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Alzheimer’s Disease Related Markers, Cellular Toxicity and Behavioral Deficits Induced Six Weeks after Oligomeric Amyloid-β Peptide Injection in Rats

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Abstract

Alzheimer’s disease (AD) is a neurodegenerative pathology associated with aging characterized by the presence of senile plaques and neurofibrillary tangles that finally result in synaptic and neuronal loss. The major component of senile plaques is an amyloid-β protein (Aβ). Recently, we characterized the effects of a single intracerebroventricular (icv) injection of Aβ fragment (25–35) oligomers (oAβ25–35) for up to 3 weeks in rats and established a clear parallel with numerous relevant signs of AD. To clarify the long-term effects of oAβ25–35 and its potential role in the pathogenesis of AD, we determined its physiological, behavioral, biochemical and morphological impacts 6 weeks after injection in rats. oAβ25–35 was still present in the brain after 6 weeks. oAβ25–35 injection did not affect general activity and temperature rhythms after 6 weeks, but decreased body weight, induced short- and long-term memory impairments, increased corticosterone plasma levels, brain oxidative (lipid peroxidation), mitochondrial (caspase-9 levels) and reticulum stress (caspase-12 levels), astroglial and microglial activation. It provoked cholinergic neuron loss and decreased brain-derived neurotrophic factor levels. It induced cell loss in the hippocampic CA subdivisions and decreased hippocampic neurogenesis. Moreover, oAβ25–35 injection resulted in increased APP expression, Aβ1–42 generation, and increased Tau phosphorylation. In conclusion, this in vivo study evidenced that the soluble oligomeric forms of short fragments of Aβ, endogenously identified in AD patient brains, not only provoked long-lasting pathological alterations comparable to the human disease, but may also directly contribute to the progressive increase in amyloid load and Tau pathology, involved in the AD physiopathology.

Introduction

Alzheimer’s disease (AD) is the most common cause of dementia in the elderly and is characterized by a progressive impairment in cognitive functions, resulting from synapse and nerve cell destruction in the brain. AD symptoms include memory loss, alteration of the individual’s personality and failure to communicate or perform routine tasks. The histopathological hallmarks of AD include the presence of extracellular senile plaques, intracellular neurofibrillary tangles (NFT), reduction and dysfunction of synapses, neuronal death and reduction in overall brain volume. Senile plaques are composed of insoluble extracellular aggregates consisting mainly of amyloid-β (Aβ) peptides, which are generated by enzymatic cleavages of the amyloid precursor protein (APP), while NFT are the result of hyper- and abnormal phosphorylation of the microtubule-stabilizing protein Tau [1,2].

There is no doubt that progressive Aβ accumulation contributes to AD. A correlation between the total amount of Aβ in human brain and cognitive decline indicates that the amount of Aβ, but not necessarily plaque formation, is important for AD progression [3,4]. Transgenic APP mice demonstrate cognitive decline before plaque formation [5], and soluble oligomers can inhibit cognitive function [6] and long-term potentiation [7,8]. In fact, it is possible that extracellular amyloid deposits are only one aspect of the larger pathological cascade and an indirect consequence of possible protective responses intended to sequester toxic soluble Aβ oligomers [4]. The degree of dementia in AD correlates better with Aβ assayed biochemically, than with the histologically determined number of plaque. The concentration of soluble Aβ species, which cannot be detected through an immunohistochemical analysis, appears to be more closely correlated with cognitive deficits [3,4]. In fact, Aβ deposits may not even carry the most aggressive toxicity, but instead represent a reserve of toxicity from where toxic oligomeric fragments could be released [9–11].

The soluble Aβ oligomers observed in AD patients contain Aβ in its most predominant sequences: Aβ1–40 or Aβ1–42 [3,11]. Nevertheless, they also contain peptides with shorter sequences such as N-truncated amyloid-β oligomers [12,13]; Aβ25–35 or Aβ25–35/40 [14–16]. Aβ25–35 (GSNKGAIIGLM) can be produced in AD patients by enzymatic cleavage of Aβ1–40 [14,15]. This Aβ peptide includes extracellular and transmembrane residues that have been reported to represent a biologically active region of Aβ.
of incubated scrambled peptide (10 μg/rat) and a third group received an icv injection of oAβ25-35 peptide (10 μg/rat) [25]. For icv injection through a Hamilton syringe (VWR, France), the animals were anesthetized with an intramuscular injection of 0.2 ml of a mixture of Ketamine hydrochloride (80 mg/kg b.w.) and Xylazine (10 mg/kg b.w.). They were then stereotaxically injected directly into the lateral ventricles at coordinates (AP: −1 mm, L: ±1.5 mm, and DV: −3.5 mm) according to Paxinos and Watson [35].

**Spatial Short-term Memory (Delayed Alternation in the T-maze)**

As previously detailed [20,36], delayed alternation was tested in the T-maze and the results were expressed as ratio of the time spent in the initially closed novel arm over the time spent in the previous arm and as a ratio of the number of entries into the novel arm over the familiar one.

**Spatial Long-term Memory (Place Learning in the Water-maze)**

As previously reported [20,25], spatial reference memory was tested using a place learning procedure in the water-maze. Training consisted of three swims per day for 5 days. Each rat was allowed a 90 s swim to find the platform and was left for a further 30 s on the platform. The median latency was determined for each training session. A probe test was performed 4 h after the last training session. The platform was removed and each rat was allowed a free 60 s swim. The percentage of time spent in the training quadrant was determined by videotracking (Viewpoint, France).

**Endocrine Stress**

Blood samples were collected after killing the rats by decapitation, as previously reported [37]. Plasma corticosterone (CORT) was assayed with a radioimmunoassay kit (Biotar, GE-Healthcare, France) in 50 μl plasma sample diluted 1:5 with the assay buffer. The intra- and inter-assay coefficients of variation were 5% and 7%, respectively. The assay sensitivity was 0.6 ng/ml.

**Oxidative Stress**

As previously described [20,28], quantification of lipid peroxidation in tissue extracts was based on Fe(III)xylenol orange complex formation according to the Hermes-Lima method [38].

