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Abstract

A promising therapeutic intervention for preventing the onset and progression of Alzheimer's Disease (AD) is to protect and improve synaptic resilience, a well-established early vulnerability associated with the toxic effects of oligomers of Ab (AbO) and Tau (TauO). We have previously reported that exosomes from hippocampal neural stem cells (NSCs) protect synapses against AβO. Here, we demonstrate how exosomes can also shield against TauO toxicity in adult mice synapses, potentially benefiting primary and secondary tauopathies. Exosomes from hippocampal NSCs (NSCexo) or mature neurons (MNexo) were delivered intracerebroventricularly to adult wildtype male mice (C57Bl6/J). After 24 hours, TauO were administered to suppress long-term potentiation (LTP) and memory, measured by electrophysiology and contextual memory deficits measured using novel object recognition (NOR) test. We also assessed TauO binding to synapses using isolated synaptosomes and cultured hippocampal neurons. Furthermore, mimics of select miRNAs present in NSCexo, were delivered ICV to mice prior to assessment of TauO-induced suppression of hippocampal LTP. Our results showed that NSC-, not MN-, derived exosomes, prevented TauO-induced memory impairment, LTP suppression, and reduced Tau accumulation and TauO internalization in synaptosomes. These findings suggest that NSC-derived exosomes can protect against synaptic dysfunction and memory deficits induced by both AβO and TauO, offering a novel therapeutic strategy for multiple neurodegenerative states.

Significance Statement: NSCexo provide an unprecedented therapeutic strategy targeting an early vulnerability driven by amyloidogenic toxic oligomers associated with multiple neurodegenerative states.

Keywords: Alzheimer’s Disease; Tau oligomers; miRNAs, Neural Stem Cells; Exosomes; Synapses

Introduction

Alzheimer's disease (AD) stands as the most prevalent form of age-related neurodegenerative dementia in the United States, with projections indicating that the number of affected individuals will more than double by 2050. Despite ongoing efforts, effective treatments for AD remain elusive, prompting a consensus that successful therapies should target prevention and/or minimization of symptoms prior to the onset of irreversible neuronal loss that characterizes the disease's advanced stages (https://www.alz.org/media/documents/alzheimers-facts-and-figures.pdf).

Over the last two decades, AD research has been significantly shaped by the identification of individuals who maintained cognitive function despite advanced AD pathology, termed Non-Demented with AD Neuropathology (NDAN).(Zolochevska and Taglialatela, 2016) This discovery highlights the brain's potential to resist or delay the neurotoxic processes that typically lead to cognitive decline in AD. Notably, our previous research uncovered distinctive proteomic profiles in hippocampal synapses of NDAN subjects,(Zolochevska et al., 2018) along with resistance to harmful amyloid β oligomers (AβO).(Bjorklund et al., 2012) As synaptic dysfunction, induced by AβO and tau oligomers (TauO), emerges as an early event in AD progression,(Klein, 2013; Spires-Jones and Hyman, 2014; Tu et al., 2014; Fa et al., 2016; Selkoe and Hardy, 2016) preserving synaptic integrity, as observed in NDAN individuals, holds promise as a therapeutic approach, albeit challenging to achieve.

New neurons are generated from neural stem cells (NSCs) via a process known as neurogenesis.(Altman and Das, 1965) Neurogenesis occurs in the mammalian brain, in the hippocampus, throughout life and is known to contribute to brain plasticity and repair.(Altman and Das, 1965) Extensive evidence indicates that hippocampal neurogenesis decreases significantly more in individuals with AD than in those experiencing normal aging.(Kuhn et al., 2007; Mu and Gage, 2011; Hamilton et al., 2013; Horgusluoglu et al., 2017),(deToledo-Morrell et al., 2007; Ohm, 2007; Appel et al., 2009; Barnes et al., 2009) Our research revealed that the brains of NDAN individuals exhibit greater numbers of hippocampal NSCs compared to the brains of healthy age-matched

controls.(Briley et al., 2016) This suggests that maintaining robust neurogenesis could mitigate cognitive decline in the presence of AD pathology.

Neural stem cells release exosomes,(Marzesco et al., 2005; Stevanato et al., 2016) small vesicles containing various molecular components, including microRNAs (miRNAs),(Raposo and Stoorvogel, 2013; Sato-Kuwabara et al., 2015) which are increasingly recognized for their role in mediating the functional effects of NSCs.(Baulch et al., 2016; Han et al., 2016; Zhang et al., 2017) These exosomes have been implicated in controlling aging processes(Zhang et al., 2017) and ameliorating synaptic dysfunction and cognitive decline in AD models.(Cui et al., 2018) Moreover, a meta-analysis reveals a connection between exosomes, synaptic plasticity, and neurodegenerative diseases, including AD.(Wang et al., 2017)

Our previous research indicated that hippocampal NSC-derived exosomes rendered central nervous system (CNS) synapses resistant to AβO by delivering specific miRNA cargo.(Micci et al., 2019) Additionally, we found that compared to individuals with AD, the brains of NDAN subjects exhibited increased numbers of NSCs in the hippocampus. Since the number of NSCs, but not neurons, positively correlated with cognitive function, we hypothesized that NSCs may support cognitive resilience through mechanisms other than neurogenesis.(Briley et al., 2016)

Building on these findings, here we investigate whether NSC-secreted exosomes (NSCexo) prevent functional synaptic deficits and memory impairments driven by TauO and whether this protection involves enhanced synaptic resistance to TauO binding. Moreover, given that miRNAs are a predominant cargo of exosomes, we aimed to identify specific miRNAs found exclusively or highly enriched in exosomes derived from NSCs that may mediate their protective effects on target neuronal synapses.

Materials and methods

Animals

Male mice (C57BL/6J; 6-8 weeks old) were purchased from Jackson Laboratories (Bar Harbor, ME). The Institutional Animal Care and Use Committee of the University of Texas Medical Branch, Galveston, Texas, approved all animal experiments, which were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The mice were housed four per cage on a 12/12/light dark cycle with free access to food and water.

Randomization and blinding procedures

Animals were randomized to the experimental groups using a simple randomization procedure. To maximize scientific rigor, a simple blinding procedure was achieved by coding exosome preparations (NSC-exo or MN-exo), vehicle, or the miRNAs to be employed in the various experiments. The correspondence of the codes was known only to Guilio Taglialatela, PhD and Maria-Adelaide Micci, PhD and was revealed only after final statistical analyses were performed.

Neural stem cell (NSC) and mature neuron (MN) cultures

Adult rat hippocampal NSCs were purchased from MilliporeSigma (Temecula, CA). NSCs were cultured as neurospheres on low-attachment plates in expansion media consisting of: EmbryoMax DMEM/F12 containing L-Glutamine without HEPES, B27-with retinoic acid, GlutaMAX™ (all from Gibco, ThermoFisher Scientific, Waltham, MA), FGF-b (20 ng/ml, MilliporeSigma, Temecula, CA) and antibiotics (PSF, Gibco, ThermoFisher Scientific, Waltham, MA). Cells were passaged every five to seven days using Neuropapain (Genlantis, San Diego, CA).

