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Hormetic effect of amyloid-beta peptide in synaptic plasticity and memory

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Abstract

One of the hot topics in Alzheimer's disease research field is the "amyloid hypothesis" postulating that the increase and deposition of beta-amyloid peptides (Aβ) is the main pathogenetic factor. However, antiamyloid-based therapies have so far been a failure and, most importantly, growing evidences suggest that Aβ has important physiologic functions. Based on our previous findings demonstrating that low concentrations of Aβ enhanced both synaptic plasticity and memory, whereas high concentrations induced the well-known impairment of cognition, here we show that Aβ acts on hippocampal long-term potentiation and reference memory drawing biphasic dose-response curves. This phenomenon, characterized by low-dose stimulation and high-dose inhibition and represented by a U-shaped or inverted-U-shaped curve, resembles the characteristics of hormesis. The Aβ double role raises important issues on the use of Aβ level reducing agents in Alzheimer's disease.

Keywords: Amyloid-beta; Hormesis; Alzheimer's disease; Synaptic plasticity; Memory; Hippocampus; Low doses

1. Introduction

The term hormesis refers to a biphasic dose-response phenomenon characterized by low-dose stimulation and high-dose inhibition represented by a bell-shaped, J-shaped, U-shaped or inverted-U-shaped curve, depending on the parameter measured (Calabrese and Baldwin, 2001, 2002). This phenomenon was shown for the first time by Hugo Schultz, over a century ago (Kendig et al., 2010), who noticed that some chemicals stimulated or inhibited yeast growth and respiration depending upon the applied doses. The general concept of low-dose-stimulation versus high-dose-inhibition was then known as the Arndt-Schulz' law but, given the poor knowledge of its scientific bases, the growing fame of classical toxicology, and the association with homeopathy, hormesis fell into disuse (Calabrese, 2009). Recently, however, hormesis has gained a renewed success and several studies have been published (for reviews see Calabrese, 2008a, 2008b, 2010). As pointed out by these studies, the definition of hormesis is not limited to the biphasic character of the response, but implies a specific adaptative biological effect of a substance on a variable acting by uni- or polymodal mechanisms and characterized by specific qualitative and quantitative features (Calabrese and Baldwin, 2002). Indeed, according to hormesis principles, biological systems are damaged by high doses of a stressor whereas the same substance, at low doses, is able to positively stimulate several physiologic functions from cell growth to cognition. Several, if not all, physiological molecules are likely to present a hormetic effect, exhibiting opposite effects at high or low concentrations. Few examples include: (1) glutamate, the principal excitatory neurotransmitter, which stimulates synaptic plasticity and memory at physiological low doses (Rezvani, 2006), whereas at higher doses becomes toxic and is involved in pathologies such as stroke (Wieloch, 1985; Choi, 1988) and Alzheimer's disease (AD) (Mattson, 2004, 2008a); (2) glucocorticoids, whose effect on memory might be described as

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an inverted-U shape curve (Lupien, 2005); (3) nitric oxide, the par excellence "Janus" molecule, which might be neurotoxic or neuroprotective (Calabrese, 2001b; Duncan and Heales 2005; Lowenstein et al. 1994; Mattson 2008b; Puzzo et al., 2006), and many others (Calabrese and Baldwin, 1998). Here, we focused on amyloid-beta (Aβ), a peptide widely known because it is produced in high amounts during AD. Aβ derives from the cleavage of amyloid-precursor protein (APP) that undergoes a complex proteolytic processing catalyzed by α-β- and γ-secretases generating several fragments (Chow et al., 2010; Mattson, 1997). The normal function of APP remains poorly understood, although some fragments might have physiological properties (Chow et al., 2010; Randall et al., 2010). Aβ, instead, is considered a toxic fragment (Hardy and Selkoe, 2002) causing synaptic dysfunction and memory impairment (Selkoe, 2002). However, the peptide is normally produced in the healthy brain and growing evidence indicates that it might have a physiologic function. Indeed, APP, and β- and γ-secretases have been shown to be involved in synaptic plasticity and memory (Dawson et al., 1999; Laird et al., 2005; Ma et al. 2007; Phinney et al., 1999; Saura et al., 2004; Seabrook et al., 1999; Wang et al., 2008) and Aβ itself, at picomolar concentrations, is likely to play a neurotrophic and neuroprotective role (Giuffrida et al., 2010; López-Toledano and Shelanski, 2004; Plant et al., 2003; Yankner et al., 1990). Interestingly, Cirrito et al. (2008) have demonstrated that synaptic transmission induces an increase of Aβ generation and release, and Kamenetz et al. (2003) suggested that endogenous Aβ might regulate synaptic plasticity with a feedback mechanism. Recently, we have demonstrated that low picomolar amounts of exogenously applied Aβ42 enhance synaptic plasticity in vitro and memory in vivo (Puzzo et al., 2008) and that endogenous Aβ is necessary for synaptic plasticity and memory (Puzzo et al., 2011). Here, we show that dose-response curves for the effect of Aβ on hippocampal synaptic plasticity and memory resemble the hormetic characteristics, raising several issues when designing effective and safe approaches to AD therapy.

2. Methods

2.1. Animals

We used 3–4 month-old wild type (WT) mice (C57BL/6), obtained from a breeding colony kept in the animal facility of the Department of Bio-Medical Sciences, Section of Physiology (University of Catania). The animals were maintained on a 12-hour light/dark cycle (with light onset at 6:00 A.M.) in temperature and humidity-controlled rooms, and food and water were available ad libitum.