**Specific Markers (GFAP, Iba1, VACHT, PSA-NCAM, Caspase-9, -12 and -3)**

Rats were sacrificed by decapitation and structures of interest were weighed, immediately frozen in liquid nitrogen and stored at −20°C. Tissues were sonicated with a VibraCell (Sonics & Materials, USA) in 2% SDS. Homogenates were then boiled (5 min) and centrifuged for 30 min at 14000 g for GFAP, pro-
Figure 1. Brain localization of Aβ25–35 and particle characterization of Aβ25–35 solutions A–I. Localization within brain structures of oAβ25–35-HLF, determined 6 weeks after its icv injection (10 μg/rat). oAβ25–35-HLF was visualized in green, while the nucleus was counterstained with DAPI (blue labeling). Abbreviations: 3V: third ventricle; alv: alveus of the hippocampus; CA1: field CA1 of hippocampus; CA3: field CA3 of the hippocampus; cc: corpus callosum; D3V: dorsal third ventricle; ec: external capsule; fi: fimbria of the hippocampus; FrA: frontal association cortex; hf: hippocampal fissure; LV: lateral ventricle; MEE: median eminence, external part; MEI: median eminence, internal part; MHb: medial habenular nucleus; PVN: paraventricular hypothalamic nucleus; PVP: paraventricular thalamic nucleus, posterior part. Arrowhead: blood vessel. Scale bar = 100 μm. J. Particle size distribution of the different fractions of Aβ25–35 solution (1 μg/μl) was determined by PCS at 25°C. Samples were prepared as described in the materials and methods section. Black curve: Aβ25–35 peptide dissolved in hexafluoroisopropanol (HFIP); Red curve: solution of aggregated Aβ25–35 peptide; Green curve: supernatant of aggregated Aβ25–35 peptide centrifuged at 1 000 g; Purple curve: re-suspended pellet of aggregated Aβ25–35 peptide obtained after centrifugation at 1 000 g; Blue curve: supernatant of aggregated Aβ25–35 peptide centrifuged at 16 000 g. Data were analyzed using a Zetasizer software 6.01 and expressed as size frequency distribution (%) in function of particles size (nm).

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and cleaved caspase-9 and -12, and pro-caspase-3). To detect cleaved caspase-3, VACHT and Iba1, tissues were homogenized using a specific lysis buffer (Triton X100 1%; Tris-HCl pH 7.5, 20 mM; NaCl 150 mM; EDTA 10 mM; Na3VO4 100 μM) previously described by Cotrufo et al. [39]. Supernatants were collected and the protein concentration was measured using the BCA Kit (Pierce, France) and 20 to 40 μg from each sample was taken for western blot analysis depending on the structure and antigen considered. Samples were boiled (5 min), separated by SDS-polyacrylamid gel (12%) and transferred to a nitrocellulose membrane (Whatman, France). The membrane was incubated overnight (4 °C) with a mouse anti-glial fibrillary acidic protein (GFAP) (1/1000; Sigma-Aldrich, France), or a rabbit anti-Iba-1 (1/750; Wako Chemicals, Japan), or a rabbit anti-VAChT (1/500; Sigma-Aldrich) or a rabbit anti-procaspase-3 and a rabbit monoclonal anti-caspase-3 (cleaved form) (1/1000 and 1/2000, respectively; Cell Signaling, France), or a rabbit anti-caspase-9 (pro- and cleaved forms; 1/1000; Cell Signaling), or a rat anti-caspase-12 (pro- and cleaved forms; 1/5000; Sigma-Aldrich, France) or a mouse anti-β-tubulin (β-tub) (1/5000; Sigma-
The number of animals in each group is indicated within the columns.

A mouse AT8 (S 199/S202/T205) and AT100 (T 212/S214/T217) primary antibodies to detect phospho-Tau epitopes (50 kDa) were used in the analysis following the same procedures detailed before. The optical density of the bands corresponding to each antibody used (control group: C, treated group: S, OAβ25-35 treated group: OAβ25-35) from each sample were taken to western blot analysis (mouse monoclonal anti-PSA-NCAM, clone 2-2B, ref AbC0019 from AbCys SA) were unable to quantify undamaged hippocampic cells [20,29,42,43]. The counting was made using images captured with Leica DFC495 high-resolution camera (Leica Microsystems, Nanterre, France) attached to Leica DM2500 microscope (Leica) and the Leica LAS Core image analysis software (Leica). For this purpose, digitized images acquired using a ×40 objective were transformed into TIFF files and brought to the same level of contrast and sharpness using the software. Four sections were studied from each brain, taken from the anterior hippocampus level (~3.0 to ~4.0 from the bregma) [35], with intervals of 250 µm. Sections were selected on a subjective random basis. Three fields were analyzed per hippocampus for CA1, one for CA2 and CA3 and two for DG. Counts of undamaged cells were made using ImageJ software on TIFF captured images. Neuron densities on slices (number of neurons in the optical field expressed as the number of cells per mm²) were calculated as the arithmetic mean number of neurons in the two hemispheres and, for each animal, as the arithmetic mean of results obtained for each of the four slices. The count of undamaged cells in the CA1, CA2, CA3 and DG fields of the hippocampus was done by two different scientists unaware of the experimental conditions and independently from each other using display projections of the images (each person performed cell count for all animals included into the experiment and no difference was observed between the two independent and unaware analyses).

APP Processing

As previously reported [20], 60 µg from each sample was taken to western blot analysis following the same procedures detailed in 2.3. The primary antibody used to detect APP (125 kDa) and C99 fragment (13 kDa) was a rabbit anti-Amyloid Precursor Protein (PA1-84165: 1/750, ABR-Thermo-Scientific, France).

Tau Phosphorylation

To determine the levels of Tau phosphorylation at specific sites, equal amounts of protein (varying from 60 and 80 µg depending on the antibody used) from each sample were taken to western blot analysis following the same procedures detailed before. The primary antibodies to detect phospho-Tau epitopes (30 kDa) were a mouse AT8 (T231/S235/T238) and AT100 (T217/S214/T217) antibodies (MN1020: 1/3000 and MN1060: 1/3000, respectively; ThermoScientific, France), and to detect total Tau (50 kDa) was a mouse anti-Tau antibody (MA1-38710: 1/5000, ThermoScientific, France).

BDNF Content

Rats were sacrificed by decapitation and structures of interest were weighed, immediately frozen in liquid nitrogen and stored at −20°C until assayed. Brain-derived neurotrophic factor (BDNF) content was measured with a conventional ELISA assay (BDNF Emax; Promega, France), as previously reported [40,41]. The assay sensitivity was 15 pg/tube. The BDNF concentration was expressed as pg/g wet weight. The intra- and inter-assay coefficients of variation were 5% and 6%, respectively.

