NeuroReport

Optimized NMDA receptor antagonist exhibits hippocampal pro-neurogenic effects in aged SAMP8 mice

--Manuscript Draft--

Manuscript Number:	NR-D-22-00397R1
Full Title:	Optimized NMDA receptor antagonist exhibits hippocampal pro-neurogenic effects in aged SAMP8 mice
Article Type:	Original paper
Keywords:	senescence; cognitive decline; neuroinflammation
Corresponding Author:	Merce Pallas, PhD University of Barcelona Barcelona, Barcelona SPAIN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Barcelona
Corresponding Author's Secondary Institution:	
First Author:	Júlia Companys-Alemany, PhD
First Author Secondary Information:	
Order of Authors:	Júlia Companys-Alemany, PhD
	Joan Roig-Soriano
	Andreea L. Turcu
	Miguel Chillón
	Santiago Vázquez
	Christian Griñán-Ferré
	Merce Pallas, PhD
Order of Authors Secondary Information:	
Abstract:	Objectives: NMDA receptor antagonists mediate adult neurogenic effects. Here, the neurogenic effect of a new NMDA receptor antagonist endowed with neuroprotective effects in AD mice model. Methods: 9-month-old SAMP8 with UB-ALT-EV were orally treated. 5-Bromo-2-deoxyuridine (BrdU) (50 mg/Kg) was 3x injected I.P. every 2 hours. Results: After 28 days of treatment, SAMP8-treated group improved working memory. Moreover, the number of BrdU + cells and DCX + cells in the SAMP8 dentate gyrus (DG) was significantly increased. GFAP + cells were not affected by treatment. Conclusions: Together, these results provided evidence that UB-ALT-EV promotes the survival and proliferation of neural progenitor cells in the aged SAMP8 hippocampus.

Section Editor's evaluation:

This is a re-review of a previously reviewed manuscript. While the authors responded to many comments of this reviewer in a satisfactory manner, there are still some outstanding issues:

(1) While the authors describe their light;dark cycle, they do not specify the start for the light phase so it is unclear whether the light:dark cycle was normal or reversed. Very important!

The authors thank the reviewer for the comment. Therefore we have modified that statement.
(Page 3 Lines 17-18): *"temperature conditions (22 ± 2 ºC) and 12h:12h (300lux/0lux) light/dark cycles (turn on the light at 8AM, turn off at 8PM)."*

(2) Since the dosage of UB-ALT-EV was measured through determining the volume the mice drank, there should be some range as it is statistically impossible that the mice would drink all the same volumes resulting in identical concentrations. So the authors may wish to represent the range based on the volumes measured or a table that would provide essentially the same information but can add a n average volume drank by individual groups and comparison in between.

The authors thank the reviewer for noticing this. The average volume of water consumed by the animals was approximately 5,5 ml/day per animal for all groups. Taking into account the volume drunk per week, the treatments were adjusted accordingly. We have clarified this information in the manuscript.

(Page 3 Lines 18-21): *"Consumption of water and weight of the animals were monitored every week; the daily average water consumption was 5 ml/day per animal showing no significant variation between groups. The drug concentration was accordingly adjusted every week based on the water consumed."*

(3) Region of interest in the hippocampal DG should be precisely defined, such as this example. We counted all tagged cells in the hilus of the DG bordered by the granule cell layer and a straight connection between the two ends of the granule cell layer. We used every 10th section starting from the beginning of corpus callosum (section counted as 1)-- or similar corresponding to your procedure. Anybody reading the manuscript should be able to reproduce the procedures

We agree with the reviewer and have accordingly added this information to the manuscript.
(Page 4 Line 38-42): *"We counted all labelled cells in the hilus of the DG bordering the granule cell layer and a straight connection between the two ends of the granule cell layer. Every 5th section from the beginning of the corpus callosum was used. Finally, the number of positive cells for each of the staining to the dentate gyrus area was quantified and the average amount per animal was obtained."*

Optimized NMDA receptor antagonist exhibits hippocampal pro-neurogenic effects in aged SAMP8 mice

Júlia Companys-Alemany[1*], Joan Roig-Soriano[2*], Andreea L. Turcu[6], Miguel Chillón[2,3,4,5], Santiago Vázquez[6], Mercè Pallàs[1] and Christian Griñán-Ferré[1]

[1]Pharmacology Section, Department of Pharmacology, Toxicology, and Therapeutic Chemistry. Faculty of Pharmacy and Food Sciences, Institut de Neurociències, Universitat de Barcelona (UB), Av. Joan XXIII 27-31, 08028 Barcelona, Spain.