For the generation of mature neurons (MN), NSC neurospheres were dissociated using Neuropapain (Genlantis, San Diego, CA), 2 mg/ml in basal media, plated out onto poly-ornithine/laminin-coated plates at a density of 8.0 x 10^5 cells/cm^2 and cultured in differentiation media consisting of expansion media without FGFb and with the addition of 1 μM retinoic acid (MilliporeSigma, Temecula, CA) and five μM forskolin

(MilliporeSigma, Temecula, CA) for five days. Immunocytochemistry and western blot analyses confirmed the purity of the cultures by identifying the presence or absence of specific NSCs (Sox2 and nestin) and neuronal markers (bIII-tubulin and NeuN) as previously reported .(Micci et al., 2019)

Tau oligomers preparation

Prepared recombinant TauO were provided by Rakez Kayed, PhD's laboratory. They were produced and characterized following established and published protocols,(Gerson et al., 2017; Sengupta et al., 2018) and labeled TauO488 using the Microscale Protein Labeling Kit from ThermoFisher (cat# A30006; lot# 2413458) following the manufacturer's instructions. Briefly, 100 µl of TauO 1 mg/ml was mixed with 1/10 volume of 1M sodium bicarbonate. Then, 11.3 µl of the Alexa Fluoro 488 TFP ester was added following the kit molar ratio recommendations and incubated for 15 m at room temperature. The labeled TauO were purified by centrifugation at 16000 x g in spin filters with yields between 60 and 90% and quantified spectrophotometrically using a Nanodrop 2000C (ThermoFisher) to be used for flow cytometry analyses. The determined degree of labeling (DOL) was higher than two.

Isolation and characterization of exosomes

Exosomes were isolated from conditioned culture media using the ultracentrifugation method.(Greening et al., 2015; Lobb et al., 2015) Comprehensive characterization of isolated exosomes was performed using electron microscopy (EM), dot blotting, and nanoparticle tracking analyses (Figure 1) according to the guidelines of the International Society of Extracellular Vesicles(Lotvall et al., 2014; Witwer et al., 2021) and as previously reported.(Micci et al., 2019) Briefly, 225 ml of conditioned media collected from approximately 60 million cultured cells (NSC or mature neurons) were centrifuged at 2,000 x g, at 4°C for 10 minutes to remove cells and debris. The resulting supernatant was transferred to new tubes and centrifuged at 10,000 x g, at 4 °C for 10 minutes. The supernatant was transferred to Beckman 60 Ti ultracentrifuge tubes and centrifuged at 100000 x g for three hours at 4°C. The resulting pellet was resuspended in 2 ml PBS containing a protease inhibitor cocktail (MilliporeSigma, Temecula, CA), and centrifuged at 182,000 x g for one hour at 4°C in a Beckman TLA110 centrifuge. The resulting pellet,

containing exosomes, was resuspended in 1X PBS containing protease inhibitors to a concentration of approximately 10^9 exosomes per microliter.

Ultrastructure analysis of exosomes was performed with 5 µl drops adsorbed on a 200-mesh coated resin grid (FCF 200 – CU Formvar/Carbon, Electron Microscopy Sciences, Hatfield, PA, USA) for 10 min at room temperature (RT). Grids were blotted with filter paper and stained with 2% aqueous uranyl acetate (cat# 541-09-3, Electron Microscopy Sciences) for negative staining for 1 min at RT. The uranyl acetate was then removed using filter paper, and the grids were dried with warm regular light for 2 min. Images were acquired with a Philips CM-100 transmission electron microscope at 60 kV with an Orius SC2001 digital camera (Gatan, Pleasanton, CA, USA). For dot blot NSCexo, MNexo, and mouse parietal cortex were lysed with RIPA buffer containing protease inhibitors and 0.5 µg of each sample was dotted on a S nitrocellulose membrane. The membrane was let dry at room temperature (RT) for 30 minutes, blocked with Intercept® (TBS) Blocking Buffer (LI-COR Bioscience) for 1 hour at RT, and probed overnight at 4 °C with primary antibodies against CD9, CD63, CD81, Hsp70 and GM130 (1:1000; EXOABSBI Biotechnology), followed by IR Dye® secondary antibodies (1:1000; LI-COR Biosciences). Nanoparticle tracking analysis was performed using the NanoSight N300 system and NTA 2.1 operating system (Malvern) according to manufacturer's instructions. Briefly, the exosome preparation was diluted in PBS and injected into the NanoSight for analysis of both particle size and concentration.

Intracerebroventricular injections

Adult (six to eight week-old) male C57BL/6J mice were anesthetized with isoflurane and subjected to ICV injections using the freehand injection method.(Clark et al., 1968; Dineley et al., 2010; Krishnan et al., 2018). One day after ICV injection of exosomes (prepared from NSC or MN conditioned media) or PBS (vehicle), mice were euthanized for the electrophysiology study or injected ICV with 3ml of 0.55mM TauO (prepared as described above) or ACSF and euthanized 24 h later (for biochemistry studies) or 48 h later (after behavioral testing) by deep usoflurane anesthesia followed by decapitation. Brains were removed and divided into two groups: 1) fresh hippocampal slices were prepared for assessment of LTP or 2) brains were further dissected into hippocampus,

frontal cortex, parieto-occipital cortex, and midbrain, snap frozen on dry ice, and stored at -80 ºC until ready to use for the synaptosome preparation.

Brain slice preparation and electrophysiological assessment of long-term potentiation (LTP)

Brain slices were prepared and electrophysiological assessment of LTP was performed according to the methods reported by Ting *et al.*(Ting et al., 2014) Mice were deeply anesthetized with isoflurane and transcardially perfused with 25-30 mL of room temperature carbogenated gas mixture (95% O_2 and 5% CO_2) and NMDG ACSF consisting of 93 mM NMDG, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 30 mM $NaHCO_3$, 20 mM HEPES, 25 mM glucose, 2 mM thiourea, 5 mM Na-ascorbate, 3 mM Na-pyruvate, 0.5 mM $CaCl_2{\cdot}2H_2O$, and 10 mM $MgSO_4{\cdot}7H_2O$, titrated to pH 7.3–7.4 with HCl.

Following perfusion, brains were carefully extracted within 1 min. Each brain was mounted using Super Glue®, on the mounting cylinder, at a transverse slicing angle, to generate 350 µM sections using the Compresstome VF-300 (Precisionary Instruments) protocols. The sliced hippocampi were allowed to recover from the procedure at 32-34 ºC for <12 min. The slices were then transferred to a new holding chamber with carbogen-saturated HEPES ACSF recovery solution (92 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 30 mM $NaHCO_3$, 20 mM HEPES, 25 mM glucose, 2 mM thiourea, 5 mM Na-ascorbate, 3 mM Na-pyruvate, 2 mM $CaCl_2{\cdot}2H_2O$ and 2 mM $MgSO_4{\cdot}7H_2O$) at pH7.3-7.4, RT.

