Appendix 1

Methods

1. Animals

All experiments were carried out in the Amylgen facility (Montpellier, France). Animal procedures were carried out in strict adherence to the European Community Council Directive of September 22, 2010 (2010/63/UE). All experiments and protocols were authorized and approved by the French Ministry of Research, as well as by the Regional Animal Welfare Committee. All efforts were made to minimize the number of animals used. Six-week-old, male Swiss mice (RjOrl:SWISS, Janvier, Saint Berthevin, France) weighing 30–35 g, were housed in Amylgen's regulated animal facility (agreement #A 34-169-002 delivered on May 02, 2014), in plastic cages with free access to food and water, except during behavioral experiments. They were kept in a regulated environment under a 12 h light/dark cycle.

2. Photobiomodulation treatment

RGn500 (REGEnLIFE, France) uses a combination of components in an unprecedented way. It is a non-ablative, non-ionizing, non-invasive and non-thermal light source working in the visible and infrared spectrum, including lasers and Light Emitting Diodes (LEDs). The device is made of a near infrared (NIR) InAlGaAs laser (850nm) combined with a NIR LED (850nm) and a red LED (625nm) surrounded with a ring-shaped magnet creating a static magnetic field (SMF) at 200mT. The photonic emissions are pulsed at a 10Hz frequency (50% duty cycle) through the SMF with a total irradiance at skin surface of 28 mW/cm^2 and a total fluence of 8.4 J/cm^2 for a 10 min exposure. As a result, the treatment is a PBM with the addition of a SMF, at the difference with more common PBM used up to now.
Considering the possibility of an abscopal effect of such stimulation based on developing evidence of brain-gut interactions, the treatment was applied on two sites:

head and abdomen.

Mice were maintained motionless by restraining them in a 30 ml Falcon tube and the photonic emitters were manually applied at 1 cm from the shaved skin of the head and/or center of the abdomen. Three applications were tested: the top of the head as a transcranial PBM through a SMF (1cm^2 surface), the abdomen (1cm^2 surface) as a transcutaneous PBM through a SMF, and both head and abdomen (2cm^2 surface) at the same time. Treatments were applied once a day (o.d.) or twice a day (b.i.d.) for 7 days after $A\beta_{25-35}$ injection for several durations (2.5 min, 5 min, 10 min, 20 min).

3. Amyloid peptides preparation and injection

Homogeneous oligomeric preparation of the $A\beta_{25-35}$ peptide was performed according to AMYLGEN's owned procedure. Male Swiss mice were anesthetized with isoflurane 2.5% (Anesteo France) and injected intra cerebroventricularly (i.c.v.) with $A\beta_{25-35}$ peptide (9 nmol/mouse) or Scramble Aβ (Sc Aβ) peptide (9 nmol/mouse), amounting to a final volume of 3 µl/mouse, according to the previously described method [1].
A 26s gauge microsyringe (#701N, Hamilton) with a 4 mm stainless-steel needle was used for the intracerebral injection. The needle was manually inserted unilaterally 1 mm to the right of the midline point equidistant from each eye, at an equal distance between the eyes and the ears and perpendicular to the plane of the skull. The 3 µl injection was performed at the speed of 1 µl/min, the needle was carefully removed from the head of the mouse which was replaced in its homecage and exhibited normal behavior within 1 min after the injection. Behavior experiments were performed 7 days later.

4. Y-maze

Mice were tested for spontaneous alternation in the Y-maze, an index of spatial working memory according to the procedure already described [2, 3]. The Y-maze is built according to the characteristics described previously [4]. Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. An alternation was defined as entries into all three arms on consecutive occasions. The number of maximum alternations were therefore the total

number of arm entries minus two and the percentage of alternation was calculated as (actual alternations / maximum alternations) x 100. Parameters included the percentage of alternation (memory index) and total number of arm entries (exploration index).

5. Step through passive avoidance

Passive avoidance performance, an index of contextual long-term memory, was tested using the apparatus described previously [2, 3] and consisting of two compartments, one white and one black separated by a guillotine door. The black compartment is equipped with a grid floor able to deliver scrambled footshocks. Each mouse was placed into the white compartment. After 5 seconds, the door was raised. When the mouse entered the dark compartment and placed all its paws on the grid floor, the door was closed and the footshocks (0.3 mA) delivered for 3 seconds. The step-through latency, i.e., the latency spent to enter the dark compartment was recorded. The retention test was carried out 24 hours post training. Each mouse was placed again into the white compartment. After 5 seconds, the door was raised. The step-through latency was recorded up to a cut-off time of 300 seconds.