[2]Institut de Neurociènces (INc), Department of Biochemistry and Molecular Biology, Universitat Autònoma Barcelona, Av. Vall Moronta s/n, 00193 Bellaterra, Spain.

[3]Vall d'Hebron Institut de Recerca (VHIR) Passeig de la Vall d'Hebron 119-129, 08035 Barcelona, Spain.

[4]Unitat producció de Vectors (UPV), Universitat Autònoma Barcelona, Av. Vall Moronta s/n, 00193 Bellaterra, Spain.

[5]Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluis Companys 23 08010 Barcelona, Spain.

[6]Laboratory of Medicinal Chemistry (CSIC Associated Unit), Department of Pharmacology, Toxicology, and Therapeutic Chemistry. Faculty of Pharmacy and Food Sciences and Institute of Biomedicine (IBUB), University of Barcelona, Av. Joan XXIII, 27-31, 08028 Barcelona, Spain

*First co-authors

Corresponding author: Christian Griñán-Ferré

e-mail: christian.grinan@ub.edu

Running title: NMDA receptor antagonist pro-neurogenesis in SAMP8

Abstract

Objectives: NMDA receptor antagonists mediate adult neurogenic effects. Here, the neurogenic effect of a new NMDA receptor antagonist endowed with neuroprotective effects in AD mice model.

Methods: 9-month-old SAMP8 with UB-ALT-EV were orally treated. 5-Bromo-2-deoxyuridine (BrdU) (50 mg/Kg) was 3x injected I.P. every 2 hours.

Results: After 28 days of treatment, SAMP8-treated group improved working memory. Moreover, the number of $BrdU^+$ cells and DCX^+ cells in the SAMP8 dentate gyrus (DG) was significantly increased. $GFAP^+$ cells were not affected by treatment.

Conclusions: Together, these results provided evidence that UB-ALT-EV promotes the survival and proliferation of neural progenitor cells in the aged SAMP8 hippocampus.

Keywords: senescence, cognitive decline, neuroinflammation

Introduction

Neurogenesis is the process by which new neurons are generated throughout embryonic brain development [1]. In particular, evidence has shown that during adulthood, the so-called adult neurogenesis occurs in two brain areas, in the anterior part of the subventricular zone and the dentate gyrus (DG) of the hippocampus, due to the presence of neural stem cells (NSCs) [2; 3]. During ageing, alterations occur in the neurogenic process [4; 5], which can be aggravated by neurodegenerative diseases such as Alzheimer's disease (AD) [6; 7]. Several studies in aged mice indicate that newly generated cells develop at a slower rate [8], and differentiate less into neurons and more into astrocytes [9]. However, the reason behind this neurogenic alteration is still largely unknown [10].

The N-methyl-D-aspartate (NMDA) receptors have an important role in neuronal function and learning and memory process [11]. It has been established that the survival of neurons born in the adult brain is regulated by neuronal activity in the DG via NMDA receptor-dependent long term potentiation [11]. However, overactivation of NMDA receptors during Alzheimer's disease is associated with neuronal loss and cognitive decline [12]. In fact, memantine, an NMDA receptor antagonist, has been shown to enhance cognitive decline through promoting proliferation in the adult mice's DG and subventricular zone[13–15].

Adult neurogenesis plays an essential role in maintaining neuronal functions such as learning and memory [16]. For this reason, therapies focused on boosting neurogenesis have been proposed as a strategy to reduce the progression of AD [17]. In fact, it has been observed that in a well-established AD mice model with cognitive decline, the senescence-accelerated mouse prone 8 (SAMP8) [18], by using neurogenesis-inducing agents, the microenvironment of the neurogenic niche can be regulated, rescuing the number of stem cells and thus reversing the pathological neurogenic phenotype [10]. Particularly, the SAMP8 model at young ages shows a considerable increase in neurogenesis compared to the healthy control mouse with normal aging and intact

cognitive function, the senescence-accelerated mouse resistant 1 (SAMR1) animals [19] that has been suggested to be a mechanism to deal with pathological alterations in the brain, such as oxidative stress, inflammation and activation of neuronal death pathways, mainly affecting the cerebral cortex and hippocampus [18; 20]. However, SAMP8 mice exhibit a severe decrease in neurogenesis with the progression of senescence [19]. For this reason and given the therapeutic potential of neurogenesis inducers during neurodegenerative processes, we decided to evaluate the effect of the new NMDA receptor antagonist UB-ALT-EV [21] an optimized NMDA antagonist that has exhibited neuroprotective effects in *Caenorhabditis elegans* and a transgenic AD mouse model on neurogenesis in the DG of the hippocampus in aged SAMP8 mice.