Recording was done in constantly flowing oxygenated (95% O_2/5% CO_2) ice-cold normal ACSF consisting of (in mM) 124 NaCl, 2.5 KCl, 1.2- $NaHPO_4$, 24 $NaHCO_3$, 5 HEPES, 13 Glucose, 2 $CaCl_2{\cdot}2H_2O$, 1.2 Mg $SO_4{\cdot}7H_2O$, at pH 7.3-7.4. Slices were perfused at a rate of approximately three mL/min. Field excitatory post-synaptic potentials (fEPSPs) were collected using an Axon MultiClamp 700B amplifier connected to a Windows computer running Clampex 8.2 software (Molecular Devices). All electrodes were placed under the visual guidance of an upright microscope (Olympus BX51WI). The slope from a single fEPSP trace was calculated from the initial slope of the fEPSP relative to the slope of the 10 ms interval immediately preceding afferent stimulation. The current magnitude was delivered through a digital stimulus isolation amplifier (AMPI, ISRAEL) and set to elicit a fEPSP of approximately 30% of maximum for synaptic potentiation experiments using

platinum-iridium tipped concentric bipolar electrodes (SUK 30200, FHC Inc, Bowdoin, ME). Using a horizontal P-97 Flaming/Brown Micropipette puller (Sutter Instruments), borosilicate glass capillaries (catalog# BF150-110-10, Sutter Instruments, Novato, CA) were used to pull electrodes and filled with ACSF to get a resistance of 1–2 MΩ.

A stable baseline (for a minimum of 10 m) was obtained by delivering a single pulse stimulation at 20 s interstimulus intervals. Field excitatory post-synaptic potentials in the CA1 were evoked by stimulating the Schaffer collaterals (SC: CA3->CA1) using a conditioning stimulus (CS) consisting of three trains of 100 pulses at 100 Hz, 20 s apart (high-frequency stimulation; HFS). Input-output experiments were conducted to measure basal dendritic excitation in response to increasing applied current in ACSF. Evoked fEPSP responses were digitized via Digidata 1550B, and the initial slope of the fEPSP was analyzed using pClamp 10.6 software (Molecular Devices). All data are represented as percentage change from the initial average fEPSP slope, defined as the average slope obtained for the 10 m prior to CS application.

Intracerebroventricular injection technique

Intracerebroventricular (ICV) injections were performed on deeply anesthetized mice using a 29-gauge needle, firmly held in place using hemostatic forceps to leave 4.5 mm of the needle tip exposed and connected to a 25 µl Hamilton syringe via 0.38 mm polyethylene tubing. Infusions were performed at the rate of 2 µl/min for a total volume of 3 ml, using an electronic programmable microinfuser (Harvard Apparatus). After ICV injection, the needle was left in place for 2 min, and the mouse was allowed to recover while lying on a heated pad under warm light.

Novel Object Recognition testing

The Novel Object Recognition test (NOR) was performed as described previously.(Taglialatela et al., 2009; Comerota et al., 2017; Krishnan et al., 2018) Each mouse was habituated to an empty NOR open field box that served to test each animal for normal locomotion. The sessions commenced with two 10 m habituation sessions spaced 24 h apart, during which the TopScan (Clever Sys. Inc., Reston, VA) video-tracking software quantified various locomotor parameters. Twenty-four hours after the last habituation session, mice were subjected to 10 m training sessions consisting of

exposure to two identical, non-toxic objects (metal or hard plastic items) in the same open field box to which the mouse had previously been acclimated, so that the mice would be more likely to maximize time exploring the objects as opposed to the environment.

The time spent exploring each object was recorded using ObjectScan (Clever Sys. Inc.). Time spent in each quadrant zone, and object zone surrounding each object, was also recorded. After the training session, the animal was returned to its home cage. Mice were returned once more to the same box after retention intervals of either two or 24 h, for the test session. In the box would be one object identical to the familiar one but previously unused (to prevent olfactory cues) and one completely unfamiliar, hence, novel, object. The animal was allowed to explore for 10 m, during which the amount of time spent exploring each object was recorded.

Objects were randomized and counterbalanced across animals. The ratio of the time spent exploring the novel object versus the time spent exploring the familiar object was reported as the Discrimination Index. (Buenz et al., 2009; Bussian et al., 2018) An index above 1 is indicative of recognition that an object is novel. Each mouse was tested at 2- and 24-hour intervals, with the intention of assessing the shorter and longer time frames in memory recall. To avoid the experience in the 2-hour test affecting the performance in the 24-hour test, different novel objects were used for the two memory recall tests.

Tissue collection and processing

Animals were deeply anesthetized using isoflurane and transcardially perfused with PBS. Brains were carefully removed and then cut in two halves along the sagittal line. One hemisphere was collected for biochemistry assays. The other hemisphere was post-fixed in 4% paraformaldehyde in 0.1 M PBS, pH 7.4 for 48 hr at 4°C, and cryoprotected by suspension in 30% sucrose solution for 48 h at 4°C. Brains were then embedded in OCT compound (Tissue-Tek, Tokyo, Japan) and frozen on dry ice prior to storage at -80°C. For immunofluorescence experiments, mouse brain blocks were removed from storage at −80°C, equilibrated at −20°C and sectioned at 12 µm onto Superfrost/Plus slides (catalog# 12-550-15, Fisherbrand, ThermoFisher Scientific, Waltham, MA, USA). Prepared slides were stored at −80°C.

Immunofluorescence staining

Slides were processed as previously described.(Fracassi et al., 2023) Briefly, slides were fixed in 4% paraformaldehyde in 0.1 M PBS, pH 7.4, for 30 m at RT. Nonspecific binding sites were blocked with 5% bovine serum albumin (BSA) (catalog# A4503-100G, Sigma-Aldrich Inc., Saint Louis, MO, USA)/10% normal goat serum (NGS, catalog# S26-100ml Sigma-Aldrich) and sections were permeabilized with 0.5% Triton X-100/0.05% Tween-20 for 1h at RT. Slides were incubated overnight at 4°C with the following primary antibodies diluted in PBS containing 1.5% NGS: chicken anti-Tau (1:100, #ab75714, Abcam – Cambridge Science Park, Cambridge, UK); mouse anti-phospho-Tau (Ser202/Thr205 - AT8) (1:100, MN1020, Thermofisher); rabbit anti-NeuN (1:200, #12943S Cell Signaling Technology, Inc., Danvers, MA, USA). Slides were washed in PBS before incubation with the appropriate Alexa-conjugated secondary antibodies (goat anti-chicken Alexa Fluor 594, 1:400, # A-11042; goat anti-mouse Alexa Fluor 488, #A-10680, goat anti-rabbit Alexa Fluor 647, 1:400, #A32733, ThermoFisher Scientific) in PBS containing 1.5% NGS/0.25% Triton X-100 for one hr at RT. Finally, slides were washed in PBS, treated with 0.3% Sudan Black B prepared in 70% EtOH for 10 m to block lipofuscin autofluorescence, washed again with deionized water, and coverslipped using Fluoromount-G containing 4′,6-diamidino-2-phenylindole (DAPI) (Cat# 0100-20, SouthernBiotech, Birmingham, AL, USA) and sealed.

Quantitative analysis of immunofluorescence

All immunoreacted sections were acquired with a Keyence BZ-X800 (Keyence Corporation, Osaka, Japan) microscope by using immersion oil 60X. Three sections were analyzed for each animal, and at least two images per section were taken at 1,920 × 1,440-pixel resolution, with the z-step size of 2 µm at 12 µm thickness. We analyzed five different regions: dentate gyrus (DG), CA1, CA3, frontal and parietal cortex. For the feasibility of the quantification, all layers from a single image stack were projected on a single slice (stack/Z projection). Quantitative analyses were performed using ImageJ software (https://imagej.nih.gov/ij, NIH, Bethesda, MD, USA). We analyzed the intensity of fluorescence for each marker (Tau and posphoTau AT8) *per* area (Integrated Density, IntDen) when the overall distribution of a specific marker was studied. The colocalization between Tau and either NeuN or Iba1 was evaluated and quantified using Pearson's correlation coefficient (PCC). Representative images were composed in an Adobe Photoshop CC2020 format.