2.2. Aβ preparation

Aβ42 was prepared as previously described (Puzzo et al., 2008). A peptide film was obtained suspending the peptide (American Peptide, Sunnyvale, CA, USA) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP; Sigma, St. Louis, MO, USA). Dimethylsulfoxide (DMSO; Sigma) was added and the compound was sonicated for 10 minutes, aliquoted, and stored at −20 °C. Twenty-four hours before the experiment, an aliquote of dimethylsulfoxide-Aβ was added, phosphate-buffered saline (PBS; 5 mM), vortexed for 30 seconds, and incubated at 4 °C. This peptide contained both monomers and oligomers, as demonstrated by routinely characterization using Western blot analysis (Puzzo et al., 2008). The following doses of peptides were prepared in artificial cerebrospinal fluid (ACSF) prior to the administration: 2 pM, 20 pM, 200 pM, 2 nM, 20 nM, 200 nM, 2 μM, and 20 μM. The final amounts of exogenous peptide injected in vivo into each hippocampus ranged from $9 \times 10^{(-15)}$ to $9 \times 10^{(-8)}$ grams.

2.3. Electrophysiological measurements

Electrophysiological recordings were performed as previously described (Puzzo et al., 2008). Briefly, transverse hippocampal slices (400 μm) were cut and maintained in a recording chamber at 29 °C, continuously bubbled with 95% O_2 and 5% CO_2 and perfused with artificial cerebrospinal fluid (composition in mM: 124.0 NaCl, 4.4 KCl, 1.0 Na2HPO4, 25.0 $NaHCO_3$, 2.0 $CaCl_2$, 2.0 $MgSO_4$, 10.0 glucose). Field excitatory postsynaptic potentials (fEPSP) were recorded by placing the stimulating electrode at the level of the Schaeffer collateral fibers and the recording electrode in the CA1 stratum radiatum. Basal synaptic transmission (BST) was assayed by plotting the stimulus voltages against slopes of fEPSP. For long-term potentiation (LTP) experiments, a 15-minute baseline was recorded every minutes at an intensity that evokes a response approximately 35% of the maximum evoked response. LTP was induced using θ-burst stimulation (4 pulses at 100 Hz, with the bursts repeated at 5 Hz and each tetanus including 3 ten-burst trains separated by 15 seconds). Responses were recorded for 2 hours after tetanus and measured as fEPSP slope expressed as percentage of baseline. Data were analyzed as residual potentiation given by the average among the last 5 recording points.

2.4. Infusion technique

Mice were implanted with a 26-gauge guide cannula into the dorsal part of the hippocampi (coordinates: Posterior = 2.46 mm, Lateral = 1.50 mm to a depth of 1.30 mm) (Paxinos, 1998). Cannulas were fixed to the skull with acrylic dental cement (3M ESPE AG, Seefeld, Germany). After a 6–8-day recovery, mice were handled once a day for 3 days before behavioral experiments. Then, 20 minutes before each test, they were bilaterally injected with different doses of Aβ42 or vehicle 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. A solution of 4% methylene blue was infused into the cannulas for histological localization at the end of the behavioral session.

2.5. Morris water maze

Morris water maze experiments were performed as previously described (Puzzo et al., 2008; Trinchese et al., 2004). Mice were trained in 2 daily sessions (4 hours apart), each consisting of 3 trials (1 minute each), for 3 days, so that they had to rely on long-term memory of platform location of previous days. Time required to reach the hidden platform (latency) was recorded. After the training, the platform was removed to test the retention of spatial memory and 4 probe trials were performed. The maze was divided into 4 quadrants and the percentage of time spent in quadrants (target quadrant [TQ] = quadrant that previously contained the platform; non-target quadrants: AL = adjacent left; AR = adjacent right; OQ = opposite quadrant) was recorded and analyzed with a video tracking system (Netsense srl, Catania, Italy).