Methods

9-month-old SAMR1 and SAMP8 mice were randomly divided into three groups (n=31): SAMR1 (n=10), SAMP8 (n=11) and SAMP8 treated with UB-ALT-EV (n=10). The animals had free access to food and water and were maintained under standard temperature conditions (22 ± 2 ºC) and 12h:12h (300lux/0lux) light/dark cycles (turn on the light at 8AM, turn off at 8PM). Consumption of water and weight of the animals were monitored every week; the daily average water consumption was 5 mL/day per animal showing no significant variation between groups. The drug concentration was accordingly adjusted every week based on the water consumed. Control groups were administered with 1.8% 2-hydroxypropyl-b-cyclodextrin. The compound UB-ALT-EV, previously designed and *in vitro* and *in vivo* tested as a new memantine analog, synthetized by us according previous reports [21], was dissolved in 1.8% 2-hydroxypropyl-b-cyclodextrin and mice were administered a dose of 5mg/Kg/day through drinking water for 28 days until euthanasia **(Fig. 1A)**. The studies were approved by the Ethical Committee on Animal Experimentation (ECAE) of the Universitat de Barcelona.

Novel object recognition tests (NORT) is a cognitive test used to evaluate short- and long-term recognition memory. The apparatus consists of a black polyvinyl chloride L-maze with two arms measuring 25 x 20 x 5 cm with a 90° orientation. The objects to discriminated were plastic and had no biting parts. The test was carried out for 5 days. On the first three days, the animals were placed at the center of the L-maze and individually habituated to the apparatus for 10 min each day. On the fourth day, the animals were exposed individually for 10 min to the apparatus and allowed to freely explore the area inside the apparatus (familiarization phase) where we had placed two identical objects (A+A or B+B) at the end of each arm. Two hours later, the first test (short-term memory) was performed. In this second phase, objects A and B were swapped (A+B or B+A) and mice were allowed to explore the maze for 10 min. Twenty-four hours after the first trial, the animals were again exposed to the apparatus, and in this case, objects A and B were replaced by two new objects with different shapes and colors (A+C or B+C), and the animals were allowed to explore them for 10 minutes **(Fig. 1B)**. The exploration time of the new object (TN) and the old object (TO) were measured.

Exploration of an object was defined as the time spent by the rodent pointing its nose towards the object at 2 cm and/or touching it with its nose. To avoid object preference biases, objects A and B were counterbalanced. Finally, to quantify cognitive function, the discrimination index (DI), defined as (TN-TO)/(TN+TO), was calculated.

