage inflammatory protein 1-alpha; mRNA, messenger RNA; RQ, respiratory quotient; Veh, vehicle; WT, wild-type. Doses of SAR110894 (SAR) are expressed in percents.

moderate AD, no real and sustained evidence of efficacy was demonstrated despite robust preclinical procognitive profile. The reporting of dose-dependent sleep disturbances was frequent across trials. The effects occurred generally early in treatment and varied in intensity and duration. Although the H3R antagonists notably differ in their PK parameters and safety profile, they are reported as safe and generally well tolerated. Only long duration trials would allow assessing whether these molecules have anti-tau/disease-modifying properties.

In conclusion, we showed that chronic administration of SAR110894 in a transgenic mouse model of tauopathy prevents cognitive deficits and inhibits tau pathology. These data suggest that H3R antagonists could have disease-modifying activity in patients with tauopathies.

Acknowledgments

The authors thank Marie-Dominique Bock, Dominique Briet, Pascal Chaillou, Anne-Laure Coutant, Elisabeth Genet, Jérémy George, Philippe Goniot, Stéphanie Hamon, Patrick Juvet, Joëlle Le Guern, Jocelyne Lolivier, Nicolas Moindrot, Alain Roccon, Sandrine Roche, and Sophie Vivier for their excellent technical assistance and collaboration; Bertrand Monneau and Luc Esserméant for the review of the statistical analysis; and Isabelle Clavier and Bill Hurst for helpful discussions in the preparation of this manuscript. Pascal Barnéoud, Véronique Blanchard-Brégeon, Philippe Delay-Goyet, Véronique Mary, Jean Ménager, Mati Lopez-Grancha, Thomas Rooney, and Nathalie Schussler are coinventors on a patent application related to the use of an H3R antagonist for the treatment of Alzheimer's disease (PCT/EP2013/057241).

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.trci.2016.10.002.

RESEARCH IN CONTEXT

1. Systematic review: We reviewed PubMed literature sources. Only one study has reported that a histamine H3-receptor antagonist inhibits tau phosphorylation in a transgenic mouse model of Alzheimer's Disease. Data on the other main hallmark of tauopathies, neurofibrillary tangles, and the demonstration of a possible functional benefit have not been previously shown.
2. Interpretation: Our findings provide evidence that chronic treatment with a new H3-receptor antagonist in a preclinical model of tauopathy reduces the formation of neurofibrillary tangles and microgliosis, in addition to the already reported reduction in tau hyperphosphorylation, and these effects are accompanied by prevention of cognitive impairment, suggesting a potential disease modifying activity.
3. Future directions: The article suggests that chronic treatment with H3-receptor antagonists could be beneficial in tauopathies. The article also provides an interest to conduct additional investigations to confirm this potential therapeutic use.

References

[1] Brioni JD, Esbenshade TA, Garrison TR, Bitner SR, Cowart MD. Discovery of histamine H3 antagonists for the treatment of cognitive disorders and Alzheimer's disease. J Pharmacol Exp Ther 2011;336:38–46.

[2] Bongers G, Sallmen T, Passani MB, Mariottini C, Wendelin D, Lozada A, et al. The Akt/GSK-3β axis as a new signaling pathway of the histamine H3 receptor. J Neurochem 2007;103:248–58.

[3] Mariottini C, Scartabelli T, Bongers G, Arrigucci S, Nosi D, Leurs R, et al. Activation of the histaminergic H3 receptor induces phosphorylation of the Akt/GSK-3β pathway in cultured cortical neurons and protects against neurotoxic insults. J Neurochem 2009;110:1469–78.

[4] Wagner U, Utton M, Gallo JM, Miller CC. Cellular phosphorylation of tau by GSK-3β influences tau binding to microtubules and microtubule organisation. J Cell Sci 1996;109:1537–43.

[5] Augustinack JC, Schneider A, Mandelkow EM, Hyman BT. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. Acta Neuropathol 2002;103:26–35.

[6] Sergeant N, Delacourte A, Buée L. Tau protein as a differential biomarker of tauopathies. Biochim Biophys Acta 2005;1739:179–97.

