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The effect of amyloid-β peptide on synaptic plasticity and memory is influenced by different isoforms, concentrations, and aggregation status

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ABSTRACT

The increase of oligomeric amyloid-beta (oAβ) has been related to synaptic dysfunction, thought to be the earliest event in Alzheimer's disease pathophysiology. Conversely, the suppression of endogenous Aβ impaired synaptic plasticity and memory, suggesting that the peptide is needed in the healthy brain. However, different species, aggregation forms and concentrations of Aβ might differently influence synaptic function/dysfunction. Here, we have tested the contribution of monomeric and oligomeric Aβ42 and Aβ40 at 200 nM and 200 pM concentrations on hippocampal long-term potentiation and spatial memory. We found that, when at 200 nM, oAβ40, oAβ42, and monomeric Aβ42 impaired long-term potentiation and memory, whereas only oAβ42 200 pM enhanced synaptic plasticity and memory and rescued the detrimental effect due to depletion of endogenous Aβ. Interestingly, quantification of monomer-like and oligomer-like species carried out by transmission electron microscopy revealed an increase of the monomer/oligomer ratio in the oAβ42 200 pM preparation, suggesting that the content of monomers and oligomers depends on the final concentration of the solution.

1. Introduction

Progressive accumulation of amyloid-beta peptide (Aβ) in brain regions involved in cognition has been considered a main pathogenic event in Alzheimer's disease (AD). The multifaceted pathological role of this protein spans from its deposition in the characteristic senile plaques, a histological hallmark of AD, to the synaptotoxic effect of oligomers leading to memory dysfunction (Walsh and Selkoe, 2007). On the other hand, low physiological concentrations of Aβ have neurotrophic and neuroprotective properties (Cárdenas-Aguayo et al., 2014), and exert a positive modulatory function on synaptic plasticity and memory (Puzzo and Arancio, 2013). Several studies aimed at understanding whether these opposite Aβ effects are due to different concentrations, species or aggregation forms of the peptide, as this might represent a crucial aspect to clarify when and how Aβ physiological function switches toward pathology.

Aβ is a β-sheet–forming protein with a high propensity to form aggregates, such as oligomers, protofibrils, and fibrils. Typically, Aβ peptides are composed of 39–43 amino acids and, among these, Aβ40 is the most represented form (about 60% of total Aβ) that, however, has a less propensity to aggregate (Vandersteen et al., 2012). The aggregation rate seems to be related to the amino acids at the C terminus (Sgourakis et al., 2007), giving Aβ42 the higher propensity to form oligomers, which have been related to AD neuronal dysfunction (Jarrett and Lansbury, 1993). Different types of oligomers seem to correlate with cognitive decline in AD (Ono

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et al., 2009; Roychaudhuri et al., 2009) and are present in the human brain or cerebrospinal fluid (CSF) decades before AD onset (Fukumoto et al., 2010; Lesné et al., 2013).

Because Aβ is thought to be secreted in a monomeric form, it is common to ascribe its physiological properties to monomers (Giuffrida et al., 2009). However, in the normal healthy brain, the concentration of soluble Aβ has been estimated in the picomolar range with species ranging from monomers to higher molecular weight oligomers (Giedraitis et al., 2007; Puzzo et al., 2008; Schmidt et al., 2005). Thus, a certain degree of oligomerization also occurs in physiological conditions for both Aβ40 and Aβ42.

Although several studies have investigated the effects of different forms and aggregation states of soluble Aβ responsible for its toxic actions in AD, few studies have evaluated these aspects under physiological conditions. We have previously demonstrated that Aβ42 exerts an opposite effect on long-term potentiation (LTP) and memory, depending on its concentration (Puzzo et al., 2012). Interestingly, both the positive and negative Aβ42 effects were attributable to a preparation containing monomers and oligomers, suggesting that oligomeric forms of Aβ are involved in normal synaptic plasticity other than synaptic dysfunction.

Here, we aimed to clarify the effects of different isoforms, concentrations, and aggregation status of the peptide on synaptic plasticity and memory.

2. Materials and methods

2.1. Animals

All experiments involving animals were approved by University of Catania (#327/2013-B, #119–2017-PR) and Columbia University (#AC-AAAO5301) in accordance with the respective regulations of local Institutional Animal care and Use Committee. Wild-type (C57Bl/6J) mice were obtained from breeding colonies kept in the animal facilities at University of Catania and Columbia University. Mice were maintained in stable hygrometric and thermic conditions (50%; 21 °C ± 1 °C) on 12 hours light/dark cycle with ad libitum access to food and water. Males and females were used in a sex-balanced fashion (5 males and 5 females; averaged weight: 30.5 ± 0.14 vs. 25.07 ± 0.18) for each condition described in behavioral experiments. Males were used for electrophysiological experiments. We used 5–7 slices for each LTP recording from 5 to 7 different animals as reported for each experiment in the result section. All mice used in our experiments were 3–6 months old.

2.2. Aβ preparation

Aβ was prepared as previously described (Puzzo et al., 2012; Ripoli et al., 2013; Stine et al., 2003). Briefly, the lyophilized peptide (American Peptide, Sunnyvale, CA, USA) was suspended in 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma, St. Louis, MO, USA) to 1 mM. After the complete evaporation of 1,1,1,3,3,3-hexafluoro-2-propanol to allow complete monomerization, the Aβ film was dissolved in dimethyl sulfoxide (DMSO; Sigma), sonicated for 15 minutes, aliquoted, and stored at −20 °C. Different protocols were used to obtain preparations of Aβ42 or Aβ40 enriched in monomers (mAβ42 and mAβ40) or oligomers (oAβ42 and oAβ40). For mAβ42 and mAβ40, the DMSO-Aβ solution was diluted in artificial CSF (ACSF) immediately before use to the final concentration (200 pM and 200 nM). For oAβ42 and oAβ40, the DMSO-Aβ solution was incubated in PBS at 4 °C for 12 hours and 1 week, respectively, to allow oligomerization. These oligomerized Aβ solutions were then diluted in ACSF to the final concentration, calculated based on the MW of the monomeric peptides. The oligomerization status of these solutions was routinely tested by western blot (WB) analysis. Scramble Aβ42 and Aβ40 (AnaSpec Inc, San Jose, CA, USA) were prepared following the same procedure.