Synaptosome isolation

We isolated synaptosomes from the hippocampi of mice previously injected ICV with PBS, NSCexo, or MNexo. The synaptosomal fraction containing both pre- and post-synaptic components was isolated using a well-established method developed in our laboratory.(Franklin and Taglialatela, 2016; Comerota et al., 2017; Franklin et al., 2019; Marcatti et al., 2022) Snap frozen hippocampus from mouse brains was lysed using SynPER lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA) with 1% protease and phosphatase cocktail inhibitors, and the homogenates were centrifuged at 1200 × g for 10 min at 4°C. The supernatants (containing the synaptosomes) were collected and centrifuged at 15,000 × g for 20 min at 4°C. The synaptosomal pellets were resuspended in HEPES-buffered Krebs-like (HBK) buffer (143.3 mM NaCl, 4.75 mM KCl, 1.2 mM MgSO4·7 H2O, 1.2 mM CaCl2, 20.1 mM HEPES, 0.1 mM NaH2PO4, and 10.3 mM D-glucose, pH 7.4). Finally, 0.5% of Pluronic F-68 non-ionic surfactant (cat# 24040-032, lot# 2275337; Thermo Fisher Scientific) was added to prevent synaptosome aggregation.

The quality and concentration (synaptosomes/µl) of isolated synaptosomes was routinely verified by flow cytometry, electron microscopy and Western blot as previously reported(Franklin and Taglialatela, 2016; Micci et al., 2019; Marcatti et al., 2022).

Enzyme-linked immunosorbent assay of total Tau

Synaptosomes isolated from the hippocampi of mice injected with PBS, NSCexo, or MNexo (ICV) were challenged with TauO (5 nM) and then digested with proteinase K (PK). Total tau content was measured by commercial Tau enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (cat# KHB0041; Thermo Fisher Scientific, Waltham, MA, USA). Ten million synaptosomes were incubated for 1h at RT with 5 nM of TauO. Half of synaptosomes were incubated with 1 mg/ml of PK (cat# 70663-4, lot# 3018798; EMD Millipore) for 30 min at 37 °C (1 mg of PK is the equivalent of 30 mAU, where AU is an Anson unit that represents the amount of enzyme that liberates 1.0 µmol (181 µg) of tyrosine from casein per min at pH 7.5 at 37 °C). The other half was subjected to the same conditions to serve as control. Synaptosomes were then washed three times with HBK buffer, and lysate in 10µl of Cell Extraction Buffer (cat# FNN0011; Thermo Fisher Scientific, Waltham, MA, USA) containing 0.3M of PMSF and

1% protease cocktail inhibitors. Finally, standards and samples were applied to the ELISA plate. After washing, a biotin-conjugated detection antibody was applied. The positive reaction was enhanced with streptavidin–horseradish peroxidase and colored by 3,3′,5,5′-tetramethylbenzidine. The absorbance at 450 nm was then measured, and the concentration of tau protein was calculated from the standard curve.

TauO binding challenge to synaptosomes and proteinase K digestion

Synaptosomes were treated with TauO for binding challenges, and the binding percentages and fluorescence intensity (MFI) were evaluated with flow cytometry. The same number of synaptosomes isolated from each mouse of the three experimental groups was pooled together. We incubated two million synaptosomes for 1 h at RT without oligomers (control) as well as with TauO tagged as described above at concentrations of 0, 0.025, 0.05, 0.25, 0.5, 1 µM. Following the challenge experiments, synaptosomes were digested with 1 mg/ml of PK (cat# 70663-4, lot# 3018798; EMD Millipore) for 30 m at 37 °C (1 mg of PK is the equivalent of 30 mAU, where AU is an Anson unit that represents the amount of enzyme that liberates 1.0 µmol (181 µg) of tyrosine from casein per m at pH 7.5 and 37 °C). Synaptosomes were then pelleted, washed three times with HBK buffer, and resuspended in HBK. Oligomer fluorescence positivity was acquired by a Guava EasyCyte 8 flow cytometer (EMD Millipore) and analyzed using Incyte software (EMD Millipore).

Tau oligomer binding to hippocampal neurons *in vitro*

Hippocampal neurons were prepared by differentiating adult hippocampal NSC as described above and plated on poly-ornithine/laminin-coated plates. Cultures were treated with exosomes (NSCexo or MNexo; $1x10^6$) or an equivalent volume of PBS for 24 h at 37°C. After washing, cultures were exposed to 2.5 mM TauO for 60 m at 37°C. One separate set of cells was treated with PBS for 24 h, followed by a vehicle to control for non-specific staining of the anti-hTau antibody. At the end of incubation, the cells were washed and fixed in 4% paraformaldehyde for 15 m. After two washes in PBS, the cells were blocked and permeabilized in PBS containing 5% normal goat serum for 30 m at room temperature. The cells were incubated with mouse anti-bIII-tubulin primary antibody (1:1000, Promega, Madison, WI) and Tau5 antibody (1:100, ThermoFisher Scientific,

Waltham, MA), diluted in 1.5% normal goat serum in PBS overnight at 4°C in a humid chamber. Following three washes in PBS, the cells were incubated with Alexa 488-conjugated anti-mouse and Alexa 594-conjugated anti-rabbit secondary antibodies (1:400; Invitrogen, Carlsbad, CA) in 1.5% normal goat serum in PBS for 1 h at room temperature in a humidified chamber. The cells were washed in PBS and coverslipped with Prolong® Gold AntiFade Reagent with DAPI (ThermoFisher Scientific, Waltham, MA). Images were acquired with an Olympus confocal microscope (FV1200, Olympus Life Science), and quantification of TauO was performed by an investigator blinded to the experimental groups by counting the number of fluorescent puncta in dendrites using ImageJ software.

Intracerebroventricular injection of miRNA mimics and target engagement validation

Mice were injected (ICV) with miRNA mimics (scrambled, miR-17, miR-322, miR-485) (ThermoFisher Scientific, Waltham, MA) dissolved in ACSF. Scrambled miRNA was delivered at one nmole per mouse, while 0.33 nmole of miR-17, miR-322, miR-485 were mixed, and a final concentration of one nmole of miRNA per mouse was administered. Four animals per group were used. Twenty-four hours after ICV injection, RNA was extracted from the hippocampi of ICV injected mice using the RNeasy Mini Kit (Qiagen, Venlo, Netherlands), and cDNA was generated with the amfiRivert cDNA Synthesis kit (GenDEPOT, Barker, TX). Quantitative RT-qPCR was performed using specific sense and antisense primers for known targets (STAT3, SYN5X, HIFa3) in a 20ml reaction volume containing 10ml of KAPA SYBR FAST qPCR Master mix (Kapa Biosystems), 0.5 ml of 10 µmol/L primer stock, 1 ml cDNA, and 8ml double-distilled H_2O. Data were normalized to b-actin.

Statistical Analysis

The sample sizes were based on a two-sided power analysis performed on the experimental groups to allow us to detect a 10-unit difference in the most variable measurement at $\alpha=0.05$ and $\beta=0.8$ based on the typical variance we have in our data. Data is expressed as mean+/- SEM or SD. Analysis of variance (ANOVA) followed by multiple comparisons post-hoc tests were performed using GraphPad Prism software.

