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Epigenetic Regulations of Immediate Early Genes Expression Involved in Memory Formation by the Amyloid Precursor Protein of Alzheimer Disease

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Abstract

We previously demonstrated that APP epigenetically regulates Egr1 expression both in cultured neurons and in vivo. Since Egr1 is an immediate early gene involved in memory formation, we wondered whether other early genes involved in memory were regulated by APP and we studied molecular mechanisms involved. By comparing prefrontal (PF) cortex from wild type (APP+/+) and APP knockout mice (APP−/−), we observed that APP down regulates expression of four immediate early genes, Egr1, c-Fos, Bdnf and Arc. Down regulation of Egr1, c-Fos and Bdnf transcription resulted from a decreased enrichment of acetylated histone H4 on the corresponding gene promoter. Further characterization of H4 acetylation at Egr1 and c-Fos promoters revealed increased acetylation of H4K5 and H4K12 residues in APP−/− mice. Whereas APP affected Egr1 promoter activity by reducing access of the CREB transcription factor, its effect on c-Fos appeared to depend on increased recruitment of HDAC2 histone deacetylase to the gene promoter. The physiological relevance of the epigenetic regulation of Egr1 and c-Fos gene transcription by APP was further analyzed following exposure of mice to novelty. Although transcription of Egr1 and c-Fos was increased following exposure of APP+/+ mice to novelty, such an induction was not possible in APP−/− mice with a high basal level of expression of these immediate early genes. Altogether, these results demonstrate that APP-mediated regulation of c-Fos and Egr1 by different epigenetic mechanisms is needed for their induction during exposure to novelty.

Introduction

One of the first steps involved in memory formation is the rapid induction of immediate early genes (IEGs) transcription in the brain. Egr1 is an IEGs member of early growth response family of zinc fingers transcription factors, widely studied for its role in reconsolidation of memory and its ability to establish long term spatial localization memories [1,2]. In mouse, Egr1 is needed for late-phase LTP, is involved in long-term memory formation and controls neuronal function in the hippocampus [3,4].

Expression of c-Fos protein in neurons is induced by a wide range of sensory stimuli [5,6] and several studies have demonstrated the role of c-Fos in the establishment of neuronal plasticity by regulating downstream gene expression [7,8]. c-Fos deficient animals show spatial and associative learning deficits correlated with decrease in synaptic plasticity [9]. Although expressed at low levels in the brain, transcription of c-Fos gene sharply increases after exposure to novelty. Both Egr1 and c-Fos mRNA levels are significantly increased 30–45 minutes after exposure to novelty, in particular in the CA1 and CA3 regions of the hippocampus as well as in the PF cortex [10].

Contrary to Egr1 and c-Fos, Arc protein is not a transcription factor but an effector synaptic protein involved in multiple neuronal pathways [11]. Arc induction occurs in the hippocampus and the cortex following exploration of a novel environment [12]. LTP and synaptic activation also induce Arc expression both at the mRNA and protein levels [13].

Bdnf is a member of neurotrophin family involved in neuronal growth and survival [14], in the development of dendritic network modulating synaptic transmission [15] and in the regulation of synaptic plasticity and memory formation [16]. In rodents, at least 22 different Bdnf mRNA are produced by alternative splicing of a primary transcript, and 9 alternative promoters control Bdnf gene transcription, but exon IV promoter is preferentially induced by neuronal activity [17,18]. Chromatin remodeling also controls Bdnf gene transcription in neurons [19].

We previously demonstrated that the amyloid precursor protein (APP) of Alzheimer disease regulates, at the epigenetic level, the transcription of the Egr1 gene [20]. Induction of IEGs expression is closely related to a final change in chromatin remodeling that allows gene expression [21]. Recruitment of CREB on the Egr1 and c-Fos gene promoters induces an increase in histones
acetylation mediated by the CBP/P300 acetyltransferase, and the concomitant depletion of HDACs [19].

Here we show for the first time that APP fosters a low level of Egr1 and c-Fos expression in mouse PF cortex, by inhibiting CREB recruitment and improving HDAC2 recruitment on the corresponding gene promoters. A low constitutive level of Egr1 and c-Fos expression mediated by APP is needed for their induction during exposure of mice to novelty.