Histology (Cresyl Violet Staining; GFAP, Iba-1, PSA-NCAM, VACHT Immunolabeling)

Animals were anaesthetized using an intramuscular injection of ketamine/xylazine solution and perfused intracardially (30 ml of NaCl 0.9% and 100 ml PO4 0.2 M containing 4% paraformaldehyde). Brains were removed and postfixed in the same fixative for 48 h (4°C) and then in a solution of sucrose (30%) for 3 days. Thereafter, tissues were included in a block of OCT compound (Tissue-Tek, Sakura Finetek, USA) and quickly frozen in acetone chilled on dry ice. Frozen brains were mounted on a cryostat (Leica, France) and serially cut into 10 µm coronal sections. For histology, they were stained with 0.2% cresyl violet reagent, dehydrated and mounted. The method used for neuronal count is a classical method used with thin brain sections (10 µm) to quantify undamaged hippocampic cells [20,29,42,43]. The counting was made using images captured with Leica DFC495 high-resolution camera (Leica Microsystems, Nanterre, France) attached to Leica DM2500 microscope (Leica) and the Leica LAS Core image analysis software (Leica). For this purpose, digitized images acquired using a ×40 objective were transformed into TIFF files and brought to the same level of contrast and sharpness using the software. Four sections were studied from each brain, taken from the anterior hippocampus level (~3.0 to ~4.0 from the bregma) [35], with intervals of 250 µm. Sections were selected on a subjective random basis. Three fields were analyzed per hippocampus for CA1, one for CA2 and CA3 and two for DG. Counts of undamaged cells were made using ImageJ software on TIFF captured images. Neuron densities on slices (number of neurons in the optical field expressed as the number of cells per mm²) were calculated as the arithmetic mean number of neurons in the two hemispheres and, for each animal, as the arithmetic mean of results obtained for each of the four slices. The count of undamaged cells in the CA1, CA2, CA3 and DG fields of the hippocampus was done by two different scientists unaware of the experimental conditions and independently from each other using display projections of the images (each person performed cell count for all animals included into the experiment and no difference was observed between the two independent and unaware analyses).

Analyses of the glial (GFAP) and acetylcholine (VACHT) markers were conducted according to a diaminobenzidine (DAB) immunohistochemistry approach, while analyses of the microglial (Iba-1) and neurogenesis (PSA-NCAM) markers were conducted according to a fluorescent immunohistochemistry approach [20,40]. Sections were incubated overnight at room temperature with a mouse anti-GFAP (1/1000, Sigma-Aldrich), or a rabbit anti-Iba-1 (1/500; Wako Chemicals, Japan), or a rabbit anti-VACHT (1/500; Sigma-Aldrich) or a rabbit anti-PSA-NCAM antibody (1/200; Abcys, France). Then sections were incubated...
for 2 h with the appropriate biotinylated (anti-GFAP and anti-VACHT) or fluorescent (Alexafluor-488; anti-Iba-1 and Alexafluor-555; anti-PSA-NCAM) secondary antibody (Sigma-Aldrich or Molecular Probes, The Netherlands). Biotinylated sections were incubated for 1 h in avidin–biotin complex (ABC kit; Vector Laboratories, USA). Signals were detected with the diaminobenzidine kit (Vector Laboratories), according to the manufacturer’s instructions, while nuclei of fluorescent sections were counterstained with 4',6-diamino-2-phenylindole (DAPI, Molecular Probes). The immunostaining specificity was determined with the same protocol but by incubating control sections with the secondary antiserum alone.

### Aβ25–35 Distribution

We used a tagged peptide with a fluorescent dye (αAβ25–35 HiLyte Fluor 488, ANASPEC/Eurogentec, France) (αAβ25–35-HLF) to analyze the distribution within brain structures of the injected αAβ25–35 fragment. The ability of the tagged peptide to form amyloid fibrils after 4 days of incubation at 37°C was previously assessed by electron microscopy and Congo red staining [20]. Six weeks following the icv injection of αAβ25–35-HLF, animals were anaesthetized using an intra-muscular injection of ketamine/xylazine solution and perfused intracardially, as previously described. Brains were removed and postfixed in the same fixative for 3 days (4°C) and serially cut with a vibrating blade microtome (Leica) into 30 μm coronal sections. Before to be mounted, sections were counterstained with DAPI to visualize nuclei.

### Statistical Analysis

Data are presented as mean±SEM. Comparisons were performed using one-way or two-way ANOVA (F values) followed by a Fisher’s multiple comparison test. P<0.05 was considered significant.
Results

Regional Distribution of Aβ25–35-HLF Showed Persistent Presence of oAβ25–35 after 6 Weeks in the Brain

The regional distribution of oAβ25–35-HLF 6 weeks after injection is presented in Fig. 1A–I. Control rat sections were treated and examined in the same conditions as injected rat sections and displayed no specific labeling. oAβ25–35-HLF labeling was relatively discreet, suggesting a progressive clearance of the peptide after 6 weeks in comparison to previous study [20]. However, oAβ25–35-HLF was again found at the injection site level where it was trapped by local cells (Fig. 1A). The fluorescent peptide was also found in brain ventricles, particularly in the lateral ventricle (LV) (Fig. 1B–C), and at the dorsal (D3V) and ventral (3V) parts of the third ventricle (Fig. 1D–F). The fluorescent peptide was found in the walls of blood vessels particularly in the amygdala, frontal (Fig. 1G) and parietal cortex, but also in hypothalamus and thalamus (Fig. 1H) regions. As previously reported, in addition to ependymal cells, oAβ25–35-HLF was found in neurons (Fig. 1H–I) and in glial cells, particularly in

Figure 6. ER stress. Variations in pro- and activated caspase-12 levels in the frontal cortex, amygdala, hippocampus and hypothalamus, determined in rats by western blot 6 weeks after oAβ25–35 icv injection (10 μg/rat). Pro-caspase-12 (50 kDa) and activated caspase-12 (25 kDa) variations were normalized with β-tubulin (β-tub, 55 kDa) variations and compared with untreated rats (control group: C). The results are expressed as means ± SEM. *p<0.05 and **p<0.01 vs. control group, +p<0.05 and ++p<0.01 vs. scrambled treated rats. Note that scrambled peptide injection (10 μg/rat) served as negative control and did not induce any modifications in pro- and activated caspase-12 levels. The number of animals in each group is indicated within the columns.

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the median eminence (Fig. 1E), but also in nerve fibers, principally in the hippocampus (Fig. 1I).

4-days Incubation of Aβ25–35 Led Almost Exclusively to Soluble Oligomers

In addition to a previous qualitative study [20], where we have evidenced the amyloid property of the aggregated Aβ25–35 peptide, we have determined in the present work the respective quantity of each particle species contained in the solution injected in rats. The characterization of the aggregated Aβ25–35 solution was realized by PCS and is presented in Figure 1J. This figure shows the size distribution of the oligomeric species in the aggregated Aβ25–35 solution. After 4-days incubation at 37°C, the sample is composed of particles with an average diameter of 103.4 nm (weighted average, min/max: 50.7/712.4 nm) (red curve). In order to determine the size of particles that populated the aggregated Aβ25–35 solution, a low-speed centrifugation was carried out at 1 000 g for 15 min. PCS analysis of the pellet resuspended in water (purple curve) indicated that the particle size extended from 295.3 to 825 nm with a peak maximum at 458.7 nm and an average diameter of 479.5 nm. For the supernatant (green curve), the weighted average diameter of particles was 60.0 nm with a peak maximum at 50.7 nm. Similar size of particles (weighted average diameter of 52.8 nm) was measured in the supernatant after centrifugation at 16 000 g (blue curve). Note that these particles had a size with at least one order of magnitude higher than the monomer form of Aβ25–35 peptide in HFIP (black curve). This suggests that the aggregated Aβ25–35 solution that has been used for icv injection (red curve) is mainly composed of a mixture of soluble oligomer species whose sizes extended from 52.8 to 295.3 nm (98.1%). Some high-density aggregates with a diameter greater of equal at 458.7 nm were also detectable but in low proportion (1.8%).