2.3. WB analysis of Aβ preparations

Aβ solutions, prepared as described previously, were incubated for 20 minutes at 29 °C to reproduce electrophysiological conditions. After this step, NuPAGE LDS sample buffer 4× was added and the samples were separated on 10%–20% Novex Tricine precast gels (Invitrogen) according to the manufacturer's protocol. Gels were transferred to 0.2 mm nitrocellulose membranes (Amersham Biosciences, Buckinghamshire, UK). Membranes were blocked for 1 hour, at room temperature (RT), in a solution of 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween-20 before incubation overnight at 4 °C with the mouse monoclonal antibody 6E10 (1:1000; Covance, Princeton, NJ, USA). Membranes were washed 3 times with Tris-buffered saline containing 0.1% Tween-20 and then incubated with the horseradish peroxidase-conjugated Ig anti-mouse antibody (1:2500; Cell Signaling Technology Inc, Danvers, MA, USA) at RT for 1 hour. In another series of experiments, only oAβ42 at the final concentrations of 200 nM and 200 pM was assessed by WB following the same procedure. Development was done by using the SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Scientific, Waltham, MA, USA). Immunoblots were documented by using UVItec Cambridge Alliance. Molecular weights were estimated using Rainbow Molecular Weight Markers (GE Healthcare Life Sciences).

2.4. Electrophysiological recordings

Extracellular electrophysiological field recordings were performed on 400 μm transverse hippocampal slices as previously described (Puzzo et al., 2008). After cutting procedure by using a manual tissue chopper, slices were transferred to a recording chamber and perfused (1–2 mL/min) with ACSF (composition in mM: 124.0 NaCl, 4.4 KCl, 1.0 Na_2HPO_4, 25.0 $NaHCO_3$, 2.0 $CaCl_2$, 2.0 $MgCl_2$, 10.0 Glucose) kept at 29 °C and continuously bubbled with an O_2/CO_2 mixture at 95% and 5%. After 120 minutes recovery, field excitatory postsynaptic potentials (fEPSPs) were recorded in CA1 stratum radiatum by a glass electrode filled with ACSF in response to Schaffer collaterals stimulation by a bipolar tungsten electrode. Baseline synaptic transmission (BST) was assessed by a 5–35 V stimulus delivery and plotted as fEPSP slope against afferent volley amplitude. Baseline was recorded every minute, by stimulating at a voltage able to evoke a response of 35% of the maximum evoked response in BST. LTP was induced by a theta-burst stimulation, that is, 3 TBS trains delivered with a 15 seconds inter-train interval with each train consisting in 10 × 100 Hz bursts with 5 pulses per burst with a 200-ms interburst interval, at the test pulse intensity. Recordings were performed and analyzed in pClamp 10 (Molecular Devices, Sunnyvale, CA, USA).

2.5. Infusion technique

Cannulas were implanted as previously described (Puzzo et al., 2017). Anesthesia was induced with an association of Tiletamine + Zolazepam (60 mg/kg) and Medetomidine (40 μg/kg). Mice were implanted with a 26-gauge guide cannula into the dorsal hippocampi (coordinates from *bregma*: posterior = 2.46 mm, lateral = 1.50 mm to a depth of 1.30 mm). After 6–8 days of recovery, drugs were bilaterally injected in a final volume of 1 μL over 1 minute through infusion cannulas that were connected to a microsyringe by a polyethylene tube. During infusion, animals were handled gently to minimize stress. After infusion, the needle was left in place for another minute to allow diffusion. In some animals,

after behavioral studies, a solution of 4% methylene blue was infused for localization of infusion cannulas.

2.6. Behavioral studies

Morris Water Maze (MWM) experiments were performed as described (Puzzo et al., 2013). The apparatus consisted of a plastic maze filled with water maintained at about 25 °C and made opaque to hide the submerged platform by the addition of nontoxic white paint. The submerged platform was located in the south-west quadrant and left there throughout the tests. Spatial cues were placed on the 4 cardinal points of the maze. We first assessed spatial learning by placing animals into the pool where they learned to locate the hidden platform beneath the surface of the water. Mice were trained for 3 days (2 daily sessions held 4 hours apart). Each session consisted of three 1-minute trials. For each trial, mice started from a different, randomly chosen quadrant. The time taken to reach the hidden submerged platform (latency) was recorded. After this acquisition training, on the fourth day, the platform was removed to perform the probe test. This allowed evaluating the retention of spatial memory. One session consisting of four 1-minute probe trials separated by 5 seconds was performed. The maze was divided into 4 quadrants: the target quadrant (TQ) previously containing the platform; the adjacent left, the adjacent right, and the opposite quadrant. The percent time spent in each quadrant was recorded and analyzed with a video tracking system (Netsense srl, Catania, Italy), and the performances of the 4 probe trials were averaged. On the fifth and sixth day, visual, motor, and motivation skills were tested in 2 sessions/day (each consisting of three 1-minute trials) by measuring the time taken to reach a visible platform (randomly positioned in a different place each time) marked with a green flag.

2.7. Transmission electron microscopy for Aβ visualization

For Aβ visualization, carbon-coated copper 200 mesh grids were processed according to negative stained technique with 2% uranyl acetate (Ahmed et al., 2010; Booth et al., 2011; Picou et al., 2010). Briefly, grids were incubated for 20 minutes with 5 μL of oAβ42 200 nM, 200 pM, and vehicle (2 grids for each condition), then washed with boiled filtered distillated water and then exposed for 30 seconds to 2% uranyl acetate. After air-dried, grids were examined in a blinded manner, with a Philips CM10 electron microscope coupled to a MegaView-II high resolution CCD camera (Soft Imaging System, Germany). Electron microscopical fields were captured at an original magnification of 34,000, 64,000 and 92,000×. For quantification of Aβ monomer-like and oligomer-like elements in Aβ 200 nM and 200 pM preparations, 200 nm × 200 nm subfields (n = 8) were randomly selected from 34,000× original acquisitions (n = 2). According to previous electron microscopy visualization of Aβ (Ahmed et al., 2010), negatively Aβ stained was deciphered by the presence of an electrondense contour displaying a variable morphology. Based on the shape and on the estimated internal diameter, monomer-like elements (round shape and diameter ≤ of 5 nm) and oligomer-like elements (oval shape and diameter ≥ of 10 nm (Ahmed et al., 2010) were noted and counted. Diameters of recognizable elements were calculated by using Image J software.

2.8. Statistics

All experiments were performed by operators blind with respect to treatment. Data were expressed as mean ± standard error mean (SEM). Statistical analysis was performed by using different tests, based on preliminary analyses of normal distribution. We have used (1) analysis of variance (ANOVA) for repeated measures to analyze LTP for 120 minutes of recording after tetanus and curves of spatial learning (independent variables time and treatment, with treatment as main effect); (2) 1-way ANOVA with Bonferroni's post hoc correction for LTP residual potentiation (treatment as main effect); (3) 1-way ANOVA with LSD post hoc correction for latency in the sixth MWM trial and percentage of time spent in TQ in the probe test; and (4) paired *t*-test to analyze percentage of time spent in TQ versus non-TQ quadrants. Given the non-normal distribution of data obtained in transmission electron microscopy (TEM) experiments (assessed by D'Agostino & Pearson normality) comparison between the monomer-like and oligomer-like elements of Aβ 200 nM and 200 pM was made by Mann-Whitney test. SigmaPlot 12.0, Systat 9 and GraphPad Prism 7 software were used. The level of significance was set at $p < 0.05$.