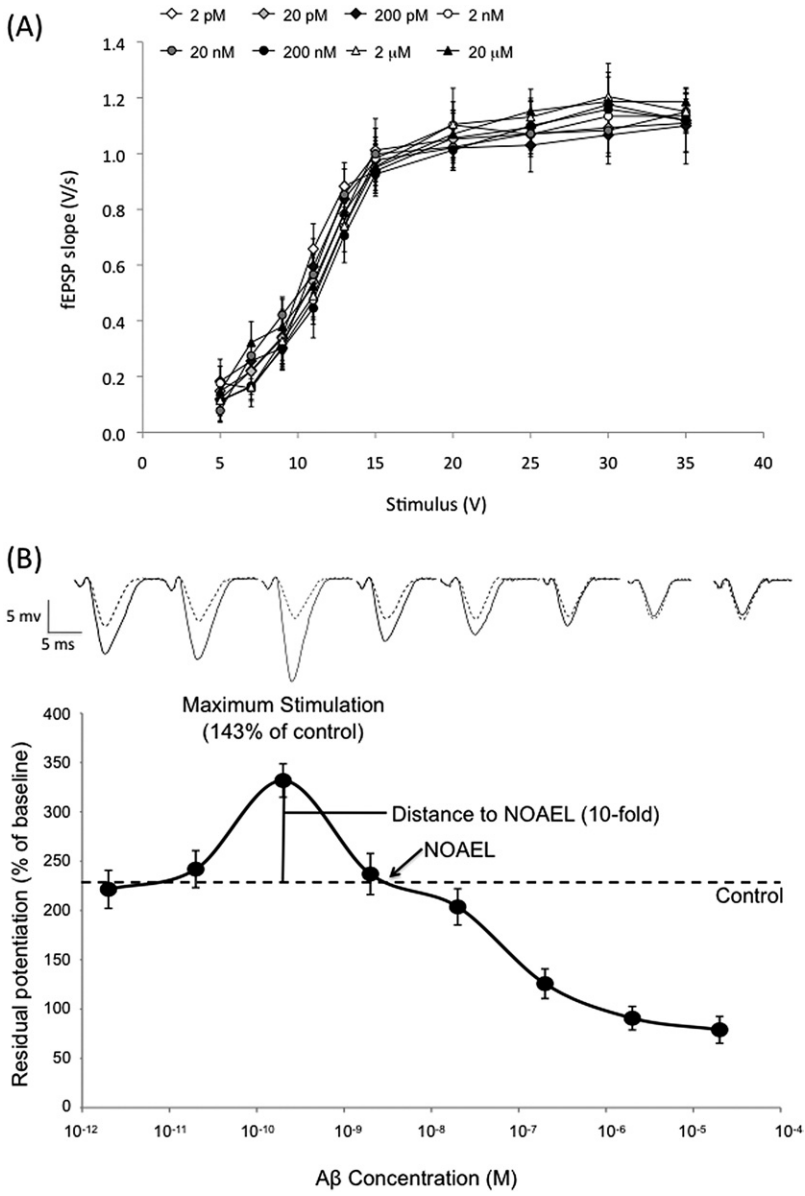

Fig. 1. Beta-amyloid peptide (Aβ)42 has a hormetic effect on hippocampal long-term potentiation (LTP). Dose-response curve for the effect of Aβ42 on CA1-LTP indicates that the peptide has a hormetic effect. (A) Basal synaptic transmission (BST) did not change after treatment with different concentrations of Aβ, whereas (B) LTP followed a hormetic distribution. The stimulatory hormetic area ranges from 20 pM to 2 nM with a maximum stimulatory response (142.46% of control) at 200 pM and distance to no observed adverse effect level (NOAEL) equal to 10-fold. In the upper part, traces of LTP before (dotted line) and after tetanus. The dotted horizontal line corresponds to treatment with vehicle in this and following figures. The residual potentiation was calculated by averaging the last 5 minutes of LTP.

After the probe trial, visual, motor, and motivation skills were also tested by measuring the time needed to reach a visible platform marked with a green flag and positioned randomly from trial to trial.

2.6. Statistics

For all experiments investigators were "blind" with respect to treatment. Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed with 2-way analysis of variance (ANOVA) (with repeated measures) for LTP and Student *t* test (pairwise comparisons) for behavioral experiments. The level of significance was set for $p < 0.05$.

3. Results

3.1. Aβ has a hormetic effect on synaptic plasticity

We have previously demonstrated that Aβ has opposite effects on LTP, a form of synaptic plasticity that is thought to underlie memory (Bliss and Collingridge, 1993), depending upon its concentration (Puzzo et al., 2008).

Here, to better understand whether Aβ acts on synaptic plasticity and memory in a hormetic fashion, we performed dose-response curves and analyzed quantitative and qualitative hormetic features (Calabrese and Baldwin, 2002) such as: (1) stimulatory and inhibitory hormetic area, because both parameters should be present in a dose-response continuum to define hormesis; (2) maximum stimulatory response, that is generally higher 2-fold of the control, not exceeding the 130%–160%; (3) width of the stimulatory response, that is typically in the 5–100-fold dosage range; (4) no observed adverse effect level (NOAEL), that, while according to the classical threshold model, represents the level of exposure to a substance at which there are not biological effect (this parameter is used to calculate the "safe" concentration of risk agents or drugs not inducing adverse effect compared with control), according to the hormesis model, represents the transition between adaptation and toxicity, to the extent that the stimulatory effect is present for doses below the NOAEL; and (5) distance between NOAEL and the maximum stimulatory response.

Hippocampal slices were perfused with concentrations of Aβ42 ranging from 2 pM to 20 μM. BST, studied by plotting the fEPSP slopes against the increasing stimulus intensity, did not change after perfusion with different doses of Aβ ($n = 5$ slices from 5 mice for each concentration; $F(7,32) = 0.148$, $p > 0.5$; Fig. 1.A).

LTP was recorded at the synapses between Schaeffer collateral fibers and CA1 neurons for 120 minutes after tetanus and residual potentiation was calculated by averaging the last 5 recording points ($n = 6$ slices from 6 mice for each Aβ42 concentration vs. $n = 9$ slices from 9 mice treated with vehicle and interleaved with dose-response experiments). The stimulatory hormetic area identified below the NOAEL (evidenced at the intersection between the hormetic curve and control levels) ranged from 20 pM to 2 nM (width = 100-fold), whereas the adverse effect started above the NOAEL and reached the maximum at 20 μM (Fig. 1B). The low-dose maximum stimulatory response was equal to 142.47% of control (Fig. 1B) and was recorded after perfusion with 200 pM Aβ. The distance between maximum stimulatory response and NOAEL was 10-fold. Altogether, mice treated with concentration of Aβ below the NOAEL showed an improvement of synaptic plasticity compared with mice treated with concentration of Aβ above the NOAEL ($F(1,10) = 75.358$; $p < 0.0001$). Analyses of doses above or below the hormetic curve showed that treatment with doses higher then the maximum inhibitory response (200 μM, 2 mM) did not produce a further LTP impairment compared with 20 μM doses ($F(1,10) = 0.039$, $p = 0.874$; $F(1,10) = 0.001$, $p = 0.985$), whereas slices perfused with doses below 2 pM (200 fM, 20 fM) showed an LTP equal to control (Fig. 1B). Thus, Aβ effect on synaptic plasticity follows a hormetic distribution. For detailed results and statistics for each concentration used see Table 1.

