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Dysregulation of histone acetylation pathways in hippocampus and frontal cortex of Alzheimer's disease patients

Running head title: Histone acetylation pathway in AD patients

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Abstract:

Memory impairment is the main feature of Alzheimer's disease (AD). Initial impairments originate in the temporal lobe area and propagate throughout the brain in a sequential manner. Epigenetic mechanisms, especially histone acetylation, regulate plasticity and memory processes. These may be dismantled during the disease. The aim of this work was to establish changes in the acetylation-associated pathway in two key brain regions affected in AD: the hippocampus and the F2 area of frontal cortex in end-stage AD patients and age-matched controls. We found that the F2 area showed more pronounced changes than the hippocampus. Indeed, CREB-Binding Protein (CBP), P300/CBP-associated protein (PCAF), Histone Deacetylase 1 (HDAC1) and HDAC2 (but not HDAC3) levels were strongly decreased in F2 area of AD compared to controls patients, whereas only HDAC1 was decreased and CBP showed a downward trend in the hippocampus. At the histone level, we detected a substantial increase in total (H3 and H2B) histone levels in the frontal cortex, but these were decreased in nuclear extracts, pointing to a dysregulation in histone trafficking/catabolism in this brain region. Histone H3 acetylation levels were increased in cell nuclei mainly in the frontal cortex. These findings provide evidence for acetylation dysfunctions at the level of associated enzymes and of histones in AD brains, that may underlie transcriptional dysregulations and AD-related cognitive impairments. They further point to stronger dysregulations in the F2 area of the frontal cortex than in the hippocampus at an end-stage of the disease, suggesting a differential vulnerability and/or compensatory mechanisms efficiency.

Keywords

Alzheimer's disease

Histones (H2B and H3)

Histone acetyltransferase (CBP, PCAF)

Histone deacetylase (HDAC1, HDAC2 and HDAC3)

F2 area of frontal cortex

Hippocampus

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia, mainly characterized by synaptic plasticity impairments and neurodegeneration starting from the hippocampal region and spreading to wider cortical regions. This is associated with the misfolding and aggregation of 2 proteins including the extracellular accumulation of amyloid- β peptide (A β) in plaques and cytoplasmic accumulation of hyper-phosphorylated tau in neurofibrillary tangles. In parallel, there are cell-signaling perturbations in the different brain structures impacted and they play an important role in the neurodegenerative processes, as well as in the memory dysfunctions observed in AD patients (Kozlov et al., 2017). Notably, AD brains present deregulated gene transcription, which may alter cognitive processes such as long-term memory formation (Alberini and Kandel, 2014). Importantly, transcriptional regulation of gene expression relies on a state of "chromatin competence", itself dependent of epigenetic regulations (Kouzarides, 2007).

Epigenetic mechanisms are involved in memory and long-term synaptic plasticity (for recent review Campbell and Wood, 2019), and it is thought that epigenetic dysregulations could also underlie the aberrant gene expression associated with the loss of synaptic plasticity and memory in Alzheimer's disease (Guan et al., 2009; Graff et al., 2012; GJoneska et al., 2015; Chatterjee et al., 2018; De Jager et al., 2018). Among these mechanisms, histone modifications and more particularly histone acetylations are key players in memory processes but also in regulating the neuroprotection / neurodegenerative balance (for review (Graff and Mansuy, 2008; Graff and Mansuy, 2009; Stilling and Fischer, 2011; Graff and Tsai, 2013; Schneider et al., 2013; Benito et al., 2015; Chatterjee et al., 2018)). Histone acetylations are regulated by two types of enzymes: histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs are responsible for histone acetylation on lysine residues, thereby opening chromatin conformation and favoring transcription factor and co-activator recruitment necessary for transcriptional activation. *A contrario* HDACs remove acetylation from the histones, thereby conferring a more closed chromatin conformation and transcriptional inactivation. It has been shown that some HATs, such as CREB-binding protein (CBP) (Alarcon et al., 2004; Korzus et al., 2004; Wood et al., 2005; Chen et al., 2010; Valor et al., 2011; Barrett et al., 2011; Bousiges et al., 2010), p300 (Oliveira et al., 2007), or p300/CBP-associated factor (PCAF) (Maurice et al., 2008), are associated with long-term memory mechanisms. Moreover, memory processes also rely on HDACs, such as HDAC2, the first HDAC for which a role in memory formation and synaptic plasticity was clearly demonstrated (Guan et al., 2009; Graff et al., 2012; Nott et al., 2008). HDAC1 was further shown to be important for fear memory extinction (Bahari-Javan et al., 2012) as well as HDAC3 which is a critical negative regulator of long-term memory formation (McQuown et al., 2011; Alaghband et al., 2017; Kwapis et al., 2017).