3. Results

3.1. Oligomeric and monomeric human Aβ42 and Aβ40 exert a different effect on hippocampal long-term potentiation

We first aimed to clarify whether the positive/negative effects of Aβ on LTP, that is, the cellular surrogate of memory (Bliss and Collingridge, 1993), were mediated by Aβ42 and/or Aβ40 in monomeric and/or oligomeric forms. Based on previous works (Puzzo et al., 2008; Ripoli et al., 2013; Stine et al., 2003), different preparations of Aβ were prepared and characterized by using WB analysis (Fig. 1A). We used (1) a fresh preparation of Aβ42 or Aβ40 enriched in monomers, named mAβ42 and mAβ40; and (2) an aged preparation of Aβ42 or Aβ40 enriched in oligomers, named oAβ42 and oAβ40. The presence of different bands corresponding to monomers (4.5 kDa), dimers (9 kDa), trimers (13.5 kDa), and tetramers (18 kDa) confirmed that although oligomers are prevalent in oAβ42 solution, they are also present in a small quantity in mAβ42. As for oAβ40 solution, it contains lower levels of oligomers compared with oAβ42, whereas only monomers and few dimers are detectable in mAβ40 preparation.

Hippocampal slices were treated with these preparations at 2 different concentrations, 200 pM and 200 nM, known to improve or impair CA3-CA1 LTP, respectively (Puzzo et al., 2012). We first confirmed that perfusion with 200 pM oAβ42 for 20 minutes before a strong theta-burst stimulation induced an improvement of LTP ($F(1,12) = 16.810$; $p = 0.001$ vs. vehicle), whereas 200 nM oAβ42 markedly reduced it ($F(1,12) = 17.512$; $p = 0.001$ vs. vehicle) (Fig. 1B). Conversely, mAβ42 at low picomolar concentrations failed to increase potentiation ($F(1,12) = 0.013$; $p = 0.911$ vs. vehicle), whereas it slightly impaired LTP when administered at 200 nM ($F(1,12) = 8.107$; $p = 0.015$ vs. vehicle; Fig. 1C). On the other hand, 200 pM concentrations of Aβ40 were ineffective either when enriched in oligomers (oAβ40: $F(1,12) = 0.032$; $p = 0.860$ vs. vehicle; Fig. 1D) or monomers (mAβ40: $F(1,12) = 3.654$; $p = 0.080$ vs. vehicle; Fig. 1E), whereas 200 nM oAβ40 ($F(1,12) = 18.154$; $p = 0.001$ vs. vehicle; Fig. 1D), but not mAβ40 ($F(1,12) = 4.015$; $p = 0.068$ vs. vehicle; Fig. 1E), impaired LTP. We confirmed these results when analyzing residual potentiation in all conditions, as summarized in Fig. 1F. In control experiments, scrambled Aβ42 or Aβ40 at 200 nM or 200 pM concentration did not affect LTP compared with tetanized slices treated with vehicle (ANOVA with Bonferroni's post hoc: $F(4,20) = 0.556$; $p = 0.697$; Fig. 1G).

Because oAβ42 in the nM range is known to inhibit LTP without affecting BST, we investigated the effect of 200 pM oAβ42 on BST. Although fEPSP slope was not modified by 200 pM oAβ42, we found a significant increase of fiber volley amplitude ($F(1,18) = 4.746$; $p = 0.043$; Fig. 1G).

Thus, only administration of exogenous 200 pM oAβ42 was capable to induce an increase of hippocampal LTP, whereas 200 nM