Materials and Methods

Animals

Five months old mice C57Bl/6J APP+/+ and APP−/− were used in this study. Mice were obtained from The Jackson laboratory and backcrossed for more than five generations in the CD1 genetic background. All animals had access to food ad libitum and were housed under controlled temperature (22°C) and with a 12 h light cycle (starting at 7 am). One week before experiments, mice were moved to the experimental platform. As previously described, the behavioral test of exposure to novelty was performed in a square openfield (60×60×40 cm) with gray plastic walls. Mice were able to explore this environment during 15 minutes and sacrificed by CO2 inhalation after a resting period of 30 minutes. Another group of mice were directly sacrificed without exposure to the open field. After brain dissection, PF cortex and hippocampus were directly placed in liquid nitrogen, frozen at −80°C or directly crushed and used in further experiments. All manipulations on mice have been approved by the local ethics committee of the catholic University of Louvain and follow the European legislation.

RNA Extraction and Quantitative Real Time PCR

Total RNA was purified using Trizol method (TriPure, Roche). Reverse transcription (RT) and quantitative (q) real time PCR (qRT PCR) were performed with the iScript cDNA synthesis Kit and the iQ SYBR Green supermix using a iCycler MyIQ2 multicolor Real-Time PCR detection system (Biorad). The relative amplification of cDNA fragments was calculated by the 2^(-ΔΔCt) method. qRT PCR primer sequences were used as follows: Egr1 Forward: TCTCTGCATCATACATGCTGTG, Egr1 Reverse: CACTCTGACACATGCTCCAG, c-Fos Forward: GTATGGTCTCGGGGTTCCTCTTCAAGG, c-Fos Reverse: GGAGAAGGAGTGCCGGCTTG, GAPDH Forward: ACCCGAGGACTGTTGGATGG, GAPDH Reverse: ACACATTTGGGCTAGGAAACA, Arc Forward: GCTGAGCTCTGCTGTAC, Arc Reverse: GGTGAGCTGTAAGCCACATAT, Bdnf Forward: GCGGACCATTGGGACTCT, Bdnf Reverse: CTGCTGCTGATGAGGACGAAGG.

Chromatin Immunoprecipitation (ChIP)

ChIP was performed using the EZ-ChIP Assay kit (Millipore) according to manufacturer instructions and as described previously [20]. Chromatin was isolated and pooled from the two PF cortices of a single mouse. ChIP experiments were performed using a minimum of 3 mice per group. Chromatin was sheared in an ice bath by a 25 cycles of 30 sec on/off sonication using the “Bioruptor UCD-20” sonicator (Diagenode). Samples were kept on ice during 30 s between two pulses. An aliquot of precleared chromatin was collected as the input. The samples were incubated overnight at 4°C with the antibodies of interest: 5 µg anti-H4Ac (Millipore), 5 µg anti-H3Ac (Millipore), 5 µl anti-H2BAc (Abcam); 10 µl anti-Tip60 (SantaCruz), 5 µl anti-HDAC2 (Abcam), 10 µl anti-CREB total (Millipore), 3 µl of acetylated H4K5, K12, K16

Figure 1. APP regulates IEGs expression in mouse PF cortex. A) Egr1 mRNA levels were quantified by q-RT PCR in APP+/+ and APP−/− PF cortex (n = 9), and in the hippocampus (n = 6). B) q-RT PCR method was used to quantify mRNA levels of c-Fos, Arc and Bdnf in APP+/+ and APP−/− PF cortex (n = 6). Values were normalized to the GAPDH mRNA, and expressed as percentage of APP+/+, mean ± SD. Student’s t-test: **p<0.01, ***p<0.001, *p<0.05. doi:10.1371/journal.pone.0099467.g001

(Active Motif). The immunoprecipitated chromatin was analyzed by quantitative PCR with primers designed to amplify short regions of the promoters of genes of interest.

qPCR primers were as follows: Egr1 Forward: GTGCCCACACACTCTTTGGAT, Egr1 Reverse: CGAATCGCTCTATTTTCAA, c-Fos Forward: GAAAGCCTGGGGCGTAGAGT, c-Fos Reverse: CCTCAGCTCGGGCGTATAGTG, Arc Forward: AGTGTGGCTACGCGCTGTCG, Arc Reverse: AGTGGCTACGCGCTGTCG, Bdnf exIV Forward: GCGGCGAAAATTCTGTTGGAAT, Bdnf exIV Reverse: GAGAGGACGCTACAGCTGACG. GAPDH Forward: AGAGAGGAGGAGGGAATG, GAPDH Reverse: AGAGAGGAGGAGGAGCAC. The quantification method used is based on the ratio between immunoprecipitated chromatin and input chromatin.
Protein Analysis