Table 1
Values of long-term potentiation residual potentiation after perfusion of hippocampal slices with different concentrations of beta-amyloid peptide (Aβ) show that the peptide displays a hormetic effect

Aβ dose [a]	Potentiation ± SEM [b]	Statistics [c]
20 fM	229.61 ± 11.81	$F(1,13) = 0.033$; $p = 0.858$
200 fM	227.86 ± 13.44	$F(1,13) = 0.074$; $p = 0.790$
2 pM	221.41 ± 19.20	$F(1,13) = 0.299$; $p = 0.594$
20 pM	241.89 ± 18.75	$F(1,13) = 0.190$; $p = 0.670$
200 pM	331.76 ± 16.89	$F(1,13) = 23.066$; $p < 0.0001$
2 nM	236.94 ± 20.92	$F(1,13) = 1.872$; $p = 0.194$
20 nM	203.61 ± 18.33	$F(1,13) = 3.102$; $p = 0.102$
200 nM	125.74 ± 14.93	$F(1,13) = 29.861$; $p = < 0.0001$
2 μM	90.72 ± 11.89	$F(1,13) = 60.858$; $p = < 0.0001$
20 μM	78.97 ± 13.62	$F(1,13) = 65.740$; $p = < 0.0001$
200 μM	75.41 ± 11.69	$F(1,13) = 75.379$; $p = < 0.0001$
2 mM	79.30 ± 10.46	$F(1,13) = 75.713$; $p = < 0.0001$

[a] Twenty-minute perfusion of hippocampal slices.
[b] Residual potentiation expressed as field excitatory postsynaptic potentials (fEPSP) (% of baseline) ± standard error of the mean (SEM) ($n = 6$ slices for condition).
[c] Fisher-Snedecor distribution (F) and Probability (P) calculated by 2-way analysis of variance (ANOVA) for repeated measures, comparing Aβ concentrations with control.

3.2. Hormetic effect of Aβ on memory

Given that LTP is thought to be associated with learning and memory, we next assessed the effect of different doses of Aβ on memory. We have previously shown that infusion of Aβ42 at 200 pM enhances memory (Puzzo et al., 2008), and it is known that high levels of Aβ, as those found in depositing mouse models, cause an impairment of memory (Trinchese et al., 2004). Here, we performed a dose-response curve to study in detail the possible Aβ42 hormetic effect on hippocampal spatial memory. Cannulas were im-