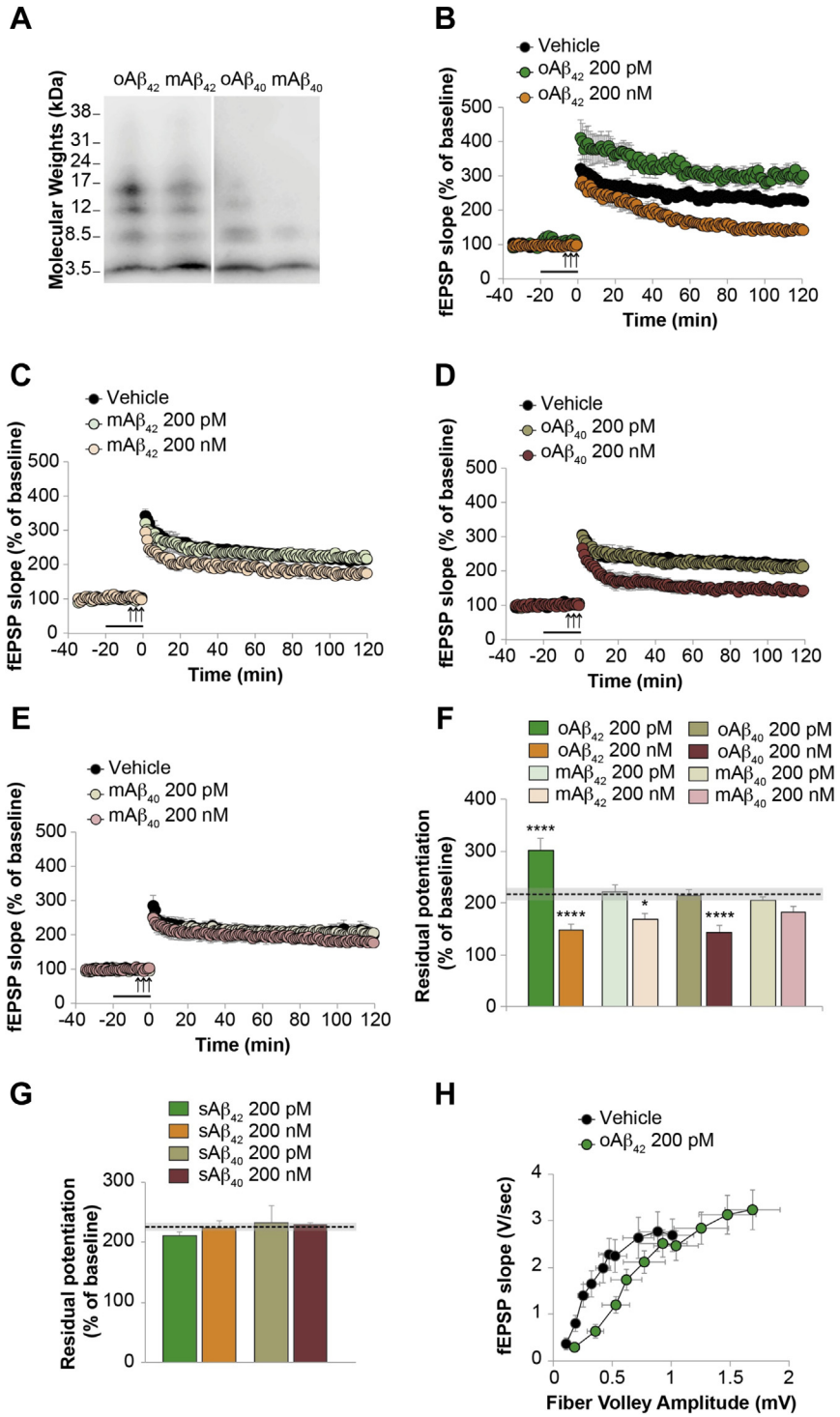

Fig. 1. LTP is impaired by nM concentrations of oAβ42, oAβ40, and mAβ42, whereas it is enhanced by pM concentrations of oAβ42. (A) WB analysis of 200 nM different human Aβ solutions shows different bands corresponding to monomers, dimers, trimers, and tetramers. (B) Hippocampal slices perfused for 20 minutes before a strong tetanic stimulation show an improvement of LTP with 200 pM oAβ42, but an impairment with 200 nM oAβ42, (C) 200 nM mAβ42, or (D) 200 nM oAβ40 (n = 7 slices for each condition from 6 to 7 animals). (E) mAβ40 does not affect LTP either at 200 pM or 200 nM (n = 7 slices for each condition from 5 to 6 animals). Arrows indicate tetanus delivery and horizontal bar Aβ perfusion. (F) Graph shows the residual potentiation obtained by averaging the last 5 recording time points of LTP (from 116th to 120th min after tetanus) from slices treated as in (B–E). Dotted line with shadow area represents the average ±SEM of residual potentiation in tetanized vehicle-treated slices. (G) Scrambled Aβ42 or Aβ40 at 200 nM or 200 pM does not affect LTP compared with tetanized slices treated with vehicle (n = 5 slices for each condition from 4 to 5 animals). (H) oAβ42 200 pM does not modify fEPSP slope but induces an increase of fiber volley amplitude during BST assessment. Data expressed as average ±SEM. **** $p < 0.0001$; * $p < 0.05$. Abbreviations: BST, baseline synaptic transmission; fEPSP, field excitatory postsynaptic potential; LTP, long-term potentiation; mAβ, monomeric amyloid-beta; oAβ, oligomeric amyloid-beta; SEM, standard error mean.

oAβ42, mAβ42, or oAβ40 impaired it. oAβ42 at 200 pM concentration also enhanced fiber volley amplitude.

3.2. Oligomeric and monomeric human Aβ42 and Aβ40 exert a different effect on hippocampal-dependent spatial memory

Because LTP represents the molecular correlate of learning and memory (Bliss and Collingridge, 1993), we next assessed the effects of different preparations of synthetic human Aβ in hippocampal-dependent memory tested by MWM, a widely used behavioral task to study spatial learning and reference memory (Puzzo et al., 2014). We implanted guide cannulas into the dorsal hippocampi and, after 1-week recovery, animals (n = 10 for each condition) underwent intrahippocampal injections of Aβ preparations as previously described (Puzzo et al., 2012).

During the first 3 days, mice were trained to find a platform hidden beneath the surface of the water and latency, that is, the time needed to reach the platform, was recorded. ANOVA for repeated measures showed a significant difference among groups ($F(4,45) = 5.145$; $p = 0.002$). When analyzing the last point of the spatial learning curve, mice treated with 200 pM oAβ42 needed less time to find the hidden platform compared with vehicle-infused mice with a significant difference in the sixth session ($p = 0.034$; Fig. 2A). On the contrary, mice treated with oAβ42 200 nM showed an impairment of spatial learning because they spent more time to find the platform compared with vehicle-treated mice in the sixth session ($p = 0.001$; Fig. 2A). Treatment with mAβ42 at low concentration did not influence latency, whereas when at 200 nM, mAβ42 was capable of impairing spatial learning ($p = 0.049$; Fig. 2A).

Then, we assessed reference memory with the probe test, performed during the fourth day. The platform was removed and mice were allowed to search for 60 seconds. The amount of time spent in each quadrant of the maze was evaluated. In each experimental group, we first compared the time spent in the TQ, where the platform was located during training, with other quadrants to verify whether mice had reference memory. All the groups spent significantly more time in the TQ compared with other quadrants ($p < 0.0001$), except when mice were treated with 200 nM oAβ42 ($p = 0.188$; Fig. 2B). Planned comparison indicated that mice treated with 200 pM oAβ42 spent more time in exploring the TQ ($p = 0.027$ vs. vehicle), whereas mice treated with 200 nM oAβ42 spent less time in exploring the TQ ($p = 0.048$ vs. vehicle), confirming the opposite effects of low and high doses of oAβ42 in reference memory (Fig. 2B). No differences were recorded in mice treated with 200 pM mAβ42 or 200 nM mAβ42, although the latter impaired LTP and spatial learning.

A visible platform trial did not reveal any significant difference in the time to reach the platform among the groups during the 4 sessions of the task ($F(4,45) = 0.282$; $p = 0.888$; Fig. 2C).

Spatial learning was different among mice previously treated with different preparations of Aβ40 ($F(4,45) = 5.052$; $p = 0.002$). In the sixth trial, mice treated with 200 nM oAβ40 spent more time to find the hidden platform ($p = 0.008$ vs. vehicle; Fig. 2D), whereas no differences were found among treatments with other Aβ40 and