6. Biochemical markers

On day 10, animals were euthanized after the last behavioral test. The brain was removed and the hippocampus and frontal cortex were dissected. The hippocampus was used to determine lipid peroxidation (LPO) levels using a colorimetric method [5] and neuroinflammation by the determination of GFAP, TNFα, IL-1β, IL-6 and apoptosis by the measurement of Bax and Bcl-2. The measurement of $A\beta_{1-42}$ and pTau (Thr 181), two surrogate markers of AD was performed in the cortex. ELISA assay kits were from USCNK (Euromedex, Souffelweyersheim, France), and from Novex (ThermoFisher, Illkirch, France).

7. Histology

Ten days after $A\beta_{25-35}$ peptide oligomers or Sc.Aβ peptide injection, 4 mice from each group were anesthetized by 200 µL intraperitoneal of a premix of ketamine (80 mg/kg) and xylazine (10 mg/kg), and quickly perfused transcardially with 100 mL of

saline solution followed by 100 mL of paraformaldehyde 4%. After fixation, samples were dehydrated using a Peloris tissue processing system(Leica Microsystemes SAS, Nanterre, France) according to an 8-hour protocol.
Sections 3 – 5 µm thick were deposited on Superfrost Plus slides (ThermoFisher Scientific, Illkirch, France). For each sample, 24 sections were performed through the entire extent of the brain hippocampus. The sections were collected according to a systematic uniform random sampling from bregma – 1.06 to -2.70 mm. and immunostained using anti-GFAP, anti MAP2 antibodies (Sigma-Aldrich,Saint Quentin Fallavier, France). Anti Iba-1 was from Wako (Neuss, Germany). Discovery FITC and Rhodamine kits were from Roche-Ventana. Spectral DAPI was from Perkin-Elmer. Immunostained slides were digitalized with the Nanozoomer scanner (Hamamatsu Photonics, Massy, France) in fluorescence conditions, at x 20 magnification. Activated astrocytes and microglial cells were numbered by morphological examination. The analysis was made possible by the labeling of nuclei by DAPI, neurons by MAP2, astrocytes by GFAP and Iba1 microglial cells by Iba1. Data were expressed as number of activated cells in the CA1 region per slice, from at least three slices from 4 brains from each group.

8. Statistical analyses

All values, except passive avoidance latencies, were expressed as mean ± S.E.M. Statistic analyses were performed on the different conditions using one-way ANOVA (*F* value), followed by the Dunnett's post-hoc test. $P < .05$ was considered as statistically significant

References

[1] Haley TJ, McCormick WG. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. British journal of pharmacology and chemotherapy. 1957;12:12-5.
[2] Meunier J, Villard V, Givalois L, Maurice T. The gamma-secretase inhibitor 2-[(1R)-1-[(4-chlorophenyl)sulfonyl](2,5-difluorophenyl) amino]ethyl-5-fluorobenzenebutanoic acid (BMS-299897) alleviates Abeta1-42 seeding and short-term memory deficits in the

Abeta25-35 mouse model of Alzheimer's disease. European journal of pharmacology. 2013;698:193-9.
[3] Villard V, Espallergues J, Keller E, Vamvakides A, Maurice T. Anti-amnesic and neuroprotective potentials of the mixed muscarinic receptor/sigma 1 (sigma1) ligand ANAVEX2-73, a novel aminotetrahydrofuran derivative. Journal of psychopharmacology (Oxford, England). 2011;25:1101-17.
[4] Hiramatsu M, Inoue K. Nociceptin/orphanin FQ and nocistatin on learning and memory impairment induced by scopolamine in mice. British journal of pharmacology. 1999;127:655-60.
[5] Hermes-Lima M, Willmore WG, Storey KB. Quantification of lipid peroxidation in tissue extracts based on Fe(III)xylenol orange complex formation. Free radical biology & medicine. 1995;19:271-80.