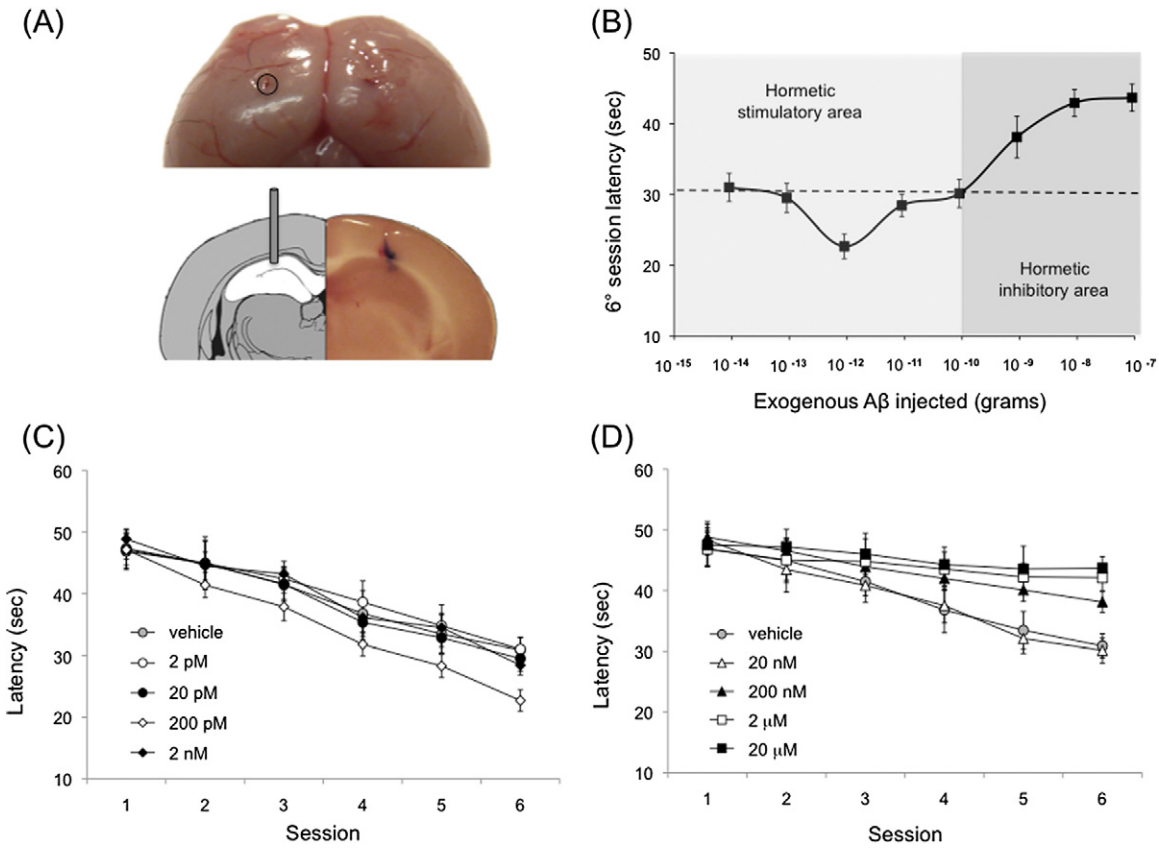

Fig. 2. Beta-amyloid peptide (Aβ)42 has a hormetic effect on cognitive performance tested through the Morris water maze hidden test. (A) Intrahippocampal cannulas were implanted according to the atlas of Paxinos and the tip location was verified through holes position (upper) and methylene blue injections (bottom). (B) Latency at 6th session shows a hormetic shape where it is possible to distinguish the 2 areas of stimulation and inhibition of memory. (C) Treatment with Aβ 200 pM for 20 minutes before training causes a decrease in the time needed to reach the hidden platform at 6th session. (D) High concentrations of Aβ induced an increase of latency, indicating a decrease of memory, at the 5th and 6th sessions.

planted bilaterally into the mouse dorsal hippocampi (Fig. 2A). After 6–8 days, animals were infused with different concentrations of Aβ42 ranging from 2 pM to 20 μm or vehicle, and after 20 minutes tested for reference memory with the Morris water maze, a widely used spatial learning test known to require hippocampal function (Schenk and Morris, 1985). During the training, mice were requested to find a hidden platform beneath the surface of the water. Plotting the results as latency versus Aβ concentration, a hormetic-shape curve was obtained (Fig. 2B). Mice treated with doses below the NOAEL (hormetic stimulatory area: 2 pM $<$ Aβ $>$ 2 nM) needed less time to find the platform compared with mice treated with higher concentrations (hormetic inhibitory area: Aβ $\geq$ 20 nM) (Fig. 2B). In particular, statistical analysis revealed a significant difference between low and high Aβ doses in the overall performance (latency: 31.73 $\pm$ 1.67 seconds vs. 38.50 $\pm$ 1.88 seconds; $t(18) = 3.636$; $p = 0.002$) and in the 5th (latency = 38.05 $\pm$ 0.67 seconds vs. 42.28 $\pm$ 1.02 seconds; $t(18) = 2.724$; $p = 0.014$) and 6th session (latency = 27.13 $\pm$ 1.47 seconds vs. 37.53 $\pm$ 1.48 seconds; $t(18) = 5.488$; $p < 0.0001$; Fig. 2B). Planned comparisons on each individual dose compared with vehicle indicated that, in the stimulatory range, the improvement of memory occurred after treatment with 200 pM Aβ42, as mice needed less time to find the platform (n = 10/10 mice; overall latency = 34.86 $\pm$ 0.94 seconds vs. 39.05 $\pm$ 0.94 seconds; $t(18) = 2.682$, $p = 0.015$; 6th session latency = 22.70 $\pm$ 1.74 seconds vs. 30.86 $\pm$ 1.98 seconds; $t(18) = 3.097$, $p = 0.006$ compared with vehicle; Fig. 2C), whereas infusion with lower concentrations (2 pM: n = 9; overall latency = 39.84 $\pm$ 0.88 seconds; $t(17) = 0.497$, $p = 0.625$; 6th session latency = 31.00 $\pm$ 1.98 seconds; $t(17) = 0.048$, $p = 0.962$; 20 pM: n = 10; overall latency = 38.53 $\pm$ 1.15 seconds; $t(18) = 0.310$, $p = 0.760$; 6th session latency = 29.50 $\pm$ 2.07 seconds; $t(18) = 0.476$, $p = 0.640$; Fig. 2C) or treatment with 2 nM and 20 nM did not determine any difference compared with vehicle (2 nM: n = 10; overall latency = 39.25 $\pm$ 0.93 seconds; $t(18) = 0.131$, $p = 0.898$; 6th session latency = 28.43 $\pm$ 1.59 seconds; $t(18) = 0.956$, $p = 0.352$; Fig. 2C; 20 nM: n = 10; overall latency = 38.71 $\pm$ 1.35 seconds; $t(18) = 0.187$, $p = 0.854$; 6th session latency = 30.13 $\pm$ 2.00 seconds; $t(18) = 0.260$, $p = 0.798$; Fig. 2D). A slight impairment of memory started to occur 200 nM

(n = 10; overall latency = 43.22 ± 1.76 seconds; $t(18)$ = 1.978, p = 0.063; 6th session latency = 38.10 ± 2.94 seconds; $t(18)$ = 2.036, p = 0.057; Fig. 2D) and worsened with higher doses (2 μM: n = 9; overall latency = 44.07 ± 1.69 seconds; $t(17)$ = 2.369, p = 0.028; 6th session latency = 42.92 ± 1.90 seconds; $t(17)$ = 3.854, p = 0.001; 20 μM: n = 8; overall latency = 45.35 ± 1.46 seconds; $t(16)$ = 3.247, p = 0.005; 6th session latency = 43.66 ± 1.92 seconds; $t(16)$ = 4.557, $p < 0.0001$; Fig. 2D).