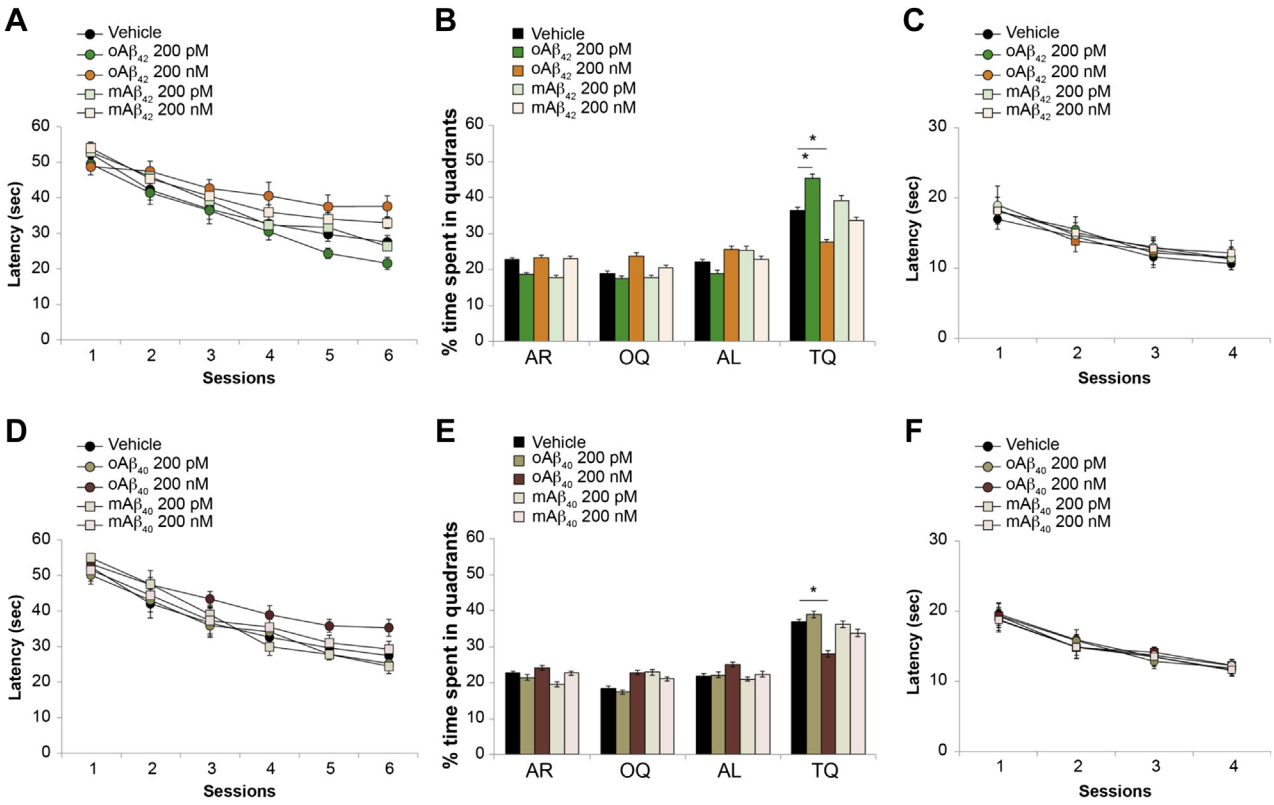

Fig. 2. High concentrations of oAβ42 and oAβ40 impair spatial learning and reference memory, whereas only low concentrations of oAβ42 enhance cognition. (A) Bilateral intrahippocampal injections of 200 nM oAβ42 and mAβ42 20 minutes before training increase the time to reach the platform in the MWM test. Conversely, latency is decreased in mice injected with 200 pM oAβ42, whereas 200 pM mAβ42 does not affect spatial learning. (B) The probe trial shows that administration of 200 nM or 200 pM oAβ42 induces a reduction or an increase of the % of time spent in TQ, respectively. mAβ does not affect reference memory regardless of the concentration used. (C) The visible platform trial does not reveal significant differences in the latency to reach the platform among groups. (D) Infusions of 200 nM oAβ40 20 minutes before training increase the time to reach the platform in the MWM test, whereas other Aβ40-based solutions do not affect latency. (E) The probe trial confirms that only 200 nM oAβ40 affects reference memory because it induces a reduction of the % of time spent in TQ. (F) The visible platform trial does not reveal significant differences among groups. Data expressed as average ±SEM. * $p < 0.05$. Abbreviations: AR, adjacent right; AL, adjacent left; mAβ, monomeric amyloid-beta; oAβ, oligomeric amyloid-beta; MWM, morris water maze; OQ, opposite quadrant; SEM, standard error mean; TQ, target quadrant.

vehicle ($p > 0.05$). Analyses of the probe trial showed that reference memory was impaired only in animals treated with oAβ40 200 nM that spent the same amount of time in TQ versus other quadrants ($p = 0.075$), whereas mice treated with vehicle or other Aβ40 preparations were able to remember the location of the platform (time spent in TQ vs. other quadrants: $p < 0.001$; Fig. 2E). Planned comparison confirmed that 200 nM oAβ40 induced a reduction of the time spent in TQ ($p = 0.027$ vs. vehicle; Fig. 2E), whereas other preparations were ineffective ($p > 0.05$).

A visible platform trial did not reveal any significant difference in the time to reach the platform among the groups during the 4 sessions of the task ($F(4,45) = 0.077$; $p = 0.989$; Fig. 2F).

Thus, administration of exogenous 200 nM oAβ40 impaired spatial learning and reference memory, whereas other Aβ40 preparations did not influence it.

3.3. Endogenous oligomers of Aβ42 are needed for synaptic plasticity and memory

After the evaluation of the effects induced by exogenous administration of human Aβ per se, we aimed to clarify the role of endogenous Aβ in synaptic plasticity. To achieve a suppression of all the endogenous Aβ species, we used a monoclonal antibody (M3.2 mAb) able to recognize rodent Aβ40 and Aβ42 with high affinity (Morales-Corraliza et al., 2009, 2013; Wesson et al., 2013) and specificity (Palmeri et al., 2017; Puzzo et al., 2011; Ricciarelli et al., 2014). Thus, we performed rescue experiments by treating hippocampal slices with M3.2 mAb concurrently with different preparations of 200 pM human Aβ, not recognized by the rodent antibody. We first confirmed that perfusion of hippocampal slices with M3.2 mAb (2 μg/mL) for 20 minutes before tetanizing Schaffer collateral fibers inhibited CA3/CA1 LTP (n = 6/6 slices from 5/4 mice; $F(1,10) = 47.954$; $p < 0.0001$ vs. vehicle; Fig. 3A). Rescue experiments (n = 6 slices from 4-5 mice for each condition) showed that only 200 pM oAβ42 was able to restore the M3.2 mAb-induced LTP deficit ($F(1,10) = 35.069$; $p < 0.0001$ vs. M3.2 mAb; $F(1,10) = 0.331$; $p = 0.578$ vs. vehicle), whereas other preparations were ineffective (M3.2 mAb + mAβ42: $F(1,10) = 0.720$; $p = 0.413$; M3.2 mAb + oAβ40: $F(1,10) = 1.383$; $p = 0.267$; M3.2 mAb + mAβ40: $F(1,10) = 0.444$; $p = 0.520$; vs. M3.2 mAb; Fig. 3A). Analyses of residual potentiation of the last 5 recording time points confirmed that only 200 pM oAβ42 rescued the M3.2-induced impairment of LTP ($F(5,30) = 26.522$; $p < 0.001$ among all; Bonferroni's $p = 0$ for M3.2 mAb vs. vehicle and M3.2 mAb + oAβ42 vs. M3.2 mAb; Fig. 3B).