Behavioral data (NOR) was analyzed using repeated two-way ANOVA with Dunnett correction for multiple comparisons correction). Immunofluorescence data was analyzed using ordinary one-way ANOVA with Tukey's post hoc test and two-way ANOVA with Šídák's multiple comparisons tests or Tukey's multiple comparisons test where appropriate. For all statistical analyses, significance was defined at $*P<0.05$.

Results

Neural stem cell-exosomes prevent Tau oligomer-induced short-term memory deficits.

Wild-type mice were injected (ICV) with ACSF, NSCexo or MNexo (1 x 10^9 exosomes) 24 h prior to receiving ICV injections of 3 mL of 0.55mM TauO or PBS. The mice underwent NOR training 4 h after treatment, followed by memory recall tests at 2h and 24h after that. The treatment schematic is shown in Fig. 2A. Throughout the training phase, animals across all groups exhibited equal exploration of the two identical objects, suggesting that the different treatments had no discernible impact on their inherent exploratory tendencies (Fig. 2B).

The ICV injection of ACSF, NSCexo or MNexo alone, akin to the PBS treatment, demonstrated no influence on the mice's capability to differentiate between the familiar and novel object after the 2 h and 24 h testing intervals (Fig. 2C). However, mice administered ACSF or MNexo, followed by ICV injection of TauO, exhibited equivalent exploration time for both the familiar and novel objects during the 2 h memory recall test, indicating memory impairment. On the other hand, mice treated with NSCexo prior to receiving TauO spent significantly more time in the novel object area, revealing preserved memory function despite the administration of the toxic TauO. During the memory recall test at 24 h mice in all four experimental groups spent significantly more time exploring the novel object, demonstrating integrity in a longer-term expression of the memory (Fig. 2C). Representative movement traces of the mice during the NOR testing are shown in Figure 3.

Neural stem cell-exosomes protect the hippocampus from Tau oligomer-induced suppression of long-term potentiation expression via select miRNAs.

To investigate the cellular underpinnings of NSCexo protective effects against TauO-induced memory impairments, we assessed LTP expression in hippocampal slices from mice injected with or without exosomes, then exposed to TauO using a well-documented protocol for TauO-mediated LTP inhibition.(Dineley et al., 2010) Wild-type mice were injected (ICV) with NSCexo or MNexo (1 x10^9 exosomes in 3 ml of PBS) 24 h before

euthanasia. Hippocampal slices were prepared and incubated in HEPES solution with or without pre-formed TauO (50 nM) for 1 h (Fig. 4A).

Following a stable 10 m baseline, slices underwent high-frequency stimulation (HFS) for 1 m, and LTP was recorded for 60 m. When comparing LTP expression after administering NSCexo or MNexo to the control treatment with PBS, there were no significant differences, with LTP expression being approximately 161% of baseline. When brain slices were treated with TauO, a clear reduction in LTP expression (to 131%) was observed in slices from animals previously treated with either PBS (131%) or MNexo (134%). However, slices from NSCexo-treated animals showed less reduction in LTP (155%) (Fig. 4B). A quantitative analysis of LTP in the final 10 m of recording (Fig. 2C) confirmed a significant decline in HFS-LTP triggered by TauO in slices from animals treated with PBS and MNexo, but not in slices from animals treated with NSCexo. Thus, given their potential role in memory protection, NSCexo appears to diminish the vulnerability of hippocampal synapses to TauO-induced LTP disruption. We also observed a trend of reduction without significance in the synaptic strength attributed to the toxic effects of TauO on the slope and on the FV as seen with MNexo (Fig. 5).

We have previously reported that specific miRNAs enriched in NSCexo as compared to MNexo (miR-17, miR-322, miR-485) render synapses physically resistant to the detrimental binding of AbO and functionally resilient to their disruptive action.(Micci et al., 2019) To test whether these miRNAs can also protect the hippocampus against TauO-suppression of LTP, wild-type mice received ICV injections of mimics for three miRNAs (miR-485, miR-17, and miR-322) or an equal amount of scrambled RNA as control. Mice were euthanized 24 h later and hippocampal slices were prepared and treated for 1 h with preformed TauO (50 nM) or PBS (Fig. 6A). After a stable baseline of 10 min, slices were subjected to high-frequency stimulation (HFS) for 1 m, and LTP was measured for 1 h after that. We found that TauO-driven reduction of LTP was absent in mice treated with miRNA mimics (Fig. 6B-C). Proper target engagement for the miRNAs was confirmed by RT-qPCR in a separate set of experiments, as previously reported.(Micci et al., 2019)

Intracerebroventricular delivery of neural stem cell exosomes prevent neuronal accumulation of Tau oligomers in the hippocampus and cortex.

At the end of NOR testing, mice were euthanized, perfused with PBS, and fixed in 4% paraformaldehyde. The brains were processed for Tau and pTau immunofluorescence analyses (refer to Fig. 1A for the experimental design). We found that significantly less Tau and pTau were present in the hippocampus (dentate gyrus, CA1, and CA3) of mice that received NSCexo ICV as compared to PBS and MNexo injected mice (Fig. 7). Moreover, we observed reduced levels of Tau and pTau in the parietal and frontal cortex of NSCexo-treated mice compared with those treated with MNexo and PBS (Fig. 8).

Neural stem cell exosomes reduce the internalization of Tau oligomers into synaptosomes.

Because of the observed protective effects of NSCexo on TauO-induced memory disruption and inhibition of LTP expression, we explored whether exosomes released by hippocampal NSCs could mitigate synaptic susceptibility to TauO. Synaptosomes were isolated from the hippocampus of mice previously injected (ICV) with PBS or exosomes (NSCexo or MNexo) and incubated for 1 h with preformed TauO (5 nM). After washing away excess Tau, synaptosomes were incubated for 30 m with PK, to remove surface-bound Tau, or vehicle. Internalized and total content of Tau was determined by ELISA (see schematic, Fig. 9A). We found that the amount of total Tau (internalized and at the surface) in hippocampal synaptosomes was not significantly different between the experimental groups (Figure 9B). However, after PK treatment, the amount of TauO measured in hippocampal synaptosomes isolated from mice treated with NSCexo was significantly reduced compared to synaptosomes from mice treated with PBS or MNexo ($p<0.01$ NSCexo vs PBS, $p<0.05$ NSCexo vs MNexo) (Fig. 9C). This suggests that NSCexo reduces TauO internalization but not binding at the synaptosome surface.

To further determine the effect of NSCexo on TauO binding and internalization in the synapses, synaptosomes were incubated with increasing concentrations of labeled TauO488 (25, 50, 250, 500, 1000 nM) in the presence or absence of PK and analyzed by flow cytometry (see schematic in Fig. 10A). Our results revealed a dose-dependent increase in both the percentage of TauO-positive synaptosomes (Fig. 10B) and the amount of TauO bound to synaptosomes expressed as Median Fluorescence Intensity (MFI) (Fig. 10D) across all experimental groups (PBS, NSCexo, and MNexo). However

following PK treatment, a greater reduction in TauO positivity was observed in synaptosomes isolated from NSCexo-treated mice at the TauO 50 nM concentration compared to synaptosomes from MNexo-treated mice (Fig. 10C). Additionally, we observed a greater reduction in TauO MFI in NSCexo-treated mouse synaptosomes compared to those from PBS-treated mice at 25 and 250 nM TauO, as well as MNexo-treated mouse synaptosomes at 25 nM TauO (Fig. 10E). When we compared the outcomes of PK-digested samples with their respective control conditions (without PK) for each TauO concentration (Fig. 11), we found that synaptosomes from NSCexo-treated mice, exposed to 25 nM TauO concentration, exhibited a significantly lower percentage of TauO-positive synaptosomes after PK digestion compared to synaptosomes treated with PBS or MNexo, relative to their respective control groups. (Fig. 11B). No differences between the groups were noted in the percentage of synaptosomes positive for TauO before and after PK treatment at higher TauO concentrations (50, 250, 500, 1000 nM) (Fig. 11C-F).