The fate of HAT and HDAC enzymes during the course of AD has been investigated in humans with contrasting results. Reduction of expression of CBP and its homolog p300 have been reported in postmortem prefrontal cortex of individuals with AD compared to age-matched

controls (Bartolotti et al., 2016), while increase in phospho-p300 has been observed in hippocampal area CA1 of human brains (Aubry et al., 2015). Graff and colleagues showed an increase of HDAC2 and no variation for HDAC1 in the hippocampus of AD patients, effects that were also observed in mouse models of neurodegeneration (Graff et al., 2012; Bie et al., 2014). By contrast, other studies showed a strong decrease of HDAC1 and HDAC2 in the cortex of AD patients (Mastroeni et al., 2011; Anderson et al., 2015). A recent in vivo study led with a new PET imaging agent capable of marking Class I HDACs ($[^{11}\text{C}]$ Martinostat) suggests an overall loss of HDAC class I enzymes in posterior cingulate, precuneus, and inferior parietal cortices of Alzheimer patients (Pascoal et al., 2018). Moreover, comparable patterns between controls and MCI patients were observed suggesting that the decrease of class I HDAC enzymes appear at the demented stage. However, such study cannot document which subtype is predominantly affected. Lastly, the sirtuins, another class of HDACs, have also been shown to be altered during the clinical course of the pathology and may play a role in the cognitive symptoms of AD (for review Cacabelos et al., 2019).

Concerning acetylation changes in AD, the literature is also divergent, with the additional observation that post-translational modifications of histones are sensitive to post-mortem delays (Barrachina et al., 2012; Jarnasz et al., 2019). In humans, Rao and colleagues (Rao et al., 2012) did not detect H3Ac changes in the frontal cortex of AD patients compared to controls, whereas Zhang and colleagues highlighted H3Ac decrease in AD patient's temporal lobe (Zhang et al., 2012). Interestingly, comparison of old and AD subjects revealed a genomic redistribution of H4K16ac, as assessed by ChIP-sequencing, in the lateral temporal lobe of AD subjects, with more losses than gains (Nativio et al., 2018). Some other studies described an acetylated H3 increase of bulk chromatin in inferior temporal gyrus and middle temporal gyrus (Narayan et al., 2015) or in occipital cortex of AD patients (Lithner et al., 2013) of post-mortem AD brain compared to those of age-matched control patients. In rodent models, many studies lead on epigenetic changes measurements have reported different results, e.g.: acetylation decrease (Bie et al., 2014; Benito et al., 2015; Chatterjee et al., 2018), acetylation increase (Lithner et al., 2013; Wu et al., 2013; Marques et al., 2012) or no change (Francis et al., 2009; Cadena-del-Castillo et al., 2014).

Thus, there is a conflicting literature on establishing how the histone acetylation pathway is altered during AD in human brains. A potent explanation could be that most studies investigated specific proteins of interest in a specific brain region in different cohorts of AD patients. Herein, we have led a more comprehensive study, evaluating a panel of acetylation-modifying enzymes (CBP, PCAF, HDAC1, 2 and 3), as well as different histone acetylation motifs, in two AD-key regions obtained from the same patients, the F2 area of frontal cortex (F2) and in the hippocampus of *post-mortem* pathological AD compared to those of healthy brain. We show that AD patients presented a dramatic decrease of several enzymes, i.e. CBP, PCAF, HDAC1 and HDAC2, in the F2 area of the frontal cortex, while changes were more moderate in their hippocampus. Histone acetylation levels changes were more complex to analyze as a global increase of the total level of H3 and H2B histones were measured in whole

tissues of frontal cortex. Further assessments specifically performed in nuclear extracts revealed that the total histone levels were in fact significantly lowered in the frontal cortex of AD patients and the net nuclear histone H3 acetylation levels were then increased, with no change in H2B acetylation. No overall change of total histone was observed in the hippocampus, but acetylation levels were also altered. These results show that epigenetic enzymes, total histone and acetylated histone levels are dramatically altered in the end-stage AD brains. This may account for dysregulated gene expression programs. They also emphasize that the frontal cortex, a region that is affected at later stage than the hippocampus within the course of the disease, shows more pronounced epigenetic dysregulations than in the hippocampus. This may account for early decreased plasticity in this structure and decrease in associated functions.

2. Experimental procedures

Human brain tissue

We have obtained control and AD post-mortem tissues from NeuroCEB (a French national organization dedicated to provide brain samples, either normal or pathological; identification number: "Neuro-CEB BB-0033-00011"). The samples were obtained from brains collected in a Brain Donation Program of the Brain Bank "Neuro-CEB" run by a consortium of Patients Associations: CSC (cerebellar ataxias), Fondation ARSEP (research on multiple sclerosis), France Parkinson, Fondation Vaincre Alzheimer. The consents were signed by the patients themselves or their next of kin in their name, in accordance with the French Bioethical Laws. The Brain Bank Neuro-CEB has been declared at the Ministry of Higher Education and Research and has received approval to distribute samples (agreement AC-2013-1887). All brains were examined by neuropathologists and classified based on neuropathological abnormalities, or lack thereof in neurologically normal controls. Cases were classified using Braak staging (I–VI) (Braak and Braak, 1995). All AD patients studied in this article were at a terminal stage (stage VI), while the controls are at 0-I Braak stage (Table 1). Hippocampal and frontal cortex samples frozen at -80°C were used for biochemical research, samples from hippocampal and frontal cortex sectioned to obtain $10\ \mu\text{m}$ thick sections, mounted on slides and frozen with isopentane cooled in liquid nitrogen were used for histological studies. As described in table 1, we analyzed 8 blocks from AD patients and 5 blocks from control patients for frontal cortex; and 6 blocks from AD patients and 5 blocks from control patients for hippocampus. For histological studies $n=3$ AD and $n=2$ controls patients were obtained for frontal cortex. Table 1 describes also in detail the available clinicopathological information for all the cases and controls (gender, age, frozen hemisphere, postmortem delay, profession, neuropathological diagnosis and potential associated pathology). The post-mortem delays for controls were 12.3 ± 7.0 hours versus 22.1 ± 11.4 hours for AD (non-significant difference, $p=0.113$) and the age at death was for controls 76.4 ± 7.5 years versus 80.4 ± 4.7 years for AD (non-significant difference, $p=0.171$).