Nuclear extracts of mouse PF cortex were prepared in 0.25 M sucrose buffer (sucrose 0.25 M, Tris 50 mM, EDTA 1 mM, imidazole 3 mM, pH 7.0 + proteases inhibitor cocktail). Samples were centrifuged 10 min at 250 g and nuclear fraction was resuspended in Laemmli buffer. All samples were sonicated before protein assay (BCA Pierce, Thermoscientific) and Western blotting was performed on 20 μg of protein lysates. Membranes were incubated overnight at 4°C with the primary antibodies; anti-HDAC2 1:1500 (Abcam), anti-H3 1:10000 (Millipore), anti-tubulin 1:4000 (Sigma), anti-CREB and phospho-CREB 1:1000 (Millipore). Washes with PBS-Tween (0.005%) were followed by incubation with secondary antibody (1:10 000 anti-mouse or anti-rabbit IgG) (GE Healthcare) coupled to horseradish peroxidase and revealed by ECL. For quantification, the membranes were stripped and reincubated with an anti-tubulin or an anti-H3 antibody. Immunoreactive bands were quantified with an electrophoresis Gel Doc 2000 imaging system coupled to a Quantity one software (Bio-Rad).

Statistical Analysis

All results were expressed as mean ± standard deviation (SD) values. Statistical significance was determined by student’s t-test for two-group comparisons or one-way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparisons test for multi-group comparison.

Results

1. APP Decreases the Transcription of 4 Different IEGs in the Mouse PF Cortex

RNA was prepared from the hippocampus and PF cortex of APP+/- and APP−/− mice, and qRT-PCR were performed to quantify IEGs mRNAs. Results presented in figure 1A indicate a 2.5 increase in Egr1 mRNA levels in PF cortex of APP−/− mice, confirming our previous results [20]. We wondered whether transcription of other IEGs involved in memory formation could also be regulated by APP. Results presented figure 1B indicate a significant increase in c-Fos, Arc and Bdnf mRNA levels in APP−/− mice, confirming our previous results [20]. We wondered whether transcription of other IEGs involved in memory formation could also be regulated by APP. Results presented figure 1B indicate a significant increase in c-Fos, Arc and Bdnf mRNA levels in APP−/− mice. Levels of IEGs mRNA were normalized to Gapdh mRNA levels, which were not regulated by APP, as 3 other housekeeping genes (Actin, peptidylprolyl isomerase A, and β-glucuronidase) (Figure S1). In addition, expression of 2 other IEGs (c-Jun and Homer-1a) was not affected by APP (Figure S1).

These results therefore suggest that APP is able to regulate transcription of several IEGs involved in memory formation.
Enrichment of acetylated H4 was not observed on the promoter. Data represent the level of enrichment normalized as enrichment of H4K12Ac, H4K5Ac, and H4K16Ac. A) Egr1 promoter. B) c-Fos promoter. Data represent the level of enrichment normalized as percentage of APP+/+. **p<0.001. All results derive from at least 3 mice per group and per antibody, and are expressed as mean ± SD. doi:10.1371/journal.pone.0099467.g003

3. APP Specifically Regulates Acetylation of Histone H4 at Lysines 5 and 12

Acetylation of histone H4 can occur at different positions including lysines 5, 12 and 16. To identify the positions that are acetylated on H4 enriched on the Egr1 and c-Fos gene promoters in APP−/− mice, ChIP experiments were performed using anti-H4K5Ac, -H4K12Ac and -H4K16Ac specific antibodies. Results presented in figure 3A indicate that histones H4 enriched on the Egr1 gene promoter in APP−/− mice were acetylated on positions K5 and K12, but not on K16. Interestingly, the same profile of acetylation was found for histone H4 enriched on the c-Fos gene promoter in APP−/− mice (Figure 3B). These results suggest that APP regulates Egr1 and c-Fos gene transcription by similar molecular mechanisms.