After the 6th hidden platform session, we assessed spatial reference memory with the probe trial (Schenk and Morris, 1985). The platform was removed from the pool and animals were given 60 seconds to search for it. The maze was virtually divided in 4 areas, and the amount of time spent in each quadrant of the maze was calculated. Performance was evaluated comparing the percentage of time spent in the TQ, where the platform had been located during training, and in the other quadrants. We found an improvement of memory in mice treated with low doses of Aβ, as demonstrated by the fact that they spent more time in the TQ compared with mice treated with high doses (41.78 ± 2.28% of time vs. 31.19 ± 3.65% of time; $t(18)$ = 2.455; p = 0.025; Fig. 3A). In particular, the maximum stimulatory response was reached at 200 pM and was equal to 124% of control (TQ = 47.70 ± 4.77% of time vs. 38.54 ± 2.37% of time; $t(18)$ = 2.829; p = 0.011 compared with vehicle and $t(18)$ = 5.940; $p < 0.0001$ compared with other quadrants; Fig. 3A and B). The maximum inhibitory response was reached at 20 μM (TQ = 25.92 ± 4.77% of time; $t(16)$ = 3.527, p = 0.003 compared with vehicle and $t(14)$ = 0.132, p = 0.897 compared with other quadrants; Fig. 3A and B). A visible platform trial performed after the probe trials did not reveal any significant difference in the time to reach the platform among low-dose and high-dose treatment during the 4 sessions of the task (latency: 13.1 ± 0.41 seconds vs. 13.3 ± 0.62 seconds; $t(18)$ = 0.275; p = 0.786). Taken together, these data indicate that Aβ42 produces a hormetic effect on hippocampal spatial memory.

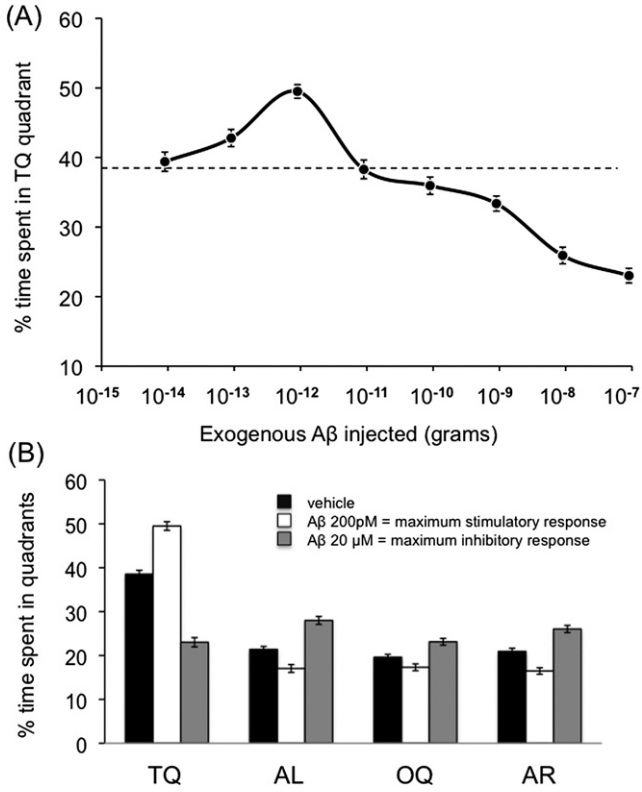

Fig. 3. Beta-amyloid peptide (Aβ)42 has a hormetic effect on cognitive performance tested through the Morris water maze probe test. (A) Hippocampal reference memory was increased by low doses of Aβ42, whereas high doses induced memory impairment as shown by longer or shorter time spent in target quadrant (TQ) where the platform was located during the training. (B) Time spent in TQ, adjacent quadrant left (AL) and right (AR) and opposite quadrant (OQ) in mice treated with vehicle, Aβ concentration evoking the maximum stimulatory (200 pM) or inhibitory response (20 μM).

4. Discussion

The present study shows that Aβ has a hormetic effect on synaptic plasticity and memory. This is consistent with previous data suggesting a double role of Aβ, with low concentrations enhancing synaptic plasticity and memory, whereas high levels induce the well-know detrimental effect on cognition (Puzzo et al., 2008); and with experiments showing that Aβ displays neurotoxic and neurotrophic effects, depending upon the concentration (Calabrese, 2001a; Giuffrida et al., 2010; López-Toledano and Shelanski, 2004; Plant et al., 2003; Yankner et al., 1990). We analyzed hippocampal LTP and spatial memory after treatment with different doses of Aβ. We observed U-shaped dose-response curves, inverted or not according to the parameter measured. It was possible to distinguish a stimulatory area below the control level, and a zone of adverse effect above it. The maximum stimulatory response was about 140% of control for Aβ effect on LTP, and 124% of control for reference memory, consistent with most studies showing that the amplitude of the hormetic effect is usually no greater than 130%–160% (Calabrese and Baldwin, 2002, 2003a; Calabrese et al., 1999). The width of the low-dose stimulatory range was 20-fold, being contiguous with NOAEL, and there was a 10-fold concentration distance between NOAEL and the maximum stimulatory effect (Calabrese and Baldwin, 2003a). Thus, a hormetic-like biphasic dose-response occurs for Aβ, as for several other peptides (Calabrese and Baldwin, 2003b; Kastin and Pan, 2008). These observations have important implications in the study of physiological mechanisms underlying memory formation and, on the other hand, they might contribute to understand the pathogenesis and therapy of diseases characterized by an increase of Aβ, such as AD. Indeed, because hormetic response might represent an adaptive response due to a perturbation of homeostasis, AD may be the consequence of a homeostatic imbalance of the physiologic feed-

back mechanism exerted by Aβ on synaptic activity that needs therapeutic intervention before the "out of control" irreversible condition.