**oAβ25–35 Injection in Rats Failed to Affect Body Weight and Physiological Rhythms**

At the beginning of the experiment, the three groups of rats presented no body weight differences (F2,15 = 0.10; p>0.05) (Fig. 2A). By contrast, body weight gain was decreased 6 weeks
Figure 8. Astrocyte activation. A. Variations in GFAP levels in the frontal cortex, amygdala, hippocampus and hypothalamus, determined in rats by western blot 6 weeks after icv injection of scrambled Aβ25–35 peptide (10 μg/rat; negative control) or oAβ25–35 (10 μg/rat). GFAP (50 kDa) variations were normalized with β-tubulin (β-tub, 55 kDa) variations and compared with untreated rats (control group: C). The results are expressed as means ± SEM. *p<0.05 and **p<0.01 vs. control group, +p<0.05 and ++p<0.01 vs. scrambled treated rats. The number of animals in each group is indicated within the columns.

B. Effects of oAβ25–35 (10 μg/rat) icv injection on astrocyte reactions using GFAP immunolabeling into the frontal and parietal cortex, amygdala, hippocampus (CA1, CA2 & CA3 regions) and hypothalamus (periventricular nucleus: PeVN & paraventricular nucleus: PVN) determined in control (C) untreated rats and 6 weeks after Aβ25–35 injection. The scrambled peptide injection (10 μg/rat) served as negative control and did not induce any modifications in the GFAP signal. 3v: third ventricle. brackets: hippocampus layer of granular cells layer. Scale bar = 60 μm.

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after the icv injection of oAβ25-35 as compared to control and scrambled peptide-injected rats (F2,15 = 5.18; p<0.05), with a comparable value as previously observed [20].

Locomotor activity and body temperature rhythms were continuously recorded using telemetric transmitters and revealed only circadian differences but not among groups (Fig. 2B). In details, the two-way repeated measure ANOVA for locomotor activity and body temperature data revealed a significantly difference between night and day values but no difference induced by oAβ25-35 icv injection (locomotor activity: F1,276 = 0.13, p>0.05 for treatment; F2,276 = 14.5, p<0.0001 for time and F2,276 = 0.63, p>0.05 for interaction; body temperature: F1,276 = 0.73, p>0.05 for treatment; F2,276 = 31.2, p<0.0001 for time and F2,276 = 0.55, p>0.05 for interaction).

6 Weeks after oAβ25-35 Injection, Rats Showed Impaired Learning and Memory Capacities

The ability of rats to perform a spatial short-term memory task was examined using delayed alternation in the T-maze (Fig. 2C). The oAβ25-35 injection induced memory deficits, since analyses of ratios of time spent and the number of visits in the novel arm over the familiar one revealed significant effects (F2,23 = 8.09; p<0.01 and F2,23 = 3.93; p<0.05, respectively), while scrambled peptide-injected rats presented no deficits as compared to control animals (Fig. 2C).

The spatial reference memory was analyzed using a water-maze procedure. When rats started training 6 weeks after peptide injection (Fig. 2D), acquisition profiles decreased with training. Two-way repeated measure ANOVA showed an effect of training trials and a treatment effect: F2,260 = 131, p<0.0001 for treatment; F4,260 = 131, p<0.0001 for trials and F4,260 = 3.96, p<0.0001 for the interaction (Fig. 2D). These data outlined an alteration of acquisition performances. However, when animals were submitted to the probe test (Fig. 2E), oAβ25-35-treated rats showed a preferential presence in the training quadrant similarly as scrambled peptide-treated and control rats. The peptide injection therefore only slowed down place learning acquisition but did not impeded it.

6 Weeks after oAβ25-35 Injection, Rats Showed a Marked Endocrine Stress

The oAβ25-35 icv injection significantly increased plasma CORT concentrations after 6 weeks when compared with control and scrambled peptide-injected rats (F2,31 = 53.8, p<0.0001) (Fig. 2F).

Analyses of Cellular Markers in oAβ25–35 Injected Rats

When compared to control and scrambled peptide-injected rats, the oAβ25-35 icv injection induced after 6 weeks an increase in lipid peroxidation level in the hypothalamus (F2,12 = 4.95, p<0.05) (Fig. 3D), while in the amygdala, oAβ25-35 induced a significant decrease in lipid peroxidation levels (F2,12 = 17.7, p<0.0001) (Fig. 3B). By contrast, no difference in peroxidized lipids was observed in the frontal cortex (F2,12 = 1.17, p>0.05) (Fig. 3A) and the hippocampus (F2,12 = 3.05, p>0.05) (Fig. 3C) in control, scrambled peptide- and oAβ25-35-injected rats.

As a major neuroprotective system, BDNF contents were analyzed in the rat brain structures. BDNF levels in the frontal cortex (Fig. 4A) and amygdala (Fig. 4B) were decreased 6 weeks after oAβ25-35-treated rats, when compared to control and scrambled peptide-injected rats (F2,41 = 3.76, p<0.05 and F2,40 = 5.33, p<0.01, respectively). By contrast, the oAβ25-35 injection induced no modification of BDNF content in the
A. Nucleus Basalis (Meynert)

B. Hypothalamus

C. Parietal cortex

D. Hippocampus
hhippocampus (F2,36 = 1.85, p<0.05) (Fig. 4C) and hypothalamus (F2,38 = 1.05, p<0.05) (Fig. 4D).

We used western blot analyses to measure the levels of pro- and activated caspase-9, an index of mitochondrial alteration, in the cerebral structures of interest, before (control rats) and 6 weeks after scrambled peptide or oAβ25–35 injection (Fig. 5). While scrambled peptide injection failed to affect pro- and cleaved caspase-9 levels, oAβ25–35 induced an increase in pro-caspase-9 levels in the amygdala (+38%; F2,35 = 9.25, p<0.001) (Fig. 5B), hippocampus (+25%; F2,34 = 5.67, p<0.01) (Fig. 5C) and hypothalamus (+21%; F2,37 = 3.43, p<0.05) (Fig. 5D), but not in the frontal cortex (F2,35 = 2.17; p>0.05) (Fig. 5A). Cleaved caspase-9 levels were increased after Aβ25–35 injection in the frontal cortex (+33%; F2,35 = 3.74; p<0.05) (Fig. 5A), hippocampus (+31%; F2,34 = 2.70; p<0.05) (Fig. 5C) and hypothalamus (+59%; F2,37 = 14.6; p<0.0001) (Fig. 5D), while no effect was observed in the amygdala (F2,35 = 2.08; p>0.05) (Fig. 5B).