Immunostaining experiments were performed as follows. All mice groups were injected with a 50 mg/Kg solution of 5-bromo-deoxyuridine (BrdU) and NaCl at 2-hour intervals, three times on the first day treatment. The animals were behavioral tested after 23 days of BrdU injection and euthanazied 28 days after injection of BrdU under Ketamine (100 mg/Kg, i.p.) and xylazine (10 mg/Kg, i.p.) anesthesia. Afterwards, mice were perfused intracardially with a phosphate buffer saline (PBS) solution followed by a 4% paraformaldehyde (PFA) solution diluted in 0.1 M PBS. The brains were then fixed in 4% PFA overnight at 4 °C. Subsequently, the brains were transferred into a solution of 4% PFA and 15% sucrose. Finally, the brains were frozen on dry ice and stored at -80 °C. Coronal brain sections of 30 μm were cut through a cryostat (Leica Microsystems CM 3050S cryostat, Wetzlar, Germany) and stored in cryoprotectant solution at -20 °C. For staining, sections were washed with three rounds of 5 min incubations in PBS. Next, an antigen retrieval was done by incubating the samples in 2N HCl at 37ºC for 20 min, followed by 10 min incubation in a Sodium borate solution (0.1M ph 8.5). After three rounds of washes with PBS (5 min each), sections were permeabilized in 0.2% PBS-Triton buffer for 5 min, followed by a one-hour blocking with 10% bovine serum albumin (BSA) in PBS with 0.2% Triton (PBST). Incubation of the primary antibody was performed at 4 °C for 48 hours. Three rounds of 10 min washes were performed with PBST at room temperature, followed by an overnight incubation at room temperature of the secondary antibody. The samples were then washed in three 10 min rounds followed by a 5 min incubation with Hoescht at a dilution of 1:500. Finally, the sections were washed and mounted with Fluoromount. Primary antibodies used were: BrdU (Abcam/Ab6326; at 1:800 dilution), Doublecortin (DCX, Abcam/Ab153668; at 1:200 dilution), glial fibrillar acidic protein (GFAP, Dako/GA524; at 1: 800), and the secondary antibodies used for the detection were, respectively, Alexa Fluor® 647 anti-rat (A-21247, Invitrogen; at 1:1000 dilution), Alexa Fluor® 488 anti-chicken (A11039, Invitrogen; at 1:1000 dilution) and Alexa Fluor® 568 anti-rabbit (A11011, Molecular Probes; at 1:500 dilution). These markers were selected because of being indicative of neuronal progenitors maturation (DCX) or the implication in the maintenance of the neurogenic niche (GFAP). Images were obtained with a Zeiss LSM880 high-speed spectral confocal microscope. Images were analyzed with ImageJ software and Imaris Microscopy Image Analysis Software. At least 5 images of the region of interest (ROI) of 5 individuals per group were analyzed. We counted all labelled cells in the hilus of the DG bordering the granule cell layer and a straight connection between the two ends of the granule cell layer. Every 5^{th} section from the beginning of the corpus callosum was used. Finally, the number of positive cells for each of the staining to the dentate gyrus area was quantified and the average amount per animal was obtained. Data acquisition and statistical analysis of data was performed using GraphPad Prism software. Data is expressed as median ± standard error of the median (SEM) of at least 5 samples per group. Statistical analysis was

performed by two-tailed Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's post-hoc analysis. Statistical significance was defined as p-value <0.05. Outliers were determined with the Grubbs test and, when necessary, removed from the analysis. The analysis of cognitive parameters was performed blindly by the experimenter

Results and discussion

It is well known that the SAMP8 strain presents age-related cognitive decline [22]. Thus, we assessed the cognitive performance by NORT, a widely used task to investigate the effects of compounds in different stages of memory formation [23]. As expected, the results revealed a significant decrease in the DI of the SAMP8 group compared to the SAMR1 mice (healthy control) group (**Fig. 1C-D**). Interestingly, the compound UB-ALT-EV showed a beneficial effect on both short- and long-term memories in the aged SAMP8 animals, indicating the beneficial effects of the UB-ALT-EV (**Fig. 1C-D**).

Then, the improvement in recognition memory induced by treatment with UB-ALT-EV once again demonstrates the therapeutic potential of the new compound on cognition, this time observed in aged SAMP8 mice. Because defects in hippocampal neurogenesis appear during and are associated with neurodegenerative disease such as AD [6], and considering the evidence demonstrated by different NMDA receptor antagonists as inducers of neurogenesis [13; 14; 24], we assessed cell survival in the DG by BdrU as well as doublecortin (DCX) immunostaining in SAMP8 treated with UB-ALT-EV.

Firstly, BrdU-positive cells were counted in the hippocampus of SAMR1, SAMP8 and SAMP8 mice treated with UB-ALT-EV. Previous studies indicated that SAMP8 display an accelerated depletion of the adult hippocampal NSC [10]. Accordingly, our results showed that SAMP8 mice presented a 50% reduction of BrdU-positive cells compared to the age-matched SAMR1 mice group. Interestingly, UB-ALT-EV treatment significantly increased cell survival in the treated SAMP8 animals (**Fig. 2**). It is worth noting that the number of BrdU-positive cells in the aged groups of rodents was extremely low, which makes the positive effect of our compound on survival rate particularly relevant.

Afterwards, we evaluated the number of neurons in the maturation process using the doublecortin (DCX), an immature neuronal marker [25]. In line with the BrdU results, aged SAMP8 animals showed a 50% decrease in this neural proliferation marker expression, indicating the loss of the stem cell population in this strain. By contrast, SAMP8 mice treated with the compound UB-ALT-EV had a therapeutic increase of 30% in proliferating cells compared to the SAMP8 group, suggesting the neuronal proliferation-induced effect of the new compound (**Fig. 3**). Another explanation of this results is that UB-ALT-EV protects all neurons, including the newly born and not not uniquely by inducing neurogenesis.