[7] Bitner RS, Markosyan S, Nikke AL, Brioni JD. In-vivo histamine H3 receptor antagonism activates cellular signaling suggestive of symptomatic and disease modifying efficacy in Alzheimer's disease. Neuropharmacology 2011;60:460–6.

[8] Griebel G, Pichat P, Pruniaux MP, Beeské S, Lopez-Grancha M, Genet E, et al. SAR110894, a potent histamine H3-receptor antagonist, displays procognitive effects in rodents. Pharmacol Biochem Behav 2012;102:203–14.

[9] Schindowski K, Bretteville A, Leroy K, Bégard S, Brion JP, Hamdane M, et al. Alzheimer's disease-like tau neuropathology leads to memory deficits and loss of functional synapses in a novel mutated tau transgenic mouse without any motor deficits. Am J Pathol 2006; 169:599–616.

[10] Belarbi K, Burnouf S, Fernandez-Gomez FJ, Laurent C, Lestavel S, Figeac M, et al. Beneficial effects of exercise in a transgenic mouse model of Alzheimer's disease-like Tau pathology. Neurobiol Dis 2011;42:486–94.

[11] Burnouf S, Martire A, Derisbourg M, Laurent C, Belarbi K, Leboucher A, et al. NMDA receptor dysfunction contributes to impaired brain-derived neurotrophic factor-induced facilitation of hippocampal synaptic transmission in a Tau transgenic model. Aging Cell 2013;12:11–23.

[12] Leboucher A, Laurent C, Fernandez-Gomez FJ, Burnouf S, Troquier L, Eddarkaoui S, et al. Detrimental effects of diet-induced obesity on τ pathology are independent of insulin resistance in τ transgenic mice. Diabetes 2013;62:1681–8.

[13] Troquier L, Caillierez R, Burnouf S, Fernandez-Gomez FJ, Grosjean M-E, Zommer N, et al. Targeting phospho-Ser422 by active Tau immunotherapy in the THYTau22 mouse model: a suitable therapeutic approach. Curr Alzheimer Res 2012; 9:397–405.

[14] Laurent C, Eddarkaoui S, Derisbourg M, Leboucher A, Demeyer D, Carrier S, et al. Beneficial effects of caffeine in a transgenic model of Alzheimer's disease-like Tau pathology. Neurobiol Aging 2014; 35:2079–90.

[15] Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav Brain Res 1988;31:47–59.

[16] Planel E, Richter KE, Nolan CE, Finley JE, Liu L, Wen Y, et al. Anesthesia leads to tau hyperphosphorylation through inhibition of phosphatase activity by hypothermia. J Neurosci 2007;27:3090–7.

[17] Sun A, Nguyen XV, Bing G. Comparative analysis of an improved thioflavin-S stain, Gallyas silver stain, and immunohistochemistry for neurofibrillary tangle demonstration on the same sections. J Histochem Cytochem 2002;50:463–72.

[18] Braak H, Braak E. Demonstration of amyloid deposits and neurofibrillary changes in whole brain sections. Brain Pathol 1991;1:213–6.

[19] Goedert M, Jakes R, Vanmechelen E. Monoclonal antibody AT8 recognises tau protein phosphorylated at both serine 202 and threonine 205. Neurosci Lett 1995;189:167–70.

[20] Lo AC, Iscru E, Blum D, Tesseur I, Callaerts-Vegh Z, Buée L, et al. Amyloid and Tau neuropathology differentially affect prefrontal

synaptic plasticity and cognitive performance in mouse models of Alzheimer's disease. J Alzheimers Dis 2013;37:109–25.

[21] Barker GR, Bird F, Alexander V, Warburton EC. Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. J Neurosci 2007;27:2948–57.

[22] Barker GR, Warburton EC. Evaluating the neural basis of temporal order memory for visual stimuli in the rat. Eur J Neurosci 2011; 33:705–16.

[23] Bussey TJ, Muir JL, Aggleton JP. Functionally dissociating aspects of event memory: the effects of combined perirhinal and postrhinal cortex lesions on object and place memory in the rat. J Neurosci 1999; 19:495–502.