We then focused on the role of endogenous Aβ in memory. As for electrophysiological experiments, mice (n = 10 for each condition) were treated with M3.2 mAb (2 μg/μL in a final volume of 1 μL over 1 minute) or vehicle. Twenty minutes after intrahippocampal injections, mice underwent MWM. As previously demonstrated, we confirmed that blocking endogenous Aβ impaired spatial learning and reference memory. A significant difference was found analyzing

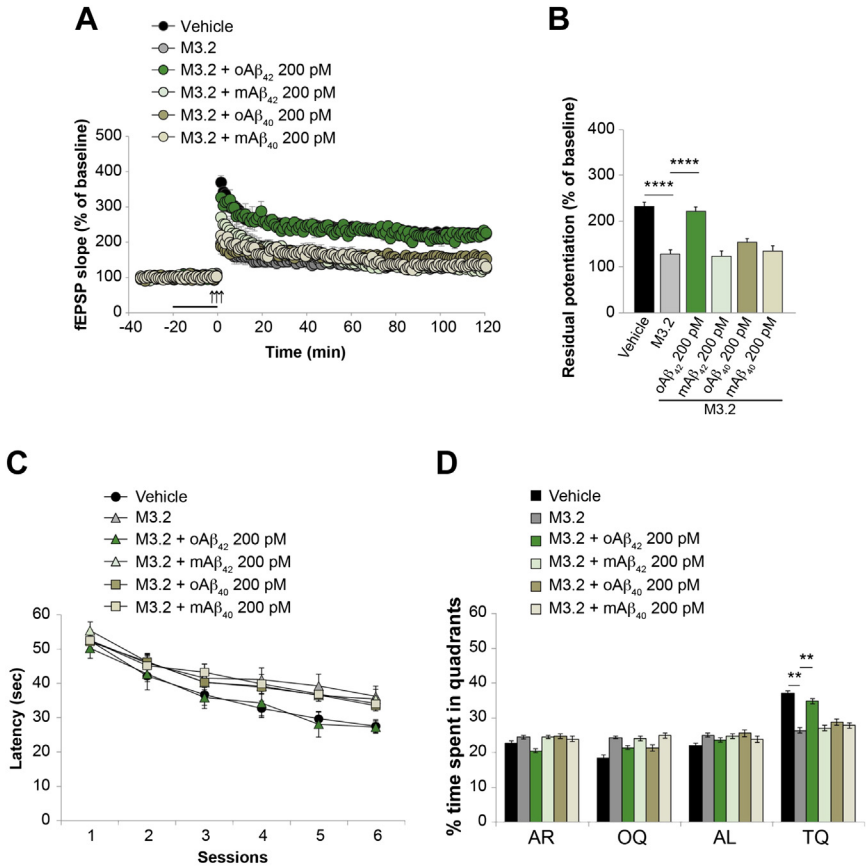

Fig. 3. oAβ42 200 pM rescues the reduction of LTP and memory induced by depletion of endogenous Aβ. (A) The reduction of LTP induced by 20 minutes perfusion with the antimurine Aβ monoclonal antibody M3.2 mAb (2 μg/μL) is rescued by a concomitant perfusion with 200 pM oAβ42 ($p = 0$), but not mAβ42, oAβ40, or mAβ40. Arrows indicate tetanus delivery and horizontal bar drugs perfusion. (B) Graph bars represent the residual potentiation (average of the last 5 recording time points). (C) Bilateral intrahippocampal injections of M3.2 mAb (2 μg/mL, in a final volume of 1 μL over 1 minute) 20 minutes before the session increase latency in the MWM test. Concomitant perfusion with oAβ42 200 pM rescues spatial learning, normalizing the time needed to find the hidden platform. Conversely, 200 pM mAβ42, oAβ40, or mAβ40 administration is not able to restore the M3.2 mAb-induced increase of latency. (D) The probe trial shows a reduction in time spent in TQ in mice treated with M3.2 mAb compared with vehicle. Memory is normal when mice are infused with M3.2 mAb and 200 pM oAβ40, but not mAβ42, oAβ40, or mAβ40. Data expressed as average ±SEM. **** $p < 0.0001$; ** $p < 0.005$. Abbreviations: AR, adjacent right; AL, adjacent left; LTP, long-term potentiation; mAβ, monomeric amyloid-beta; oAβ, oligomeric amyloid-beta; MWM, morris water maze; OQ, opposite quadrant; SEM, standard error mean; TQ, target quadrant.

the overall training among groups ($F(5,54) = 4.380$, $p = 0.002$). In particular, analyses of the last session showed that mice previously treated with M3.2 mAb spent a longer time to find the hidden platform compared with vehicle-infused mice ($p = 0.007$; Fig. 3C). The probe trial showed that reference memory was impaired in M3.2 mAb-treated animals as they spent the same time in TQ versus other quadrants ($t(18) = 0.920$, $p = 0.370$); consistently, the time spent in TQ was significantly different when compared with that of vehicle-infused mice ($p = 0.001$ vs. vehicle; Fig. 3D).

To understand whether different species and aggregation status of the peptide specifically mediated the learning impairment, mice were infused with 200 pM oAβ42, mAβ42, oAβ40, or mAβ40, in addition to M3.2 mAb. Consistently with our electrophysiological findings, only 200 pM oAβ42 was capable to rescue behavioral deficits both during the hidden training ($p = 0.006$ vs. M3.2 mAb; $p = 0.448$ vs. vehicle; Fig. 3C) and the probe trial (TQ vs. other quadrants: $t(18) = 6.897$, $p < 0.0001$; % time spent in TQ: $p = 0.006$ vs. M3.2 mAb; $p = 0.448$ vs. vehicle; Fig. 3D). Conversely, mAβ42, oAβ40, or mAβ40 did not rescue M3.2 mAb-induced spatial learning and reference memory. In fact, the memory deficit persisted in the hidden test during the fifth and the sixth session ($p > 0.05$ when comparing M3.2 mAb + mAβ42 or oAβ40 or mAβ40 vs. M3.2 mAb; Fig. 3C), and the probe trial ($p > 0.05$ when comparing TQ vs. other quadrants in mice treated with M3.2 mAb + mAβ42 or oAβ40 or mAβ40; $p > 0.05$ when comparing % time spent in TQ between in M3.2 mAb vs. M3.2 mAb + mAβ42 or oAβ40 or mAβ40; Fig. 3D).

3.4. The content of oligomers and monomers depends on the concentration of Aβ solutions

Given that only oAβ42 was able to either stimulate or impair LTP and memory depending on concentration, we investigated whether this biphasic effect was associated with a difference in Aβ total concentration and/or changes in the relative content of monomers and oligomers.

A preliminary sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the 2 synthetic preparations confirmed that when at 200 pM, both monomers and oligomers were present although in different proportions (Fig. 4A).