4. Regulation of Egr1 Gene Transcription by APP is CREB Dependent

A schematic representation of the Egr1 gene promoter is given in figure 4A [22]. The histone acetyltransferase Tip60 is able to acetylate histone H4 at positions H4K5 and H4K12 [23] and interacts with AICD [24]. Therefore, an interaction between Tip60 and APP could inhibit H4K5 and H4K12 acetylation in APP+/+ mice. To test this hypothesis, ChIP experiments were performed using anti-Tip60 antibodies. Tip60 is able to bind to the promoter of KAI1 gene and regulates its transcription [25,26]. In Chip experiments, we indeed demonstrated an enrichment of Tip60 on the promoter of the KAI1 gene (Figure 5). However, results presented in figure 4B do not show any enrichment of Tip60 on the Egr1 gene promoter in APP−/− mice, allowing us to rule out the implication of Tip60 in this regulation. On the other hand, acetylation of H4K5 and H4K12 is mediated by HDAC2 [28,29]. As HDAC2 epigenetically regulates transcription of several IEGs [30], we also attempted to immunoprecipitate CREB on Egr1 promoter and we confirmed that this transcription factor was significantly more enriched on the Egr1 gene promoter in APP−/− mice (Figure 4C). To test whether APP was able to down regulate CREB expression or to inhibit its phosphorylation, nuclear extracts from APP+/+ and APP−/− mice were analyzed in Western blotting using anti-CREB and anti-S133PhosphoCREB specific antibodies. Results presented in figure 4D indicate the same CREB/PhosphoCREB ratio in APP+/+ and APP−/− mice, ruling out modification of CREB expression and phosphorylation by APP. The recruitment of CREB on a gene promoter is often followed by local depletion of several HDACs [28,29]. As HDAC2 epigenetically regulates transcription of several IEGs including Egr1 [30], we also performed ChIP experiments using HDAC2 antibodies. Results presented in figure 4E do not show any significant enrichment of HDAC2 on the Egr1 gene promoter in APP−/− mice. Altogether, these results indicate that APP fosters low level of Egr1 expression in mouse brain by inhibiting recruitment of CREB without affecting enrichment of HDAC2 on the Egr1 gene promoter.

5. Regulation of c-Fos Gene Transcription by APP is HDAC2 Dependent

The c-Fos gene promoter also contains a single CRE site (Figure 5A). The same profile of acetylation of histone H4 weaker up regulation of Bdnf expression in APP−/− mice (Figure 1) led us to focus on the molecular mechanisms involved in the regulation of Egr1 and c-Fos expression by APP.

2. APP Affects Enrichment of Acetylated Histone H4 on Genes Promoters

We previously observed that the control of Egr1 gene transcription by APP is independent of the APP intracellular domain (AICD) but occurs at the epigenetic level [20]. Therefore, we wondered whether APP could control transcription of other IEGs by the same mechanism. To that aim, ChIP experiments were performed using anti-acetylated histones antibodies. Results presented in figure 2A clearly demonstrated a specific enrichment of acetylated H4, but not H3 and H2B, on the Egr1 promoter in APP−/− mice, confirming our previous results [20]. Interestingly, the same specific enrichment of acetylated H4 was measured on both the c-Fos (Figure 2B) and Bdnf (Figure 2C) gene promoters in APP−/− mice. No modification in H3Ac, H2Bac and H4Ac enrichment on the GAPDH gene promoter was observed in APP+/+ and APP−/− experimental conditions (Figure S2). Enrichment of acetylated H4 was not observed on the Arc gene promoter in APP−/− mice (Figure 2D), indicating that a different molecular mechanism is involved in the APP-mediated regulation of Arc gene expression. This conclusion, together with a much
Enrichment of CREB on the c-Fos and Egr1 gene promoters in APP+/− mice (Figure 3B) suggested that APP might regulate Egr1 and c-Fos gene transcription by similar molecular mechanisms. Therefore, we first measured recruitment of CREB on the c-Fos gene promoter in APP+/+ and APP−/− mice. Results of ChIP experiments (Figure 5B) show an enrichment of CREB on c-Fos promoter, but no difference between APP+/+ and APP−/− mice, suggesting that basal regulation of c-Fos gene transcription by
Figure 5. HDAC2 is enriched on the c-Fos gene promoter in APP−/− mice. A) Schematic representation of the structure of c-Fos promoter containing only one CREB binding site and the localization of the primers utilized for q-PCR. (CREB: cAMP response element, CRE: cAMP response elements SRF: Serum Response factor, SRE: Serum Response Element, AP1: Activator protein 1, SIE: sis-inducible element, STAT: Signal Transducer and
APP is not CREB dependent although c-Fos is regulated by CREB [31]. Since HDAC2 was previously demonstrated to inhibit c-Fos gene transcription [30], we performed ChIP experiments using anti-HDAC2 antibodies. Results presented in figure 5C clearly indicate a significant enrichment of HDAC2 on the c-Fos gene promoter in APP+/+ mice. Western blot analysis of nuclear extracts prepared from the PF cortex of APP+/+ and APP−/− mice with anti-HDAC2 antibodies did not show any modification of HDAC2 nuclear or cytoplasmic content, indicating that APP does not modify neither HDAC2 expression nor its nuclear localization (Figure 5D). Altogether, these results indicate that APP fosters a low level of c-Fos expression in mouse brain by increasing enrichment of HDAC2 on the c-Fos gene promoter.