Secondly, as previously described, we found a decrease in GFAP marker levels at the dentate gyrus of 10-month-old SAMP8 mice compared to SAMR1 mice (Fig. 4)[19], which was not reversed by UB-ALT-EV treatment. In this regard, it has been suggested that this decrease in astrocytic activation in SAMP8 animals could be responsible for the impairment of the neurogenic niche during senescence [19]. In our case, the advanced age of the animals could be the cause of the lack of effect of the compound UB-ALT-EV.

Overall, our results are very encouraging and suggest a neurogenesis-inducing effect of the new compound UB-ALT-EV, in advanced senescent SAMP8 mice, which did not has been tested previously.

Fundings

This study was supported by *Spanish Ministerio de Economía y Competitividad* (PID2019-106285/AEI/10.13039/501100011033; PDC2021-121096/AEI/10.13039/501100011033), María de Maeztu Unit of Excellence (Institute of Neurosciences, University of Barcelona) MDM- 2017- 0729 and 2017SGR106 (AGAUR, Catalonia). J.C-A acknowledges *Spanish Ministerio de Economía y Competitividad* for her FPI (MDM-2017-0729) fellowship. ALT acknowledges a PhD fellowship (FPU grant) from the *Spanish Ministerio de Educación, Cultura y Deporte*.

Competing interests: The authors declare no competing interests.

References

1. Götz M, Nakafuku M, Petrik D. Neurogenesis in the Developing and Adult Brain-Similarities and Key Differences. *Cold Spring Harb. Perspect. Biol.* 2016; **8** (**7**): a018853.
2. Ihrie RA, Álvarez-Buylla A. Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain. *Neuron* 2011; **70** (**4**): 674–686.
3. Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. *Cell* 2008; **132** (**4**): 645–660.
4. Lee SW, Clemenson GD, Gage FH. New neurons in an aged brain. *Behav. Brain Res.* 2012; **227** (**2**): 497–507.
5. Luo J, Daniels SB, Lennington JB, Notti RQ, Conover JC. The aging neurogenic subventricular zone. *Aging Cell* 2006; **5** (**2**): 139–152.
6. Lazarov O, Marr RA. Neurogenesis and Alzheimer's disease: at the crossroads. *Exp. Neurol.* 2010; **223** (**2**): 267–281.
7. Mu Y, Gage FH. Adult hippocampal neurogenesis and its role in Alzheimer's disease. *Mol. Neurodegener.* 2011; **6** (**1**): 85.
8. Trinchero MF, Buttner KA, Sulkes Cuevas JN, Temprana SG, Fontanet PA, Monzón-Salinas MC, et al. High Plasticity of New Granule Cells in the Aging Hippocampus. *Cell Rep.* 2017; **21** (**5**): 1129–1139.
9. Encinas JM, Michurina T V, Peunova N, Park J-H, Tordo J, Peterson DA, et al.