TEM carried out by negative staining technique (Ahmed et al., 2010) was then performed to visualize oAβ42 solutions at 200 nM and 200 pM. Both preparations exhibited negatively stained Aβ with variable size and morphology resembling monomer-like (round shape and diameter ≤ of 5 nm) and oligomer-like (oval shape and diameter ≥ of 10 nm) profiles of Aβ (Fig. 4B). Quantification of monomer-like and oligomer-like species showed that 200 nM and 200 pM solutions displayed different proportions of monomers and oligomers (Fig. 4C). In particular, comparisons of monomer-like and oligomer-like species of the 2 preparations revealed that the percentage of identifiable monomer-like elements in 200 pM solution was significantly higher than that in 200 nM solution (78.21 ± 2.88 vs. 63.49 ± 4.00%; Mann-Whitney test: $p = 0.018$), whereas the percentage of identifiable oligomer-like species was significantly lower in 200 pM solutions than that in 200 nM solutions (21.78 ± 2.88 vs. 36.50 ± 4.00%; $p = 0.018$), as confirmed by the increase of the monomers/oligomers ratio in 200 pM oAβ42 solution (1.91 ± 0.22 vs. 4.17 ± 0.65; Mann-Whitney test: $p = 0.005$). Thus, the content of monomer and oligomer forms depended on Aβ concentration.

4. Discussion

This work was inspired by previous observations indicating that Aβ exerts an opposite effect on synaptic plasticity and memory depending on its concentration (Puzzo et al., 2012). Here we have

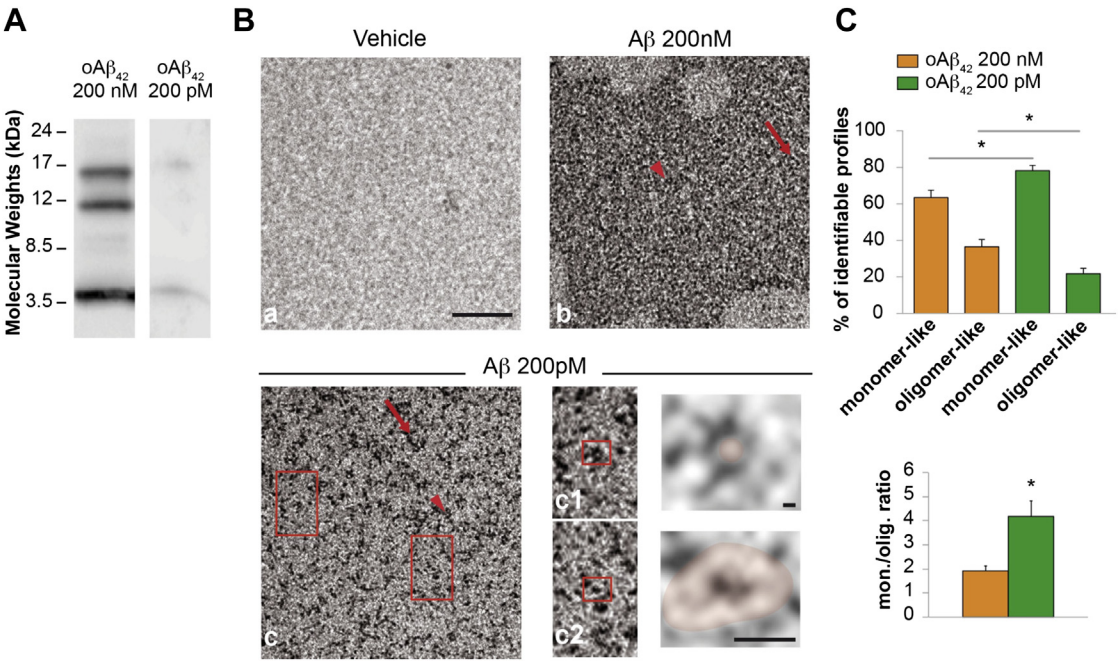

Fig. 4. oAβ42 synthetic preparations at 200 nM and 200 pM show a different content of monomers and oligomers. (A) WB of synthetic human 200 pM oAβ42 shows the presence of both monomers and oligomers. (B) TEM showing ultramicroscopic fields of grids incubated with vehicle, 200 nM or 200 pM solutions of oAβ42 and processed with negative staining technique. In b and c, arrowheads and arrows indicate monomer-like and oligomer-like elements, respectively. Enlarged framed regions showing representative examples of a monomer-like (round shape and diameter ≤ of 5 nm; c1) and an oligomer-like element (oval shape and diameter ≥ of 10 nm; c2). Colored shadows in c1 and c2 resemble the round and oval shape of monomer-like and oligomer-like elements, respectively. Bars: 100 nm for a, b, d; 1 nm for c1 subpanel; and 5 nm for c2 subpanel. (C) The % of identifiable monomers is higher, whereas the % of identifiable oligomers is lower in the 200 pM versus the 200 nM oAβ42 solution. Monomers/oligomers ratio is higher in the 200 pM solution. Data expressed as mean ± SEM. * $p < 0.05$. Abbreviations: oAβ, oligomeric amyloid-beta; SEM, standard error mean; TEM, transmission electron microscopy; WB, western blot.

demonstrated that this Aβ biphasic effect is influenced by different isoforms or aggregation status of the peptide.

While it is commonly accepted that high levels of Aβ42 oligomers are detrimental for synaptic activity (Hayden and Teplow, 2013), monomeric forms of Aβ42 are thought to exclusively have neuroprotective functions (Giuffrida et al., 2009). However, in this article, we showed that when at high concentration, Aβ42 monomers impair synaptic plasticity and memory. This suggests that monomers are also able to exert a neurotoxic action when at high concentrations, in agreement with findings suggesting a role for monomers in AD (Guglielmotto et al., 2014; Manassero et al., 2016). Although we cannot exclude that the few oligomers present in our mAβ42 preparations might be responsible of the synaptotoxic effect, or that mAβ42 might undergo further oligomerization once injected into the hippocampus, our data suggests that caution is needed when designing anti-Aβ therapies exclusively directed against oligomers sparing monomers, as they might not be a safe and effective strategy as expected.