6. Exposure of Mice to Novelty Induces IEGs Transcription in APP+/+ but not in APP−/− Mice

Egr1 and c-Fos are inducible transcription factors that are needed for synaptic plasticity and establishment of long term memory. We reasoned that induction of IEGs transcription needs a constitutive low level of expression, as measured for Egr1 and c-Fos in APP+/+ mice. APP+/+ and APP−/− mice were exposed to novelty during a spatial exploration of an open field, in an experimental protocol that was previously described to enhance Egr1 expression in mouse brain by increasing HDAC2 on the c-Fos gene promoter.

We extended our observation of the epigenetic regulation of Egr1 gene transcription by APP to c-Fos, Bdnf and Arc. All of those genes belong to the IEGs family of transcription or neurotrophic factors needed for memory formation [27,36,37]. The ability of APP to increase gene transcription by an AICD dependent mechanism has been previously demonstrated, although still debated [38–43]. Here, we show for the first time that APP represses transcription of a group of genes, all related to synaptic plasticity. The moderate effect of APP on IEGs expression that we measured could explain why these IEGs have never been reported as APP target genes in microarray experiments in which the cut off of differences in gene expression is usually higher.

Induction of IEGs through acetylation of histones H3, H4 and H2B is well documented [44], and acetylation of histone H3K14, H4K12 or phosphorylation of H3S10 via ERK activation are associated with chromatin relaxation leading to IEGs transcription and memory formation [45–47]. We have previously demonstrated that trichostatin A, a specific HDAC inhibitor, was able to induce Egr1 gene transcription in both APP+/+ and APP−/− neurons, although induction was significantly higher in APP−/− neurons, in agreement with higher H4 acetylation at the Egr1 gene promoter in these cells [20].

Although induction of IEGs has been widely studied, little is known about basal regulation of IEGs transcription in the hippocampus and the cortex. Basal expression of IEGs in the brain is referred as an expression induced by physiological synaptic input [48]. We demonstrate here that APP fosters a low level of expression of the four IEGs studied. APP represses transcription of Egr1, c-Fos, and Bdnf by decreasing enrichment of acetylated H4, but not H3 nor H2B histones on the corresponding gene promoters. APP-mediated repression of Arc gene transcription appears to be controlled by other molecular mechanisms. SAHA, a nonspecific HDAC inhibitor, was demonstrated to up regulate c-Fos and Bdnf expression, while Arc induction was much more moderate [49], indicating that Arc expression should be regulated by other mechanisms than histone modification.

We observed a very similar acetylation profile of H4K5 and H4K12, but not H4K16, on Egr1 and c-Fos gene promoters, which suggested that APP might regulate both genes by similar mechanisms. H4K5 and H4K12 are known to be acetylated by Tip60 and CBP/P300 histone acetyltransferase activities [23]. As Tip60 interacts with AICD and the adaptor protein Fe65 [24], APP could inhibit H4K5 and H4K12 acetylation in APP+/+ mice.
We analyzed a possible interaction of Tip60 with Egr1 promoter but failed to detect any significant interaction or any enrichment in APP+/− mice. Therefore, the contribution of CBP/P300 in the acetylation of H4K5 and H4K12 present on the Egr1 and c-Fos gene promoters was further investigated.