Division-coupled astrocytic differentiation and age-related depletion of neural stem cells in the adult hippocampus. *Cell Stem Cell* 2011; **8** (**5**): 566–579.
10. Díaz-Moreno M, Armenteros T, Gradari S, Hortigüela R, García-Corzo L, Fontán-Lozano Á, et al. Noggin rescues age-related stem cell loss in the brain of senescent mice with neurodegenerative pathology. *Proc. Natl. Acad. Sci. U. S. A.* 2018; **115** (**45**): 11625–11630.
11. Åmellem I, Yovianto G, Chong HT, Nair RR, Cnops V, Thanawalla A, et al. Role of NMDA Receptors in Adult Neurogenesis and Normal Development of the Dentate Gyrus. *eNeuro* 2021; **8** (**4**): ENEURO.0566-20.2021.
12. Doraiswamy PM. Alzheimer's disease and the glutamate NMDA receptor. *Psychopharmacol. Bull.* 2003; **37** (**2**): 41–49.
13. Hirasawa T, Wada H, Kohsaka S, Uchino S. Inhibition of NMDA receptors induces delayed neuronal maturation and sustained proliferation of progenitor cells during neocortical development. *J. Neurosci. Res.* 2003; **74** (**5**): 676–687.
14. Nacher J, Alonso-Llosa G, Rosell DR, McEwen BS. NMDA receptor antagonist treatment increases the production of new neurons in the aged rat hippocampus. *Neurobiol. Aging* 2003; **24** (**2**): 273–284.
15. Nacher J, Rosell DR, Alonso- Llosa G, McEwen BS. NMDA receptor antagonist treatment induces a long- lasting increase in the number of proliferating cells, PSA- NCAM- immunoreactive granule neurons and radial glia in the adult rat dentate gyrus. *Eur. J. Neurosci.* 2001; **13** (**3**): 512–520.
16. Yau S, Li A, So K-F. Involvement of Adult Hippocampal Neurogenesis in Learning and Forgetting. *Neural Plast.* 2015; **2015**: 717958.
17. Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. *Lancet* 2006; **368** (**9533**): 387–403.
18. Morley JE, Farr SA, Kumar VB, Armbrecht HJ. The SAMP8 mouse: a model to develop therapeutic interventions for Alzheimer's disease. *Curr. Pharm. Des.* 2012; **18** (**8**): 1123–30.
19. Gang B, Yue C, Han N, Xue H, Li B, Sun L, et al. Limited hippocampal neurogenesis in SAMP8 mouse model of Alzheimer's disease. *Brain Res.* 2011; **1389**: 183–193.
20. Sureda FX, Gutierrez-Cuesta J, Romeu M, Mulero M, Canudas AM, Camins A, et al. Changes in oxidative stress parameters and neurodegeneration markers in the brain of the senescence-accelerated mice SAMP-8. *Exp. Gerontol.* 2006; **41** (**4**): 360–367.
21. Turcu AL, Companys-Alemany J, Phillips MB, Patel DS, Griñán-Ferré C, Loza MI, et al. Design, synthesis, and in vitro and in vivo characterization of new memantine analogs for Alzheimer's Disease. *Eur. J. Med. Chem.* 2022; **236**: 114354.
22. Griñan-Ferré C, Palomera-Ávalos V, Puigoriol-Illamola D, Camins A, Porquet D, Plá V, et al. Behaviour and cognitive changes correlated with hippocampal neuroinflammaging and neuronal markers in female SAMP8, a model of accelerated senescence. *Exp. Gerontol.* 2016; **80**: 57–69.
23. Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn. Process.* 2012; **13** (**2**): 93–110.
24. Okuyama N, Takagi N, Kawai T, Miyake- Takagi K, Takeo S. Phosphorylation of extracellular- regulating kinase in NMDA receptor antagonist- induced newly generated neurons in the adult rat dentate gyrus. *J. Neurochem.* 2004; **88** (**3**): 717–725.
25. Francis F, Koulakoff A, Boucher D, Chafey P, Schaar B, Vinet MC, et al.

Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. *Neuron* 1999; **23** (**2**): 247–256.

LEGENDS FOR FIGURES

Figure 1. Experimental design (A). Scheme of Novel Object Recognition Test (NORT) (B). NORT results at 10-month-old SAMR1, SAMP8 and SAMP8 mice treated with UB-ALT-EV. Discrimination index for short-term memory. Data are presented as mean ± standard error of the mean (SEM). **p<0.01, ***p<0.001. F(2, 34) = 10,90, P=0,0002 (C). Discrimination index for long-term memory. Data are presented as mean ± standard error of the mean (SEM). **p<0.01, ***p<0.001. F (2, 34) = 9,223 P=0,0006 (D). The analysis of cognitive parameters was performed blindly by the experimenter. Data acquisition and statistical analysis of data was performed using GraphPad Prism software (One-way ANOVA followed by Tukey post-hoc analysis); SAMR1 (n=10), SAMP8 (n=11) and SAMP8 treated with UB-ALT-EV (n=10).