Interestingly, when we compared the amount of TauO (MFI) before and after PK treatment, we found significantly lower levels of TauO after PK in synaptosomes from NSCexo-treated mice but not in those from PBS- and MNexo-treated mice at all Tau concentrations tested (Fig. 11G-K). These data suggest that synaptosomes isolated from PBS or MNexo-treated mice, internalize most, if not all, TauO; while synaptosomes from NSCexo-treated mice do not internalize TauO, so most remains bound to the outer surface. Taken together, the ELISA and flow cytometry data suggest that NSCexo reduces TauO internalization at the synapse without affecting TauO binding at the surface.

NSCexo reduce TauO internalization in hippocampal neurons *in vitro.*

To test whether NSCexo-mediated reduction of TauO internalization at the synapse resulted from a direct effect on neurons, we exposed cultures of mature hippocampal neurons, generated from NSC differentiation, to NSCexo or MNexo for 24 h. After removing excess exosomes, TauO (2.5 mM) was added to the cultures for 60 m, and the cells were washed, fixed, and stained with anti-bIII-tubulin and anti-hTau (Tau5) antibodies. Confocal images revealed the presence of Tau bound to the dendritic

552 processes and within the cell bodies (as previously reported)(Puangmalai et al., 2020)
553 (Figure 12A-C). Quantitative analysis showed that, while TauO did not reduce the number
554 of neurons (Fig 13A), NSCexo treatment significantly reduced the amount of Tau found
555 inside the neurons, specifically in the nucleus (Fig. 12D), and increased the number of
556 neurites without TauO puncta (Fig. 6E). The number of Tau puncta per neurites was
557 reduced in both NSCexo- and MNexo-treated neurons as compared to those treated with
558 PBS (Fig. 12F). No puncta were visible in cultures not treated with TauO (Ctrl) and stained
559 with anti-hTau antibody (Fig. 13B). These results suggest that NSCexo reduced the
560 internalization of Tau into neurons (in accordance with the ELISA and flow cytometry data
561 and the in vivo immunofluorescence data).
562

Discussion

Neural stem cells (NSC) are critical for neurogenesis in the hippocampus, a process necessary for synaptic plasticity, learning and memory.(Kempermann et al., 2018) Previously, we showed that aged individuals who remained cognitively intact despite the presence of Aβ plaques and tau tangles in the brain, had high numbers of NSCs in the hippocampus.(Briley et al., 2016) Consistent with this discovery, we showed that exosomes released by hippocampal NSCs reduced the binding of AβO at synapses and prevented AβO-induced suppression of LTP and memory recall deficits.(Micci et al., 2019)

Here, we report, in wild-type mice, that NSCexo reduced internalization of TauO into hippocampal neurons, protected synapses from TauO-induced suppression of LTP expression, and prevented memory deficits driven by ICV injection of TauO. and that these effects may occur via delivery of specific miRNA exosomal cargo. Most importantly, we show that this mechanism is specific to NSCexo because exosomes secreted by MN, generated from the differentiation of hippocampal NSCs, were unable to provide similar protections. Using exosomes from MNs derived from differentiation of NSCs, enables the comparison of exosomes specifically associated with both a "stem" state and a mature neuronal phenotype within the same cell lineage. Consequently, this approach excludes the potential of non-specific effects of exosome vesicles, such as scavenging of exogenously-added TauO.(An et al., 2013; Yuyama et al., 2015)

Our data show that both synaptic plasticity (LTP) and memory (NOR) can be protected against TauO-induced deficits in mice treated with NSCexo prior to challenging with TauO. These results indicate that exosomes released by hippocampal NSCs play a key role in promoting or maintaining synaptic resistance to the damaging effects of TauO that ultimately result in memory deficits. Comparable trend of fEPSP reduction was observed following TauO application in PBS or NSCexo-treated animals, which was greater in the MNexo group where a significant effect is observed with the fiber volley that will be explored in the future. This data suggests other mechanisms than a direct effect on synaptic strength via action of NSCexo is involved with improvement of cognitive response observed.

Our data show that ICV injection of TauO impair short-term memory but not long-term memory in the NOR test. This may be explained by their preferential impact on hippocampal synaptic plasticity and function, which are critical for short-term memory consolidation.

Tau oligomers are known to disrupt synaptic integrity, impair long-term potentiation (LTP), and alter neuronal communication in the hippocampus. These disruptions can selectively affect the encoding and retrieval of short-term memory, which relies heavily on intact hippocampal circuits. Conversely, long-term memory may involve broader cortical networks and compensatory mechanisms that are less immediately impacted by acute toxic effects of TauO ICV injection.

Our data show that ICV injection of NSCexo, but not MNexo, prior to TauO delivery, reduces Tau and pTau accumulation in the hippocampus and cortex. Our previously published data showed that exosomes are taken up by neurons. Here we show that NSCexo can reduce TauO internalization when administered to neuronal cultures. The possibility that NSCexo are also taken up by glial cells when injected ICV cannot be excluded. Further studies are necessary to determine whether this is the case and what the functional significance would be.

TauO are known to accumulate in synapses resulting in disrupted synaptic transmission, ultimately leading to dendritic spine retraction and cognitive impairments.(Tu et al., 2014), (Lacor et al., 2004) Our data show that NSCexo reduce TauO internalization into isolated synaptosomes and cultured hippocampal neurons. The mechanisms remain unclear, they may involve the low-density lipoprotein receptor-related protein 1 (LRP1). LRP1 is abundantly expressed at synapses and has been recently shown to function as a major transporter for TauO allowing rapid internalization of Tau and its subsequent spreading.(Rauch et al., 2020; Cooper et al., 2021) Our data are consistent with the idea that an action of the cargo of NSCexo on the LRP1-Tau complex could be responsible for reduced Tau internalization but unaffected tau surface binding in synaptosomes of NSCexo-treated mice.