We then studied the effects of Aβ40, which is known to be the most represented Aβ species in the brain but with low propensity to oligomerize (Sgourakis et al., 2007). In our experimental conditions, 200 nM oAβ40 impaired synaptic plasticity and memory, whereas when monomers prevailed (mAβ40), the solution was ineffective. Thus, oAβ40 was capable of inhibiting LTP in a comparable manner to what observed for oAβ42, suggesting an isoform effect, even if the oAβ40 solution contains lower levels of oligomers in respect to oAβ42. Although we cannot affirm that oAβ40 oligomers are more toxic than those of oAβ42, it appears that Aβ solutions inducing a greater LTP impairment (oAβ42 and oAβ40) contain a higher amount of dimers, as shown in Fig. 1A. Conversely, mAβ42, which induces a weaker neurotoxic effect, contains few dimers and a higher quantity of trimers and tetramers. This is consistent with previous studies reporting a different oligomerization behavior for Aβ40 and Aβ42, with a distinct distribution of low order oligomers due to differences in their dimer equilibrium structures (Côté et al., 2012). Photoinduced cross-linking of unmodified proteins experiments showed that Aβ42 tends to form “paranuclei” (e.g., pentamers and examers) which, in turn, can oligomerize to generate structures of higher order. Conversely, Aβ40 does not form paranuclei and is more prone to exist as a mixture of monomers, dimers, and tetramers, rather than trimers and larger oligomers (Bitan et al., 2001, 2003), as confirmed by studies exploiting ion mobility coupled with mass spectrometry (Bernstein et al., 2009). Dimers and tetramers thus represent a key aggregation status for Aβ40 due to their stability and resistance to further monomer or dimer addition in the tetrameric state. This might be related to the fact that Aβ40 has a more closed planar angle in the tetrameric form compared with Aβ42. Consequently, the latter is able to add another monomer to its tetrameric structure leading to pentameric paranuclei formation (Bernstein et al., 2009). Based on these observations, we can speculate that dimers are mainly responsible of the negative effect exerted by oAβ40 on synaptic plasticity and memory. Accordingly, Aβ40 dimerization increased β-strand propensity and toxicity (Ono et al., 2009) and Aβ dimers have been recognized as the smallest oligomeric species increasing in the AD brain (Mc Donald et al., 2010; Shankar et al., 2008). Moreover, a recent work has evidenced that an increased production of soluble Aβ dimers, but not monomers, plaques or other forms of insoluble Aβ, is sufficient to exert a detrimental effect on synaptic plasticity and memory in TgDimer mice (Müller-Schiffmann et al., 2016). In any case, the oligomerization and the consequent toxicity of the peptide might be altered by different physicochemical conditions and further works, out of the scope of the present article, are needed to better understand the different effects exerted by single Aβ aggregates in synaptic plasticity and memory.

On the other hand, when studying the positive effect of Aβ at picomolar concentrations, we found that only 200 pM oAβ42 enhanced LTP and memory, in agreement with previous findings (Puzzo et al., 2008), whereas oAβ40 or monomer enriched solutions, that is, mAβ42 and mAβ40, did not exert any effect. Interestingly, oAβ42 also exerted an enhancing effect on fiber volley amplitude, which is an index of presynaptic recruitment, in agreement with previous works indicating that low concentrations of oAβ42 enhances neurotransmitter release (Koppensteiner et al., 2016; Puzzo et al., 2008). Consistently, the suppression of LTP and memory induced by the murine anti-Aβ antibody M3.2 mAb, was specifically rescued by 200 pM oAβ42 (Garcia-Osta and Alberini, 2009; Morley et al., 2010; Puzzo et al., 2011), excluding that mAβ42 or Aβ40 at these conditions and concentrations played a role in synaptic plasticity and memory in the healthy brain.

Thus, our study highlighted a different contribution of soluble Aβ in synaptic plasticity and memory depending on its species, aggregation state, and concentration. This is a crucial point, considering that soluble Aβ aggregates extracted from AD brains are highly heterogeneous and it is not clear whether synthetic preparations used for in vitro and in vivo research reproduced the physicochemical characteristics of Aβ in the human brain. Our functional results might be due to the structural differences between Aβ42 and Aβ40. In fact, the difference in Aβ42 and Aβ40 monomeric structures are thought to induce oligomers formation via different pathways, resulting in larger Aβ42 oligomers. However, whether this different oligomeric conformation is responsible for the different effects exerted by our preparations is unknown and out of the scope of the present article. Here, we can confirm that Aβ42 is much prone to oligomerization, consistently with previous studies demonstrating that the presence of 2 additional amino acid residues at Aβ42 C terminus confers more rigidity to the peptide structure stimulating oligomers formation (Sgourakis et al., 2007). Moreover, our experience indicates that, in a 200 nM solution, the quantity of oligomers increased after few hours of incubation in ACSF and that the oligomerization process is faster at RT. To exclude that the detection of oligomers was due to the presence of sodium dodecyl sulfate, which has been shown to enhance ex vivo aggregation of Aβ (Esparza et al., 2016), we have performed WB in nondenaturating nonreducing conditions and confirmed our findings by TEM analyses.

Notably, in our conditions, both monomers and oligomers are present when synthetic Aβ42 or Aβ40 were dissolved in ACSF, even if in different proportions. Indeed, the dynamic rearrangement of monomers/oligomers in the physiological brain environment is still a matter of debate (Bemporad and Chiti, 2012). The most diffuse opinion is that Aβ is secreted in monomeric form during synaptic activity, which strengthens the idea to restrict its physiological effects to monomers. On the other hand, the aggregation state is likely to depend on different physicochemical characteristics that are not perfectly reproducible in in vivo and in vitro studies (Hayden and Teplow, 2013). However, some works have demonstrated that a certain degree of oligomerization is likely to occur whenever the peptide is present in a solution at 37 °C and pH 7.4 (El-Agnaf et al., 2000), as in the physiological brain environment. On the contrary, recent studies performed by single-molecule fluorescence imaging have evidenced that Aβ remained in monomeric forms up to a 3 μM concentration, which is much higher than the one detected in AD brains, leading to the conclusion that, when present, oligomers should be released as such and are not the result of monomers aggregation (Nag et al., 2011).

TEM analyses confirmed that our oAβ42 preparations contained both monomers and oligomers, although their quantity depended on the final concentration of the solution with an increase of the monomers/oligomers ratio when at 200 pM concentration. This,

together with the biphasic effect exerted by oAβ42, raises 2 major points: (1) the presence of oligomers is inversely proportional to the concentration of the solution; (2) the presence of oligomers, even if limited, is necessary for Aβ to exert its positive effect on the synapse, challenging the theory that oligomers only have a deleterious effect. Intriguingly, oligomers have been detected in the CSF and brain of healthy people throughout life (Lesné et al., 2013), with a prevalence of trimers during childhood and adolescence, when plastic changes are highly effective.

As for monomers, our findings suggest that (1) in our experimental conditions, it is not possible to obtain a pure monomeric preparation, in contrast with previous studies (Giuffrida et al., 2009); (2) Aβ42 solutions enriched in monomers impaired synaptic plasticity and memory when at high concentrations, whereas they do not elicit any effect when at low physiological concentrations. This does not exclude that they might exert a physiological function through different mechanisms, for example by interfering with cellular mechanisms aimed at controlling apoptosis, survival, and energetic metabolism of the cell.

5. Conclusion

Our findings have demonstrated that the presence of oligomers is crucial either in physiological or pathological conditions. Intriguingly, we have previously demonstrated that exposure to 200 pM Aβ42 exerts opposite effects depending on the time of exposure, with short exposures enhancing synaptic plasticity and memory, and longer exposures reducing them (Koppensteiner et al., 2016). Thus, it is plausible that Aβ42 oligomers are not toxic per se but only when present in excessive quantities or for a prolonged time.

Although we cannot exclude that different methods of Aβ preparation or different concentrations might induce different effects on synaptic plasticity, these findings should be taken into consideration either when studying the physiological role of the peptide or designing therapies targeting Aβ in AD.

Disclosure statement

The authors have no actual or potential conflicts of interest.

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