We used western blot analyses to measure the levels of pro- and cleaved caspase-12, one of endoplasmic reticulum (ER) stress induction, before (control rats) and 6 weeks after scrambled peptide or oAβ25–35 injection (Fig. 6). The scrambled peptide injection failed to affect pro- and cleaved caspase-12 levels, but oAβ25–35 induced significant effects (Fig. 6). In detail, oAβ25–35 injection increased pro-caspase-12 only in the frontal cortex (+98%; F2,32 = 24.6, p<0.0001) (Fig. 6A) and hippocampus (+55%; F2,32 = 10.0, p<0.0001) (Fig. 6C), while no effect was observed in the amygdala (F2,33 = 0.16, p>0.05) (Fig. 6B) and hypothalamus (F2,36 = 1.97, p>0.05) (Fig. 6D). Cleaved caspase-12 was not increased after Aβ25–35 injection in the hypothalamus (F2,36 = 1.67, p>0.05) (Fig. 6D), while a marked increase was observed in the frontal cortex (+70%; F2,32 = 10.9, p<0.001) (Fig. 6A), amygdala (+56%; F2,33 = 3.86, p<0.05) (Fig. 6B) and hypothalamus (+81%; F2,32 = 10.8, p<0.0001) (Fig. 6C).

We used western blot analyses to measure the levels of pro- and cleaved caspase-3, one of the apoptotic effective caspases, before (control rats) and 6 weeks after scrambled peptide or oAβ25–35 injection (Fig. 7). Scrambled peptide injection failed to affect pro- and cleaved caspase-3 levels, but oAβ25–35 induced an increase in pro-caspase-3 levels in the amygdala (+32%; F2,36 = 3.42, p<0.05) (Fig. 7B) and hippocampus (+39%; F2,34 = 17.0, p<0.0001) (Fig. 7C), but no effect in the frontal cortex (F2,34 = 2.84, p<0.05) (Fig. 7A) and hypothalamus (F2,37 = 1.70, p>0.05) (Fig. 7D). oAβ25–35 injection induced a marked increase in cleaved caspase-3 levels in the frontal cortex (+79%; F2,37 = 19.1, p<0.0001) (Fig. 7A) and amygdala (+68%; F2,37 = 19.1, p<0.0001) (Fig. 7B), while no effect was observed in the hippocampus (F2,35 = 0.03, p>0.05) (Fig. 7C) and hypothalamus (F2,37 = 1.54, p>0.05) (Fig. 7D).

6 Weeks after oAβ25–35 Injection, Astroglial and Microglial Reactions Revealed Neuroinflammation

GFAP level, a marker of astroglial reactivity, was modified 6 weeks after oAβ25–35 injection in the frontal cortex (F2,37 = 3.35, p<0.005) (Fig. 8A), amygdala (F2,37 = 4.62, p<0.05) (Fig. 8B), and hypothalamus (F2,34 = 16.7, p<0.0001) (Fig. 8D), but not in the hippocampus (F2,34 = 1.70, p>0.05) (Fig. 8C), as compared to control and scrambled peptide icv-injected rats. In the amygdala and hypothalamus, astroglial activity increased by 25 and 46%, respectively, 6 weeks after Aβ25–35 injection. By contrast in the frontal cortex, GFAP level was decreased by 20%, 6 weeks after Aβ25–35 injection (Fig. 8A).

Increased GFAP immunoreactivity, indicative of astroglial activation was observed in the amygdala and hypothalamus (Fig. 8E), while no modification was observed in the parietal cortex and CA1 region of the hippocampus and a decrease was noted in the frontal cortex and other hippocampal regions (DG and CA3). Scrambled peptide icv injection did not induce any astroglial modification in the structures of interest (Fig. 8E).

Increased Iba1 immunoreactivity, indicative of microglial activation, was essentially observed in the amygdala, frontal cortex and hippocampus 6 weeks after icv injection of oAβ25–35 (Fig. 9A). By contrast, no modification was observed in the hypothalamus of oAβ25–35 injected rats or in any of the structures considered in control and scrambled peptide icv injected rats (Fig. 9A). These immunohistochemistry observations were confirmed by western blot analysis. Indeed, microglial activity (Iba1 level) was increased 6 weeks after Aβ25–35 injection in the frontal cortex (F2,37 = 6.66, p<0.01; +40% vs. control) (Fig. 9B), amygdala (F2,37 = 14.6, p<0.01; +204% vs. control) (Fig. 9C), and hippocampus (F2,34 = 19.4, p<0.01; +83% vs. control) (Fig. 8D), but not in the hypothalamus (F2,34 = 0.79, p>0.05) (Fig. 8E), as compared to control and scrambled peptide icv-injected rats.

Decrease Density of Cholinergic Neurons and Terminals was Observed 6 Weeks after oAβ25–35 Injection

In control rats, large numbers of VACHT-positive cell bodies were seen in the nucleus basalis (Meynert) (Fig. 10A). In the hypothalamus, a very dense plexus of VACHT immunoreactive fibers were present in the external layer of the median eminence and weakly VACHT-positive cell bodies were noted in the arcuate nucleus (Fig. 10B). A dense network of VACHT-positive nerve fibers was seen in the parietal cortex, with the highest density in layers I, IV and V (Fig. 10C). In the hippocampal formation (Fig. 10D), the highest density of VACHT-positive fibers was seen in the pyramidal cell layer of CA1-CA3 regions. No modification was observed after scrambled peptide icv injection. By contrast, oAβ25–35 icv injection appeared to induce a pronounced decrease in VACHT immunolabelling in the nucleus basalis (Fig. 10A), parietal cortex (Fig. 10C) and hippocampus (Fig. 10D), while no effect seemed to be induced by oAβ25–35 injection in the hypothalamus (Fig. 10B). These immunohistochemistry observations were confirmed by western blot analysis particularly in well-defined structures, i.e. hypothalamus (Fig. 10B; F2,34 = 0.02, p>0.05) and hippocampus (Fig. 10D; F2,35 = 5.39; p<0.01; –23% vs. control).

Hippocampus Integrity was also Impaired 6 Weeks after oAβ25–35 Injection

Loss of pyramidal cells in the hippocampus was determined using Cresyl violet staining before (control) and 6 weeks after scrambled peptide or oAβ25–35 injection (Fig. 11A). No modifica-
A.

B.