Figure 1. BrdU-positive cells in the hippocampal dentate gyrus of 10-month-old SAMR1 and SAMP8 animals. Graph represents % of BrdU-positive cells in comparison to SAMR1 animals. Data acquisition and statistical analysis of data was performed using GraphPad Prism software (One-way ANOVA followed by Tukey post-hoc analysis). Values represented as mean ± standard error of the mean (SEM). F (2, 12) = 4,974 P=0,0267 (n = 5 mice/group) *p<0.05. Scale represents 50μm

Figure 3. DCX-positive cells in the hippocampal dentate gyrus of 10-month-old SAMR1 and SAMP8 animals. The graph represents % of DCX-positive cells in comparison to SAMR1 animals. Data acquisition and statistical analysis of data was performed using GraphPad Prism software (One-way ANOVA followed by Tukey post-hoc analysis). Values represented as mean ± standard error of the mean (SEM). F (2, 11) = 7,339 P=0,0094 (n = 5 mice/group) *p<0.05;*p<0.01. Scale represents 50μm

Figure 4. GFAP-positive cells in the hippocampal dentate gyrus of 10-month-old SAMR1 and SAMP8 animals. Graph represents % of GFAP-positive cells in comparison to SAMR1 animals. Data acquisition and statistical analysis of data was performed using GraphPad Prism software (One-way ANOVA followed by Tukey post-hoc analysis). Values represented as mean ± standard error of the mean (SEM). F (2, 11) = 3,858 P=0,0537 (n = 5 mice/group) *p<0.05. Scale represents 50μm