An interesting Aβ target might be represented by acetylcholine receptors (AChRs) because it has been widely demonstrated that central AChRs, as nicotinic (nAChRs) and muscarinic (mAChRs), are crucial in learning and memory both in physiological and pathological conditions (Albuquerque et al., 2009; Levey, 1996; Levin, 2002). The loss of cholinergic neurons or AChRs has been reported for AD patients and, consequently, the increase of acetylcholine (Ach) transmission by the use of cholinesterase inhibitors is still 1 of the therapeutic strategies to improve memory in AD patients (Clader and Wang, 2005; Oddo and LaFerla, 2006), even though the modest effect of these drugs and the cholinergic side effects. Both mAChR and nAChR are involved in AD. In particular, it has been demonstrated that the loss of M1-mAChRs exacerbated Alzheimer's disease-like pathology (Medeiros et al., 2011) whereas their activation reduced AD-like pathological features and enhanced cognition in AD transgenic models (Caccamo et al., 2006), reduced Aβ levels in cerebrospinal fluid (CSF) (Beach et al., 2001), and APP levels in the cortex and hippocampus (Seo et al., 2002). nAChRs are also involved in synaptic plasticity and memory, boosting LTP and enhancing transmitter release in several brain regions including the hippocampus (Gray et al., 1996; Radcliffe and Dani, 1998). The hippocampus is an important target for nicotinic influences over memory and targeting the α7-nAChRs subtype might reduce AD symptoms (for a review, see Ondrejcak et al., 2010). Furthermore, a loss of α7-nAChRs has been evidenced in a triple transgenic mouse model of AD (Oddo and LaFerla, 2006) and an association between a genetic variant of the α7-nAChRs subunit and different forms of dementia, including AD, has been recently found (Fehér et al., 2009). We have recently shown that endogenous Aβ is needed for synaptic plasticity and memory (Puzzo et al., 2011). Interestingly, both the beneficial effect of exogenous application of low doses of Aβ and the endogenous role of the peptide are mediated by α7-nAChRs (Puzzo et al., 2008, 2011). The link between Aβ and α7-nAChRs has been also demonstrated in studies showing that Aβ is likely to bind to α7-nAChRs (Wang et al., 2000) or might regulate nAChR function, possibly through binding with membrane lipids (Small et al., 2007). Intriguingly, Aβ might both act as an α7-nAChRs agonist (Fodero et al., 2004) or have inhibitory actions on α7-nAChRs (Grassi et al., 2003), probably due to the used concentration, with low concentrations activating and high concentrations inhibiting α7-nAChRs (Dineley et al., 2001, 2002), pointing at an interesting parallel between Aβ and α7-nAChRs.

It remains to clarify whether this double positive-negative effect of Aβ on nAChRs might be due to changes in the composition of the Aβ as the concentration increases, because higher concentrations would favor oligomer formation. Although we used an aged preparation containing both monomers and oligomers, it is extremely difficult to know the exact composition of Aβ solutions and, consequently, the Aβ species and molarities to which hippocampi, both in vitro and in vivo, are exposed. In fact: (1) Aβ can easily change its conformation by the time it reaches the brain tissue after its initial preparation; (2) in vivo the injected dose will be diluted by the cerebrospinal fluid but it will also add to the baseline levels of endogenous murine Aβ and might increase concentrations beyond those identified as beneficial (or detrimental); (3) even if our experiments have always been performed using polypropylene materials that have been considered reliable to perform accurate Aβ (Lewczuk et al., 2006), a small amount of Aβ could stick to the tubes altering the real concentration. However, in a previous report (Puzzo et al., 2011) we have demonstrated that low concentrations of monomeric Aβ did not affect LTP. In particular, when we used a solution enriched in monomers, we did not find any rescue of LTP impairment by depletion of endogenous Aβ, suggesting that oligomers are likely to be responsible for the rescue effect. Thus, in our experimental conditions, low concentrations of a mixed Aβ preparation rescue synaptic plasticity and memory by acting through nAChRs. It remains to be determined whether these effects are mediated by a direct physical interaction of the peptide with the nAChRs, and whether a single species of Aβ might be responsible for these effects. Further experiments beyond the scope of the present work will be necessary to clarify these issues. However, based on these findings, it is possible to hypothesize that during neuronal activity in physiologic conditions Aβ acts on α7-nAChRs boosting synaptic plasticity and memory (Fig. 4A) and that the increase in Aβ levels occurring in AD might be due to a failure of Aβ effect on the synapse. If Aβ physiologically stimulates synaptic plasticity through α7-nAChRs and if, for reasons to be determined, this mechanism fails, a feedback mechanism would determine an increase of Aβ release to compensate this situation. When Aβ is produced in high amounts, it will become toxic to neurons, stimulate microglia reactions, and form senile plaques devastating the brain structure. In the late stage of the disease, Aβ production will decrease due to neuronal functional loss (Fig. 4B). Indeed, it has been demonstrated that Aβ concentrations in brain interstitial fluid increased as neurological status improved and decreased when neurological status declined (Brody et al., 2008). Moreover, an increase of Aβ cerebrospinal fluid levels and β-secretase activity has been shown in mild cognitive impairment patients who progressed to AD (Williams et al., 2011), whereas low Aβ levels have been found in AD patients (Blennow, 2004), probably due to a decrease of the peptide when pathogenic mechanisms overwhelm the amyloid response.