C.
Figure 11. Hippocampus integrity. Variations in hippocampus pyramidal cell numbers determined in rats 6 weeks after icv injection of scrambled Aβ25–35 peptide (10 μg/rat; negative control) or oAβ25–35 (10 μg/rat). A. Representative microphotographs of coronal sections of Cresyl violet stained hippocampus CA1, CA2, CA3 and DG subfields, obtained in control untreated rats and after scrambled Aβ25–35 peptide or Aβ25–35 icv injection. Scale bar = 300 and 100 μm. B. Average numbers of hippocampus pyramidal cells determined in untreated control rats and 6 weeks after icv injection of scrambled Aβ25–35 peptide (10 μg/rat; negative control) or oAβ25–35 (10 μg/rat). The results are expressed as means ± SEM (with n = 4 per group). *p<0.05 and **p<0.01 vs. control rats, +p<0.05 and ++p<0.01 vs. respective scrambled peptide-treated rats. C. Effects of oAβ25–35 (10 μg/rat) icv injection on hippocampus dentate gyrus (DG) neurogenesis using PSA-NCAM immunolabeling determined in untreated control rats and 6 weeks after Aβ25–35 scrambled amyloid peptide (10 μg/rat; negative control) or oAβ25–35 injection. Neurogenesis was visualized within coronal sections of the DG with Alexafluor555-labeled specific antibody against PSA-NCAM (red immunolabeling), while the nucleus was counterstained with DAPI (blue labeling). Scale bars = 200 μm.
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Discussion

Long-term AD-like Toxicity after oAβ25–35 Peptide Injection in Rats

The main finding of this study is that a single icv injection of oAβ25–35 provoked important physiological, behavioral, biochemical and morphological alterations 6 weeks after injection. The results revealed a clear similarity with numerous relevant signs of the pathology and were in line with the amyloid cascade hypothesis, while also suggesting the possible involvement of soluble oligomeric Aβ fragments in the etiology of AD [3,44].

The long-term effects of oAβ25–35 seemed to be generalized since the peptide injection provoked measurable short- and long-term memory deficits, hypersecretion of glucocorticoids, BDNF deficit in the frontal cortex and amygdala, modification of APP processing and Tau phosphorylation, alteration of ER and mitochondrial homeostasis, apoptosis in the frontal cortex and amygdala, neuroinflammation processes, cholinergic deficits, hippocampal cell loss and an apparent decrease in hippocampal neurogenesis. Inflammation was maintained through astroglial and/or microglial activation in all structures considered. This observation is in accordance with AD hallmarks [45–49], insofar as neuroinflammatory processes were observed in AD patients at all pathological stages, processes which could participate in the amplification of the Aβ peptides-induced toxicity [50–53].

The impact of oAβ25–35 on cholinergic neurons observed at earlier stages [20] was maintained after 6 weeks. VACHT immunoreactivity was decreased in the hippocampus, parietal cortex and basal nuclei of Meynert, but not in the hypothalamus. The cholinergic deficits induced by oAβ25–35 injection therefore seemed consistent with the well-characterized pathological hallmarks described in AD [54,55]. An effect that could be explained in part by the high levels of circulating glucocorticoids evidenced here that was shown to increase Aβ1-42 and NMDA-induced neurodegeneration in cholinergic neurons from the nucleus basalis in rat [56].

In the hippocampus, oAβ25–35 icv injection induced cellular loss after 6 weeks, but only in pyramidal cells of all CA regions, while no effect was observed in granular cells of DG. In a prior study, we showed an early cellular loss in the DG [20]. This particular difference between each hippocampus region evidenced over the time deserves a very precise quantification using a stereological approach, but it could be explained in part by neurogenesis modifications between the 3rd and 6th week following oAβ25–35 injection. PSA-NCAM immunolabeling seems to be more markedly decreased during the first weeks after injection than observed here after 6 weeks. This interesting observation that DG neurogenesis could finally be able to mitigate hippocampus cell loss must be accurately characterized including proliferation, migration and maturation of newborn cells with adequate markers [57], as previously reported between 5 to 30 days by Li and Zhuo [58]. In other hippocampal regions, several hypotheses must be
considered. Since glucocorticoids act synergistically with excitatory amino acids, particularly with glutamate [59,60], chronic overstimulation could be extremely toxic [61]. Indeed, a hypersecretion of glucocorticoids is observed 6 weeks after oAβ25–35 injection at levels that could effectively result in a deleterious effect of glucocorticoids. No other data is available at present on the effect of Aβ peptide injection on glucocorticoid regulation. However, several studies demonstrated that glucocorticoids modulated APP processing [62], increased, as previously described, Aβ1–42- and NMDA-induced neurodegeneration in cholinergic neurons from the nucleus basalis in rat [56] and Aβ25–35 toxicity in hippocampus neurons [63]. These observations are also coherent with AD symptoms, since a hypersecretion of glucocorticoids was frequently observed in AD patients [64,65].

The hippocampus cell loss is likely the result of apoptotic processes. An increase in pro- and cleaved forms of caspase-9 and -12 was observed in the hippocampus, which reflects mitochondrial and ER stress. However, these increases in initiator caspases were not associated with an activation of the effector caspase, caspase-3. Since initiator caspase-9, which can be induced by caspase-12 [66–68], also activates caspase-6 [69], it is possible that the cell loss in the hippocampus could be in part due to an activation of caspase-6 in this model. As an alternative, necrosis cell death could also be envisaged since, in vivo, the complete elimination of apoptotic cells prevents an inflammatory response, whereas necrosis often results in inflammatory reactions [70]. Moreover, in cell culture models, oAβ25–35 was reported to induce apoptosis at lower concentrations (5, 10 μmol/l) and necrosis at higher concentrations [20, 40 μmol/l] [71]. In the frontal cortex and amygdala, oAβ25–35 injection induced BDNF deficits after 6 weeks. These deficits were in line with previous results, since BDNF deficits were already observed from 2 weeks in the frontal cortex and 3 weeks in the amygdala [20]. In addition, a significant increase in caspases 9, 12 and 3 was observed in these two structures 6 weeks after oAβ25–35 injection. As previously reported in several studies [72–74], BDNF deficits could be associated with a decrease in survival pathways and therefore participate in the initiation of apoptotic processes. The expression of BDNF receptors, TrkB isoforms and p75, and other neurotrophins, particularly nerve growth factor (NGF), must therefore be analyzed to clarify the involvement of trophic factors in the toxic effects induced by oAβ25–35 injection. Consequently, the oAβ-induced toxicity, notably apoptotic processes, hippocampic cell loss, glucocorticoids increase, BDNF impairment and cholinergic deficits came with and could be considered as responsible for the delayed learning and memory impairments observed in oAβ25–35 treated rats.

It must be noted that the oAβ25–35 effects measured here on various markers led to variations that could appear as not correlated within a particular brain region, particularly at the 6 weeks time-point used in the present study. These often subtle changes are likely due to the particular time-course of the toxicity.
Figure 13. Tau phosphorylation. Effects of oAβ25–35 (10 μg/rat) icv injection on Tau phosphorylation in the frontal cortex, amygdala, hippocampus and hypothalamus, determined by western blot in untreated control rats and 6 weeks after Aβ25–35 scrambled amyloid peptide (10 μg/rat; negative control) or oAβ25–35 icv injection. The Tau hyperphosphorylation (AT8; 50 KDa) and the abnormal Tau phosphorylation (AT100; 50 KDa) variations were expressed in function of total Tau expression (50 KDa) and compared with non-injected rats (control group: C). The results are expressed as means ± SEM. *p<0.05 and **p<0.01 vs. control non-injected rats (control group: C) and +p<0.05 and ++p<0.01 vs. respective scrambled peptide-treated rats. The number of animals in groups is indicated within the columns.