MiRNAs are abundant in exosomes and, in addition to proteins and lipids, represent their main bioactive cargo.(van den Boorn et al., 2013; Bayraktar et al., 2017) Indeed,

compelling evidence indicates that many of the effects elicited by exosomes (including NSCexo) can be ascribed to the action of their specific miRNA cargoes, including modulation of aging,(Zhang et al., 2017) cognition and synaptic function(Hebert et al., 2009; Konecna et al., 2009; Cohen et al., 2011) and neuroprotection.(Properzi et al., 2015) Importantly, miRNAs have been shown to regulate transcriptional modulation of key synaptic proteins within the synaptic compartment itself.(Schratt, 2009; Garza-Manero et al., 2014) Furthermore, our own previous findings show that resilient hippocampal synapses in NDAN individuals with high numbers of NSCs(Briley et al., 2016) have a unique proteomic signature,(Zolochevska et al., 2018) suggesting that selective changes in synaptic protein expression mark synaptic resistance to toxic AβO and TauO in these humans. This is consistent with upstream regulation by specific miRNAs, as revealed by IPA analysis of our proteomic data. We have previously shown that, compared to MNexo, NSCexo contain a unique set of miRNAs that are known to modulate the expression of proteins involved in synaptic function.(Micci et al., 2019) Most importantly, we also found that when injected ICV in mice, mimics of such unique NSCexo-derived miRNAs render synapses physically resistant to the detrimental binding of AβO and functionally resilient to their disruptive action. Here we demonstrated that these unique NSCexo-derived miRNAs protect mice from TauO-induced LTP and memory dysfunctions. These data further support the notion that selected miRNAs uniquely present in NSCexo mediate their protective action on synapses of target neurons and further suggest the exciting possibility of new drug discovery to promote cognitive resilience in patients with Alzheimer's disease.

Our data point to NSC-secreted exosomes, and their miRNA cargo, as critical elements of the, yet poorly understood, neurobiological basis of the relationship between brain reserve, cognitive resilience, and resistance to Alzheimer's disease neuropathology. While the relative quantity of exosomes released by NSC in the hippocampus is not known, we know that exosomes injected in the dentate gyrus can spread to other regions of the hippocampus and to the cortex.(Zheng et al., 2017) An intriguing possibility is that a decrease in the number of NSCs in the hippocampal dentate gyrus, such as that which occurs during aging,(Kuhn et al., 2007; Ben Abdallah et al., 2010; Lugert et al., 2010) could lead to increased synaptic vulnerability to the toxic binding of AβO and TauO,

because of reduced NSCexo in the hippocampus. Such a mechanism could be one factor that links aging to increased risk of Alzheimer's disease.(Querfurth and LaFerla, 2010)

The future pursuit of this novel concept has the potential to open the door to new therapeutic strategies for Alzheimer's disease centered on NSC-secreted exosomes and their bioactive cargo. Indeed, our previous reports and the results of this work, demonstrating the effect of NSC-secreted exosomes in increasing synaptic resistance to AbOs and TauO, support this possibility. NSC-secreted exosomes could delay the onset and/or mitigate the severity of A□O- and TauO-dependent synaptic and cognitive dysfunctions associated with age-dependent decreases in neurogenesis.

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664 **Availability of data and materials:** Raw data is available from the corresponding
665 authors upon reasonable request.

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Figure legends

Figure 1. Figure 1 Characterization of isolated exosomes. Schematic representation of the methods used to characterize the exosome preparations. **(Left)** Representative high-resolution transmission electron microscopy image of NSCexo and MNexo, isolated using the ultra-centrifugation method (scale bar = 0.1 µm). **(Middle)** Representative NTA profile for NSCexo and MNexo showing the size and concentration of particles. **(Right)** Representative dot blot comparing exosome markers expression in NSCexo, MNexo and total homogenate from mouse parietal cortex (mPC-H). NSCexo = Neural stem cells exosomes; MNexo = Mature neurons exosomes; NTA = Nano tracking analysis

Figure 2. TauO-induced memory deficit is rescued by NSCexo. (A) Schematic of the experimental design. Mice were injected (ICV) with either 1 x 10^9 exosomes (NSCexo or MNexo) or ACSF 24 h before injection (ICV) of 3 mL of 0.55mM TauO or PBS. Four hours later, they were subjected to the novel object recognition (NOR) memory test. (**B**) Discrimination index during the training phase when the mice are exposed to two identical objects. One-way ANOVA (F=1.580) and Tukey's multiple comparisons test. *n*=7-10 mice/group. Data is mean +/- SD. (**C)** Box plot representation of the Discrimination Index calculated as the ratio between time spent with the novel object and the time spent with the familiar object during the 2h and 24h memory recall tests. Two-way ANOVA of repeated measure data with Dunnett multiple comparisons test versus Training. **P*<0.05, ***P*<0.01, ****P*<0.005. *n*=7-10 mice/group. Data is min-max.

NSCexo = neural stem cell exosomes; MNexo = mature neuronal exosomes; PBS = phosphate-buffered saline; TauO = Tau oligomers; ACSF = artificial cerebrospinal fluid; SD = Standard deviation

Figure 3 Representative movement traces of mice during the NOR testing. Representative movement traces of mice ICV injected with either PBS, NSCexo, or MNexo prior treatment with TauO or ACSF. Left panels show data from the training phase of the test; middle panels show data from the 2h memory recall test; right panels show data from the 24h memory recall test. N = novel object; PBS = phosphate-buffered saline; NSCexo = Neural stem cells exosomes; MNexo = Mature neurons exosomes; TauO = Tau oligomers; ACSF = artificial cerebrospinal fluid

Figure 4. TauO-induced suppression of LTP expression in the hippocampus is abolished by ICV injection of NSCexo. (A) Schematic of the experimental design: Mice were administered ICV injections of either NSCexo, MNexo, or PBS. After 24 hours, they were euthanized. Brain sections from these mice were subsequently treated with or without TauO. Field recordings of LTP were then conducted in the Schaffer collateral region of the hippocampus. **(B and D)** Field potential recording of LTP, compared to the percent of baseline, from brain slices pretreated with NSCexo but not with MNexo, in the presence or absence of TauO, revealed differences in the slope of excitatory postsynaptic potentials. **(C)** For each experimental condition, the amplitude of fEPSP during the final 10 m (from the 50th -60th min post high frequency stimulation) were averaged. TauO significantly reduced LTP in brain slice taken from mice injected with either PBS or MNexo. Conversely, this suppressive effect was not seen in slices from mice that received the NSCexo injection. PBS: n = 7, NSC-exo: n = 7, and MN-exo: n = 6 (1-4 slices per mouse). *P < 0.05 Two-way ANOVA followed by Tukey's multiple comparisons test.

NSCexo = neural stem cells exosomes; MNexo = mature neurons exosomes; PBS = phosphate-buffered saline; TauO = Tau oligomers; LTP = long term potentiation; fEPSP = functional excitatory post-synaptic potential

Figure 5 Application of TauO reduces the slope while application of MNexo affects the fiber volley. The input-output (IO) plotted as slope (mV/ms) on the Y-axis as a function of fiber volley (FV in mV) on the X-axis is provided prior to high-frequency stimulation (pre-, clear circles) and after (post-, filled circles) for (A) PBS treated, (B) NSCexo treated, (C) MNexo treated. The panel on the left is untreated, while the panel on the right is from TauO treated. Slope for (D) PBS treated, (E) NSCexo treated, (F) MNexo treated and FV as a function of stimulation (mA) for (G) PBS treated, (H) NSCExo treated, (I) MNexo treated provide additional insight into the role of TauO and MNexo treatment on synaptic strength.