Understanding the physiopathological role of Aβ is crucial when designing effective and safe approaches to AD therapy. Despite several studies have been performed to

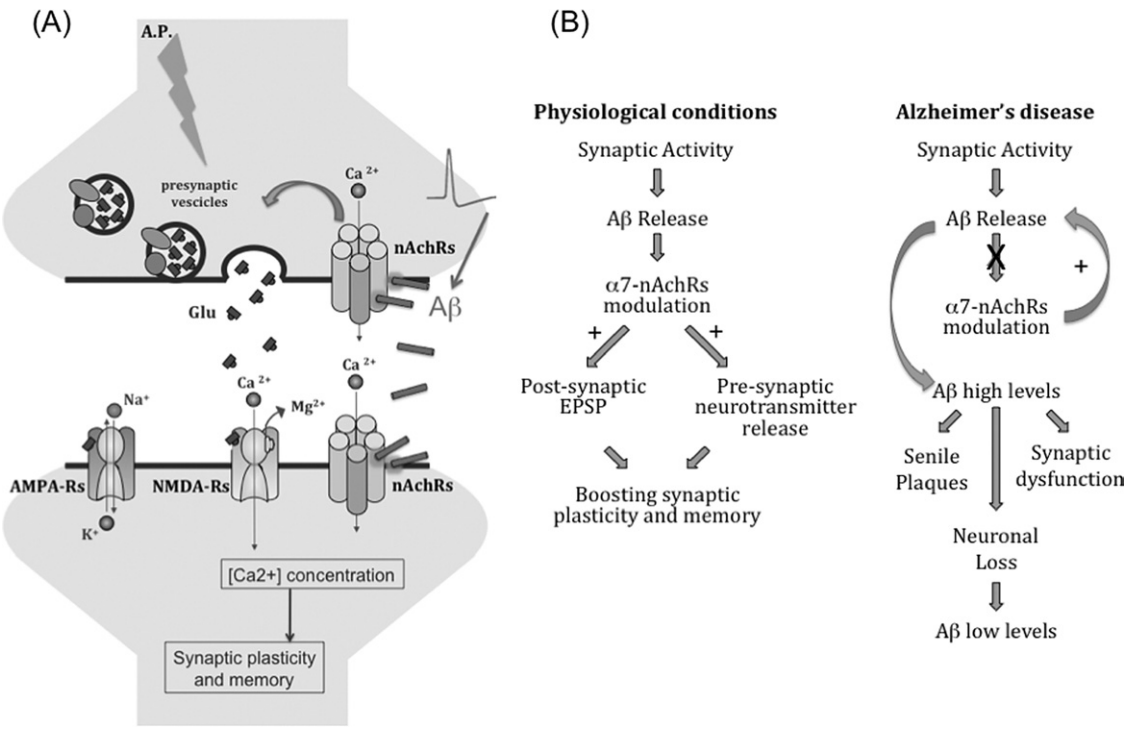

Fig. 4. Beta-amyloid peptide (Aβ) between physiology and pathology. (A) Schematic representation of a theoretic model indicating that during neuronal activity the release of Aβ acts on pre- or postsynaptic α7-nicotinic acetylcholine receptors (nAChRs), boosting synaptic plasticity and memory. (B) The failure of Aβ to modulate nicotinic signal triggers a feedback mechanism leading to the accumulation of Aβ which, in turn, causes the Alzheimer's disease (AD) pathologic features. Abbreviations: A.P., action potential; AMPA-Rs, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors; Glu, glutamate; NMDA-Rs, N-Methyl-D-aspartate receptors.

better understand the pathogenesis of the disease and to improve therapeutic strategies, the available drugs mainly aim to slow down the progression of the symptoms. Indeed, major drug discovery efforts are ongoing to develop strategies to decrease Aβ load, or by the use of treatments, such as Aβ immunization, that appear to augment the removal of Aβ from the brain (Dominguez and De Strooper, 2002). Unfortunately, the pharmacological approach aimed at decreasing Aβ load resulted so far in a therapeutic failure. Recently, the multicenter trials to asses the efficacy of the γ-secretase inhibitor Semagacestat (Eli Lilly, Indianapolis, IN, USA), aimed at inhibiting Aβ production, have been terminated, also because control healthy patients under treatment with the inhibitor showed an impairment of cognition (Cummings, 2010).

AD primum movens is still unknown, probably due to the fact that it is a multifactorial pathology, involving both genetic and environmental intricate aspects.

Finally, the knowledge of both the physiological role of Aβ described in previous studies (see Ondrejcak et al., 2010 for a review) and the hormetic effect of Aβ described in this investigation, together with the clinical failure of anti-Aβ based therapy, raises several criticisms to the approaches aiming to decrease Aβ load, especially when suggested as prevention of the disease in healthy subjects. Paradoxically, our research line suggests that very low doses of the peptide might serve to enhance memory at appropriate concentrations and conditions, at least in healthy animal models.

Disclosure statement

D.P. discloses a patent, "Methods and composition for enhancing memory" (12/414,160).

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