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and intrinsic vulnerability of each brain structure, as previously reported at shorter times [20] and as observed in the pathology [75]. For instance, the hippocampus that is precociously damaged after αβ25–35 [20], shows in the present work, after 6 weeks, some signs of recovery in terms of lipid peroxidation, BDNF levels, or inflammation. In the amygdala, the lipid peroxidation decrease observed 6 weeks after αβ25–35 was in the continuity and very coherent with the measures made at earlier times in this brain region [20]. Indeed, this apparent sign of recovery could be simply explained by the fact that after the massive and rapid oxidative stress observed during the 1st and 2nd weeks after icv injection, endogenous neuroprotective mechanisms operate, limiting the extension of lipid peroxidation by increasing activity of the enzymes involved in clearance of peroxidized lipids. Such enzymes include glutathione peroxidase activity that has been recently reported to be modified after Aβ [25–35]. For the first time in this study, we have characterized the particles composition of the aggregated Aβ25–35 solution injected icv in rats. Indeed, while in previous study [20] we detailed qualitatively each component of the injected solution, here we showed that the majority of particles (more than 98%) were small and insensitive to oAβ25–35 injection (oAβ25–35), an important lifespan within brain tissues, but also in circulation. In vivo, the particles were bigger than those obtained in vitro, indicating differences in their synthesis and assembly. Involvement of Soluble Aβ Short Fragments in the AD Physiopathology

For the first time in this study, we have characterized the particles composition of the aggregated Aβ25–35 solution injected icv in rats. Indeed, while in previous study [20] we detailed qualitatively each component of the injected solution, here we showed that the majority of particles (more than 98%) were small soluble particles, suggesting that the toxicity observed after the icv injection of this peptide (oAβ25–35) could be due to a mixture of soluble oligomers. However, in a previous study [25], we have showed that the injection of a non-aggregated solution of Aβ25–35 induced less toxicity than the aggregated solution, suggesting that bigger particles would be necessary to the toxicity. This hypothesis is reinforced by a previous result using an electronic microscopy approach and showing that bigger particles (amyloid fibrils) seemed to stabilize the smaller soluble particle forms [20].

In addition, the long-lasting presence of oAβ25–35-HLF tagged peptide in the brain not only showed that such short fragments have in vivo, an important lifespan within brain tissues, but also strongly suggested that they could participate in the maintenance of the toxicity as observed here.

Six weeks after oAβ25–35 injection, APP processing, and particularly the amyloidogenic pathway (C99 levels), was increased in the frontal cortex, amygdala and hippocampus. This long-term effect of oAβ25–35 could contribute to the global toxicity always observed after 6 weeks. Interestingly, the increase in C99 level is accompanied with a decrease in APP level in the hippocampus. In other structures examined, both APP and C99 levels were concomitantly increased. This difference could be due to a specific activation of BACE in the hippocampus, as previously suggested [92], while, in the other structures, αβ25–35 could only induce an increase in APP expression and processing. Furthermore, as previously discussed [56,62,63], the relation between the high corticosterone levels induced by oAβ25–35 and the activation of amyloidogenic pathway must therefore be further analyzed to clarify the contribution of glucocorticoids deregulation in the amyloid toxicity and more largely in AD etiology.

oAβ25–35 injection also modified Tau phosphorylation. Previous studies showed an increase of Tau phosphorylation up to 3 months after intra-amygala injection of Aβ25–35 or 4 weeks after icv injection [92,93]. In these studies, the authors did not perform the distinction between the different phosphorylation epitopes of Tau. Here, we used two antibodies directed against AT8 and AT100 epitopes, both considered as markers of AD-related Tau phosphorylation [84]. We evidenced clearly a difference of sensitivity to oAβ25–35 among the brain regions considered. An increase of AT8 phosphorylation was noted only at the frontal cortex and amygdala levels, while it was decreased in hippocampus and unchanged in the hypothalamus. Comconitantly, in the same rats, the AT100 phosphorylation was increased in the amygdala and hippocampus and unchanged in the frontal cortex and hypothalamus. In fact, one explanation of these differences of sensitivity to amyloid peptide comes from recent study, where the authors evidenced an intrinsic specific regulation of Tau phosphorylation. Indeed, it seems that Tau phosphorylation occurred in a sequential order of events and that feedback mechanisms exist within neurons where the phosphorylation of certain sites would induce the dephosphorylation of other sites, in order to constantly maintain a phosphorylation level [65]. Thus in our study, it could be suggested that each brain regions could be at a different stage of Tau phosphorylation, and for instance at the hippocampal level that the phosphorylation decrease observed of the AT8 epitope could be under a negative feedback loop exerted by the phosphorylation of the AT100 epitope shown in this structure 6 weeks after oAβ25–35 injection.

There is no doubt that progressive Aβ accumulation contributes to the AD pathology and that extracellular amyloid deposits are a hallmark of AD. However, the view that the mature amyloid fibril was the only toxic species of Aβ is now challenged. Indeed, experimental studies have shown for a range of peptides and proteins that amyloid fibril formation is preceded by the appearance of organized molecular assemblies, usually termed protofibrils [86]. In addition, detailed biophysical studies are currently identifying the formation of smaller oligomeric species at earlier stages of the aggregation process, in vitro, in animal models and in patient brains. In fact, it appears highly conceivable that amyloid deposits may only be one aspect of a larger pathological cascade and indirect consequences of protective responses geared towards sequestering toxic soluble Aβ molecules within plaques, from which oligomeric toxic fragments could be released by proteinolysis [4,9,10,87]. The peculiar potent aggregative ability and neurotoxicity of oAβ25–35, its capability to induce dephosphorylation of the Aβ1–40/42 protein synthesis and the abnormal phosphorylation of Tau, now even discovered physiologically to be present in elderly people
resulted in long-term biochemical, morphological and behavioral fragment resulting from Aβ underestimated, as recently reviewed [87]. Remarkably, this to the maintenance of the progressive neurodegeneration processes could significantly contribute to the overall toxicity and therefore to the maintenance of the progressive neurodegeneration processes observed in AD, through particularly an inhibition of BDNF, an increase of apoptotic processes, a glucocorticoid hypersecretion, and the induction of the amyloid pathway and abnormal phosphorylation of Tau.

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Author Contributions
Conceived and designed the experiments: LG TM GI. Performed the experiments: CZ AB EM CM BD LG. Analyzed the data: LG TM. Wrote the paper: LG TM.