CBP and p300 are two highly related histone acetyltransferases that share many biological functions and interact with phosphorylated cAMP response element binding protein (CREB) [50]. CRE sites are present on Egr1 and c-Fos gene promoters, and regulation of Egr1 and c-Fos gene transcription by CREB is well established [19,21,31,51]. We demonstrate that regulation of Egr1 gene transcription by APP is CREB dependent. In APP−/− mice, CREB is better recruited on the Egr1 gene promoter, arguing for a CBP/P300-mediated increase in H4K5 and H4K12 acetylation of H4 present on the Egr1 promoter. The presence of CREB on a gene promoter has been associated with the removal of HDACs [29]. HDAC2 deacetylates H4K5 and H4K12, and is described as a negative actor in memory formation and synaptic plasticity [30]. Even if we were able to detect enrichment of HDAC2 on Egr1 gene promoter, the level of HDAC2 detected was the same in APP+/+ and APP−/− mice.

Surprisingly, regulation of c-Fos gene transcription by APP occurs by a different mechanism, since the same recruitment of CREB on the c-Fos gene promoter was measured in both APP+/+ and APP−/− mice but less HDAC2 was found on the c-Fos gene promoter in APP−/− mice. APP decreased CREB recruitment on Egr1 gene promoter and increased HDAC2 recruitment on c-Fos gene promoter, without any modification of either CREB and HDAC2 expression or CREB phosphorylation. The presence of two CRE sites in the Egr1 gene promoter could explain why CREB-mediated regulation of this gene is more dependent on CREB recruitment.

Our results indicate that APP fosters a low expression of a group of IEGs involved in memory formation. Consequently, overexpression of APP could have important consequences on memory formation. Interestingly, it was previously demonstrated that in APP transgenic mice [52–54] could be related to their impairment in synaptic plasticity and memory formation [30]. HDAC2 deacetylates H4K5 and H4K12, and is described as a negative actor in memory formation and synaptic plasticity [30]. Even if we were able to detect enrichment of HDAC2 on Egr1 gene promoter, the level of HDAC2 detected was the same in APP+/+ and APP−/− mice.

Supporting Information

Figure S1 Housekeeping genes and other IEGs expressions are not regulated by APP. GAPDH mRNA levels were quantified by q-RT PCR in APP+/+ and APP−/− PF cortex (n = 6) and normalized versus Actin B) q-RT PCR method was used to assess mRNA levels of the housekeeping genes Actin, peptidylproline isomerase A (PpiA) and β-glucuronidase (Gusb) in APP+/+ and APP−/− PF cortex (n = 6), C) mRNA levels of the IEGs c-Jun and Homer-1a were quantified by q-RT PCR in in APP+/+ and APP−/− PF cortex (n = 6). Values were normalized to the GAPDH mRNA, and expressed as percentage of APP+/+, mean ± SD. Primers sequences used were as follows: PpiA FOR: CAGAGGCCACTCGTGCTT; PpiA REV: TGTTTGGAGACACCTTTGCTGAA, Gusb FOR: ACTGACACCTCCATGTATCCCAAG, Gusb REV: CAGTAGGTCACCAAGCCCGATG, c-Jun FOR: TGAAGCTGTGTTCCCTGGTC; c-Jun REV: ATGCAGCACTGCAACACTTT, Homer1a FOR: GAAGTCGCCAGAGGATG, Homer1a REV: TGAATGCTGAATGATGTTGAC.

Figure S2 Analysis of histone acetylation by ChIP assays on GAPDH promoters. ChIP experiments were performed on chromatin obtained from APP+/+ and APP−/− PF cortex. Immunoprecipitation was completed with antibody recognizing normal mouse IgG as negative control, anti H3Ac, H2BAc and H4Ac. The quantification of immunoprecipitated DNA and the normalization versus total DNA (input) was assessed by real-time qPCR with primers designed on GAPDH. All results were obtained from at least 3 or more mice per group and per antibody, and are expressed as mean ± SD.

Figure S3 Tip60 is enriched in KAI1 promoter. Tip60 binding to KAI1 gene promoter in APP+/+ and APP−/− PF cortex was assessed by ChIP using anti-Tip60 antibody, with primers designed in KAI1 promoter region. Primers sequences used were as follows: KAI1 FOR: ACCGTTAGGCAACGCCGTGAG; KAI1 REV: CTTTTGGGAGCCGGTGGCCGTC. IgG was used as negative control. Results show a significant enrichment of Tip60 in APP+/+ mice, **p<0.01. Enrichment values were normalized to input values and are the average of three or more experiments per group. Results are expressed as mean ± SD.

Author Contributions

Conceived and designed the experiments: AH JNO. Performed the experiments: AH BT OS. Analyzed the data: JNO AH NP PKC CDS. Wrote the paper: AH JNO.

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