Figure 6 TauO-induced LTP suppression in the hippocampus is abolished by mimics of miRNAs enriched in NSCexo. (A) Schematic of the experimental design. MicroRNAs (scrambled or mimics of miR-322, miR17 and miR-485) or ACSF (vehicle)

were injected ICV into adult mice 24 hours before euthanasia. **(B)** Schaffer collateral field recording of LTP (indicated as percent of baseline in the slope of fEPSPs) was performed on brain slices in the presence of TauO (50 nM) or ACSF. TauO abolished LTP in ACSF-injected mice and in scrambled-treated mice but not in miRNAs combo-treated mice. **(C)** Average for each condition of the fEPSP amplitude for the final 10 minutes (time points 50–60 minutes post high frequency stimulation). TauO significantly reduced LTP in brain slices from mice injected with vehicle or with scrambled miRNA, but not in brain slices from mice treated with combo miRNAs mimics. N=4 mice/group (3 slices per mouse). ***P*<0.01; *****P*<0.0001 one-way ANOVA followed by Dunnet's multiple comparisons test. ACSF = artificial cerebrospinal fluid; ICV = intracerebroventricular; LTP = long-term potentiation; fEPSPs = functional excitatory post-synaptic potentials; TauO = Tau oligomers

Figure 7. NSCexo reduce Tau and pTau accumulation in the hippocampus. Representative images and quantitative analyses of triple immunofluorescence staining for NeuN (magenta) in combination with total Tau (red) and pTau (AT8-green) revealed a significant decrease in Tau and pTau levels after treatment with NSCexo in DG, CA1 and CA3. Scale bar 20 mm. Statistical analyses were made using one-way analysis of variance, following Tukey's multiple comparisons test. Values are expressed as the mean ± SD. **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001.

NeuN = neuronal nuclei; NSCexo = neural stem cells exosomes; DG = dentate gyrus

Figure 8 NSCexo reduce Tau and pTau accumulation in the cortex. Representative images and quantitative analyses of triple immunofluorescence staining for NeuN (magenta) in combination with total Tau (red) and pTau (AT8-green) revealed a significant decrease in Tau and pTau levels after treatment with NSCexo in both parietal and frontal cortex. Scale bar 20 μm. Statistical analyses were made using one-way analysis of variance, following Tukey's multiple comparisons test. Values are expressed as the mean ± SD. **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001. NeuN = neuronal nuclei; NSCexo = neural stem cells exosomes; SD = standard deviation

Figure 9. NSCexo reduce hippocampal synaptic vulnerability to TauO. Exosomes (NSCexo or MNexo) or PBS were injected ICV 24hr before sacrifice. Brain synaptosomes were prepared from the hippocampus and challenged with preformed 5 nM TauO for 1h followed by treatment with PK or vehicle for 30 minutes. After washing, the amount of TauO bound to synaptosomes was quantified by ELISA. **(A)** Schematic of the experimental design; **(B)** quantification of total tau; **(C)** quantification of internalized tau after PK treatment. *n*=8 mice/group (average of two independent experiments). **P*<0.05; ***P*<0.01 vs. PBS Unpaired T-test. Data is median.

NSCexo = neural stem cells exosomes; MNexo = mature neurons exosomes; PBS = phosphate-buffered saline; TauO = Tau oligomers; PK = proteinase K

Figure 10. Effect of NSCexo on synaptic TauO binding and internalization. Exosomes (NSCexo or MNexo) or PBS were injected ICV 24 hours before sacrifice. Synaptosomes were isolated from the hippocampus and challenged with preformed labeled TauO488 at increasing concentrations or PBS for 1 hour. After washing, synaptosomes were treated with PK or vehicle for 30 minutes, washed and analyzed by flow cytometry. **(A)** Schematic of the experimental design: (**B-C)** Percentage of synaptosomes positive for TauO488 with and without PK treatment. **(D-E)** Median Fluorescence Intensity (MFI) of TauO488-positive synaptosomes with and without PK. Comparisons were performed within each group between data in the presence or absence of PK. *p<0.05 (Multiple unpaired T-test). Synaptosomes were pooled from 8 individual mice per group. N=3 flow cytometry runs. Data is mean +/- SD.

NSCexo = neural stem cells exosomes; MNexo = mature neurons exosomes; PBS = phosphate-buffered saline; TauO = Tau oligomers; PK = proteinase K; SD = standard deviation

Figure 11. NSCexo reduce the internalization of TauO into synaptosomes. (A) Schematic of the experimental design. **(B-F)** Flow cytometric analysis of the percentage of TauO488-positive synaptosomes (after challenged with increasing concentration of TauO) derived from NSCexo-, MNexo-, or PBS-treated mice before and after PK digestion. **(G-K)** Flow cytometric analysis of the MFI of TauO488-positive synaptosomes

derived from NSCexo-, MNexo-, or PBS-treated mice before and after PK digestion. *P<0.05 2-way ANOVA followed by Tukey's multiple comparison tests. Synaptosomes were pooled from 8 individual mice per group. n=3 flow cytometry runs. Data is mean +/- SD.

NSCexo = neural stem cells exosomes; MNexo = mature neurons exosomes; PBS = phosphate-buffered saline; TauO = Tau oligomers; PK = proteinase K

Figure 12. NSCexo reduce TauO internalization into hippocampus neurons *in vitro*. Hippocampal neurons generated by differentiation of adult rat hippocampus NSC were treated with NSCexo, MNexo or PBS for 24 hours before being challenged with TauO (2.5 mM) for 1 hour. **(A)** Representative confocal images (60X w/ 2 zoom) of neurons pre-treated with PBS, NSCexo, and MNexo (hTau in red, bIII-tubulin in green, and the nuclei in blue; scale bar 20 μm). **(B)** Representative confocal images (60X w/7.5 zoom) showing the presence of hTau inside the neurites (scale bar is 2 μm). **(C)** Representative confocal images (60X w/7.5 zoom) showing the presence of hTau in the neuronal cell bodies (scale bar 5μm). **(D)** Quantification of the number of hTau puncta inside neurons (5 images acquired from 8 independent experiments). *p<0.05; **p<0.01 one-way ANOVA (F = 6.701) followed by Tukey's multiple comparisons test. **(E)** Quantification of the number of neurites without hTau puncta (5 images acquired from 5 independent experiments). *p<0.05; ****p<0.0001 one-way ANOVA (F = 22.82) followed by Tukey's multiple comparisons test. **(F)** Quantification of the number of hTau puncta on neurites (5 images acquired from 5 independent experiments). *P<0.05; **P<0.01 one-way ANOVA (F = 7.975) followed by Tukey's multiple comparisons test. Data is mean +/- SEM.

NSCexo = neural stem cells exosomes; MNexo = mature neurons exosomes; PBS = phosphate-buffered saline; TauO = Tau oligomers; SEM = standard error of the mean

Figure 13 (A) Hippocampal neurons generated by differentiation of adult rat hippocampus NSC were treated with PBS for 24 hours before being incubated with PBS for 1 hour. Neurons were fixed and processed for immunofluorescence analysis using anti-hTau antibody and anti-□III-tubulin. **(Left**) Representative confocal images (460X w/ 2 zoom) of neurons pre-treated with PBS (hTau in red, βIII-tubulin in green, and the nuclei in blue; scale bar 20 μm). **(Middle)** Representative confocal images (60X w/7.5 zoom) showing

the absence of hTau inside the neurites (scale bar 2μm). **(Right)** Representative confocal images (60X w/7.5 zoom) showing the absence of hTau in the neuronal cell bodies (scale bar is 5 μm). NSC = neural stem cells; PBS = phosphate-buffered saline

(**B**) Quantification of the number of DAPI+ cells across the experimental groups (5 images acquired from 8 independent experiments). One-way ANOVA (F = 1.035) followed by Tukey's multiple comparisons test.