References
1. Mattson MP (2004) Pathways towards and away from Alzheimer’s disease. Nature 430: 631–639.
2. Selkoe DJ (2001) Alzheimer’s disease: Genes, proteins, and therapy. Physiol Rev 81: 741–766.
3. Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer’s amyloid-β peptide. Nat Rev Mol Cell Biol 8: 101–112.
4. Watson D, Castano E, Levybranco Y, et al. (2005) Physicochemical characteristics of soluble oligomeric Aβ and their pathologic role in Alzheimer’s disease. Neurol Res 27: 869–871.
5. Hungenb L, Glei M, McGowan E, Yu X, Benkovic S, et al. (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. Nat Med 4: 97–100.
6. Gruden MA, Davidova TB, Malisauskas M, Sewell RD, Voskresenskaya NI, et al. (2001) Drastic neuronal loss in vivo by microinjection of Aβ1–40 and Aβ1–42 proteolysis appears extremely toxic, with an important lifespan in brain tissues and could significantly contribute to the overall toxicity and therefore to the maintenance of the progressive neurodegeneration processes.
7. Klyubin I, Walsh DM, Lemere CA, Cullen WK, Shankar GM, et al. (2005) Amyloid-β protein immunotherapy neutralizes Aβ oligomers that disrupt synaptic plasticity in vivo. Nat Med 11: 536–541.
8. Lamberti MP, Barlow AK, Chien EA, Edwards C, Freed R, et al. (1998) Diffusible, nonfibrillar ligands derived from Aβ1–42 are potent central nervous system neurotoxins. Proc Natl Acad Sci USA 95: 6448–6453.
9. Ferreira ST, Vieira MN, De Felice FG (2007) Soluble protein oligomers as emerging toxins in Alzheimer’s and other amyloid diseases. IUBMB Life 59: 332–345.
10. Wahl DM, Selkoe DJ (2007) Aβ oligomers - A decade of discovery. J Neurochem 101: 1172–1184.
11. Glennner GG, Wong CW (1984) Alzheimer’s disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 120: 885–890.
12. Russo C, Schettini G, Saido TC, Hulette C, Lippa C, et al. (2000) Presenilin-1 mutations in Alzheimer’s disease. Nature 405: 531–532.
13. Tekirian TL (2001) Commentary: Aβ1–42 and Aβ1–40 –sheet potential [12–16,18,20], which could have been largely reinforced its potential involvement in the pathogenesis of AD. [14–16], reinforce its potential involvement in the pathogenesis of AD. [14–16], reinforce its potential involvement in the pathogenesis of AD.
14. Tekirian TL (2001) Commentary: Aβ1–42 and Aβ1–40 –sheet potential [12–16,18,20], which could have been largely reinforced its potential involvement in the pathogenesis of AD. [14–16], reinforce its potential involvement in the pathogenesis of AD.
15. Kaneko I, Morimoto K, Kubo T (2001) Drastic neuronal loss in vivo by microinjection of Aβ1–40 and Aβ1–42 proteolysis appears extremely toxic, with an important lifespan in brain tissues and could significantly contribute to the overall toxicity and therefore to the maintenance of the progressive neurodegeneration processes.
16. Klyubin I, Walsh DM, Lemere CA, Cullen WK, Shankar GM, et al. (2005) Amyloid-β protein immunotherapy neutralizes Aβ oligomers that disrupt synaptic plasticity in vivo. Nat Med 11: 536–541.
17. Lamberti MP, Barlow AK, Chien EA, Edwards C, Freed R, et al. (1998) Diffusible, nonfibrillar ligands derived from Aβ1–42 are potent central nervous system neurotoxins. Proc Natl Acad Sci USA 95: 6448–6453.
18. Pike CJ, Verleger J, Keller E, Alkam T, Nitta A, et al. (2006) Amnesia induced in mice by centrally administered amyloid-β peptides involves cholinergic dysfunction. Brain 706: 181–193.
19. Maurice T, Lockhart BP, Privat A (1996) Amnesia induced in mice by centrally administered amyloid-β peptides involves cholinergic dysfunction. Brain 706: 181–193.
20. Zussy C, Brureau A, Delair B, Marchal S, Keller E, et al. (2011) Time-course evaluation of anti-dementia drugs. Pharmacol Ther 88: 93–113.
Masugi F, Ogihara T, Sakaguchi K, Otsuka A, Tsuchiya Y, et al. (1989) High

Davis KL, Klyubin I, Shankar GM, Townsend M, Fadeeva JV, et al. (2005)

Catania C, Sotiropoulos I, Silva R, Onofri C, Breen KC, et al. (2007) The

Krugers HJ, Koolhaas JM, Bohus B, Korf J (1993) A single social stress-

Von Bohlen Und Halbach O (2007) Immunohistological markers for staging

Blennow K, de Leon MJ, Zetterberg H (2006) Alzheimer's disease. Lancet 368:

Stepanichev MY, Zdobnova IM, Zarubenko II, Moiseeva YV, Lazareva NA, et

Rogers J, Luber-Narod J, Styen SD, Civin WH (1988) The inflammatory response

Rozenmuller JM, Eikelenboom P, Pals ST, Stam FC (2008) Continuous icv infusion

Vanzani MC, Iacono RF, Caccuri RL, Berria MI (2005) Immunochemical and

Eikelenboom P, Verheun R, Schepet W, Rozenmuller AJM, van Gool WA, et al.

Van de Nes J, Konermann S, Nafe R, Swaab D (2006) Studies of the effects of central

Li X, Zuo P (2005) Effects of Aβ(25–35) on neurogenesis in the adult mouse

Kraeges HJ, Krolhaas JM, Bohns B, Korf J (1993) A single social stress-

Lowy MT, Gauthi, Yamamoto BK (1993) Adrenoceptor antagonism in stress-

McEwen BS (2008) Central effects of stress hormones in health and disease:

Catania C, Sotropoulos I, Silva R, Onofri C, Breen KC, et al. (2007) The

Goodman Y, Bruce AJ, Cheng B, Matteon MP (1996) Estrogens attenuate and

Davis KL, Davis BM, Greenwald BS, Mols RC, Mathe AA, et al. (1986) Cortisol and Alzheimer’s disease. I. Basal studies. Am J Psy 143: 100–203.

Masug F, Ogharira T, Sakaguchi K, Otsuka A, Tischuya Y, et al. (1989) High

Bazan-Peregrino G, Gutierrez-Kohel L, Moran J (2007) Role of brain-derived

Liu, F, Xie B, Cai F, Wu G (2012) Protective effect of Puerarin on β-amyloid-

Thal DR, Rub M, Orantes MM, Braak HM (2002) Phases of Aβ-deposition in

Lin HH, Yang XM, Li TJ, Cheng YF, Zhang HT, et al. (2009) Memory deficits and

Sugirdson EM, Lee JM, Dong WX, Hejna MJ, Lorens SA (1997) Bilateral

Shindoskovski K, Bretteville A, Leroy K, Bégard S, Brion JP, et al. (2006)

Alzheimer’s disease-like Tau neuropathology leads to memory deficits and loss of

Janitorial features of hypothalamic plaques in Alzheimer's disease. Am J Pathol

Long-term Effects of Oligomeric Amyloid-β Peptide