Figure 1

Companys-Alemany, Figure 1

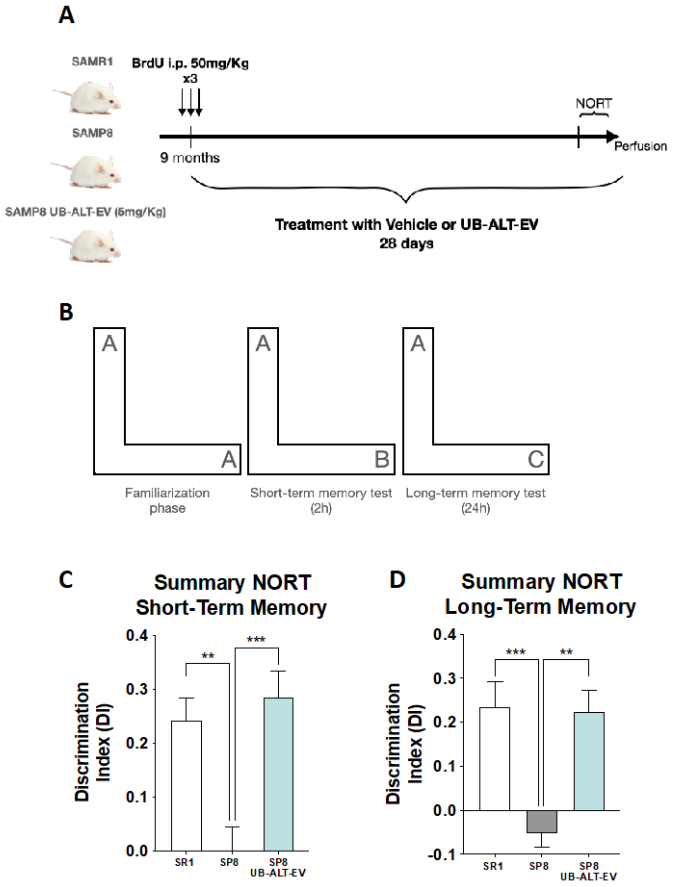

Figure 2

Companys-Alemany, figure 2

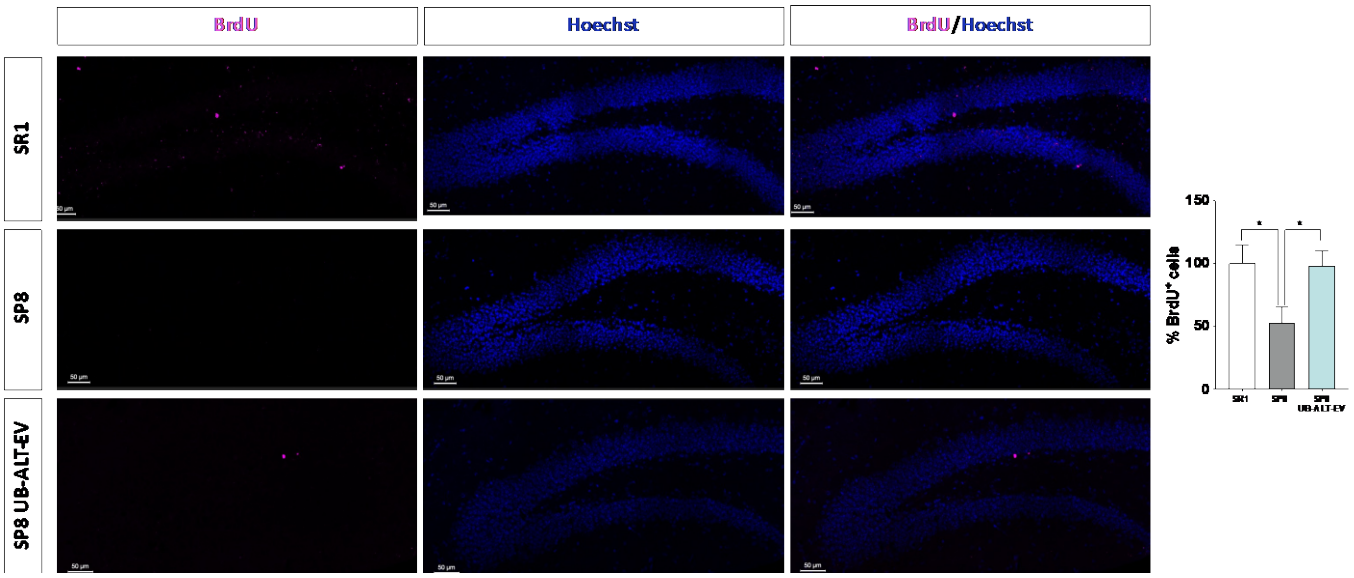

Figure 3

Companys-Alemany, figure 3

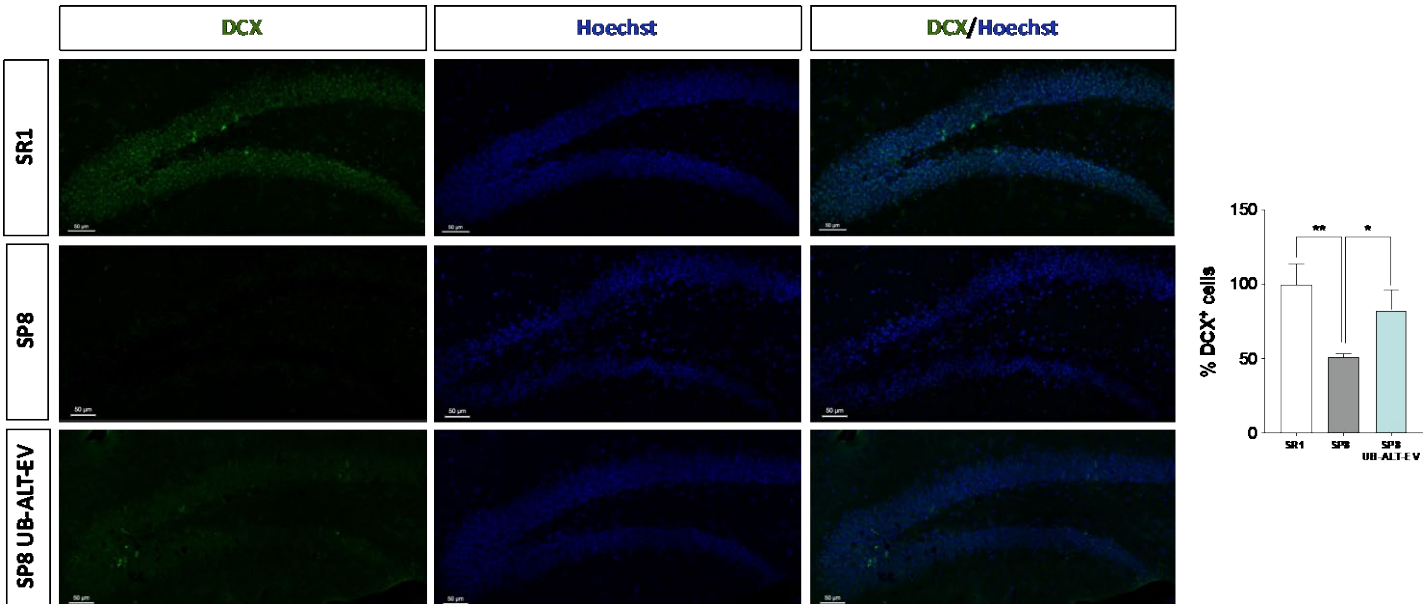

Figure 4

Companys-Alemany, figure 4