[24] Ennaceur A, Neave N, Aggleton JP. Neurotoxic lesions of the perirhinal cortex do not mimic the behavioural effects of fornix transection in the rat. Behav Brain Res 1996;80:9–25.

[25] Mumby DG, Pinel JP. Rhinal cortex lesions and object recognition in rats. Behav Neurosci 1994;108:11–8.

[26] Norman G, Eacott MJ. Impaired object recognition with increasing levels of feature ambiguity in rats with perirhinal cortex lesions. Behav Brain Res 2004;148:79–91.

[27] Winters BD, Forwood SE, Cowell RA, Saksida LM, Bussey TJ. Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. J Neurosci 2004;24:5901–8.

[28] Barker GR, Warburton EC. When is the hippocampus involved in recognition memory? J Neurosci 2011;31:10721–31.

[29] Bussey TJ, Duck J, Muir JL, Aggleton JP. Distinct patterns of behavioural impairments resulting from fornix transection or neurotoxic lesions of the perirhinal and postrhinal cortices in the rat. Behav Brain Res 2000;111:187–202.

[30] Ma J, Tanaka KF, Shimizu T, Bernard CC, Kakita A, Takahashi H, et al. Microglial cystatin F expression is a sensitive indicator for ongoing demyelination with concurrent remyelination. J Neurosci Res 2011;89:639–49.

[31] Van der Jeugd A, Ahmed T, Burnouf S, Belarbi K, Hamdame M, Grosjean ME, et al. Hippocampal tauopathy in tau transgenic mice coincides with impaired hippocampus-dependent learning and memory, and attenuated late-phase long-term depression of synaptic transmission. Neurobiol Learn Mem 2011;95:296–304.

[32] Van der Jeugd A, Vermaercke B, Derisbourg M, Lo AC, Hamdane M, Blum D, et al. Progressive age-related cognitive decline in Tau mice. J Alzheimers Dis 2013;37:777–88.

[33] Marciniak E, Faivre E, Dutar P, Alves Pires C, Demeyer D, Caillierez R, et al. The chemokine MIP-1α/CCL3 impairs mouse hippocampal synaptic transmission, plasticity and memory. Sci Rep 2015; 5:15862.

[34] Hu YS, Xu P, Pigino G, Brady ST, Larson J, Lazarov O. Complex environment experience rescues impaired neurogenesis, enhances synaptic plasticity, and attenuates neuropathology in familial Alzheimer's disease-linked APPswe/PS1DeltaE9 mice. FASEB J 2010; 24:1667–81.

[35] Nathan PJ, Boardley R, Scott N, Berges A, Maruff P, Sivananthan T, et al. The safety, tolerability, pharmacokinetics and cognitive effects of GSK239512, a selective histamine H_3 receptor antagonist in patients with mild to moderate Alzheimer's disease: a preliminary investigation. Curr Alzheimer Res 2013; 10:240–51.

[36] Hannesdottir K, Minkwitz M, Raudibaugh K, Vis P, Ackaert O, Alexander R. A phase II safety and tolerability study to investigate the effect of AZD5213 on sleep in subjects with mild cognitive impairment and mild Alzheimer's disease. Alzheimers Dement 2013; 9(Suppl):P891–2.

[37] Jones R, Vellas B, Gauthier S, Cognet B, Baurand A, Schneble HM, et al. Two phase IIb trials of S 38093 in Alzheimer's Disease patients at mild to moderate stage. J Prev Alzheimers Dis 2015;2:282–3.

[38] Stemmelin J, Kirkesseli S, Martincova R, Bejuit R, Corp-dit-Genti V, Morrison G, et al. A phase 2 study to investigate the effects of SAR110894 on cognition, daily function, apathy and sleep in mild to moderate Alzheimer's disease patients. J Nutr Health Aging 2013; 17:804–5.

[39] Haig GM, Pritchett Y, Meier A, Othman AA, Hall C, Gault LM, et al. A randomized study of H3 antagonist ABT-288 in mild-to-moderate Alzheimer's dementia. J Alzheimers Dis 2014; 42:959–71.