All the patients (controls and AD) were controlled for different Alzheimer hallmarks, such as phospho-Tau (Ser396, Thr231, Thr212/ Ser214, Ser404), amyloid β by both western blot and immunohistochemistry analysis. AD patients also showed, as expected at this age, a significant loss of NeuN in parallel with increased GFAP labeling (Figure 1).

Protein Preparation and Western Blot Analyses

Total protein preparation. Tissues were lysed and homogenized in Laemmli buffer. After 10 min at 70°C, samples were sonicated two times for 10 s (Ultrasonic Processor, power 40%), boiled 5 min and centrifuged (14 000 g, 5 min). The supernatant was frozen at -20°C.

Nuclear protein extractions were adapted from Dignam et al (1983) (Dignam et al., 1983). The samples were suspended in five packed pellet volumes of buffer A (0.32M sucrose, 1mM MgCl₂, 0.25% Triton). Samples were lysed by 10 strokes with a dounce homogenizer (tight pestle). The homogenate was centrifuged for 10 min at 1,000 g to pellet nuclei. The pellets were kept and washed with buffer B (buffer A without triton). The homogenates were again centrifuged for 10 min at 1,000 g to remove residual cytoplasmic material. The nuclear pellets were then collected in buffer C (TRIS 10mM and EDTA 13mM pH7.4) supplemented with protease inhibitor cocktail and sodium butyrate (1mM). They were then centrifuged, the soluble nuclear fractions were homogenized in Laemmli buffer and samples were sonicated two times for 10 s (Ultrasonic Processor, power 40%), boiled 5 min and centrifuged (14 000 g, 5 min). The supernatant was frozen at -20°C. The synaptic protein Neurogranin is detectable in total but not in nuclear extracts confirming the quality of nuclear extraction (Figure 1).

Western blots analyses. Protein concentration was measured using the RC-DC Protein Assay (Bio-Rad). Western blots were performed as described previously (Bousiges et al., 2013) with polyclonal antibodies against CBP (sc-369 or sc-7300), HDAC1 (sc-81598), neurogranin (sc-50401) (Santa Cruz Biotechnology), HDAC2 (05-814), phospho-Tau S396 (05-885), acetyl-histone H2B (H2B tetra-Ac, 07-373), acetyl-histone H3 (06-599) (Upstate Biotechnology, New York, NY, USA), phospho-Tau Thr2012/Ser214 (AT100, MN1060), phospho-Tau S404 (44-758G) (Thermo Fisher Scientific, France), GAPDH (ab22555), acetyl-histone H3 K27 (ab4729), histone H3 (ab24834) (Abcam, Cambridge, UK), PCAF (C14G9) (Cell Signaling Technology Europe, Leiden, The Netherlands), histone H2B (H2-2A8) (Euromedex, Strasbourg, France). Secondary antibodies were a horseradish peroxidase-conjugated whole-goat anti-rabbit IgG (111-035-003), or a horseradish peroxidase-conjugated whole-goat anti-mouse IgG (115-035-003) (Jackson Immunoresearch, Suffolk, UK). Western blots have been performed using 2 different technologies: Precast Criterion Gels, wet transfer to nitrocellulose followed by ECL, film exposure and autoradiography for the studies in the Frontal Cortex (except for HDAC3 blots). Other blots in hippocampus (and including HDAC3 in the frontal cortex) were performed using Precast Criterion Tgx stain-free gels® (#5678095, Bio-Rad) and after proteins transfer on nitrocellulose membrane with Trans Blot® Turbo™ transfer system (Bio-Rad) the proteins

were revealed with Clarity ECL (170-5061, Bio-Rad) and exposed in Chemidoc Touch® (BioRad). When blots have been revealed by autoradiography, proteins of interest (enzymes, total histones and modified histones) have been normalized on beta Actin levels present in each corresponding sample. When the blots have been revealed with the ChemiDoc Touch, results were quantified using Image Lab® software: bands of interest were normalized relative to the total amount of protein detected on the nitrocellulose after transfer for each sample.

Immunohistochemistry. Frontal cortex sections were sent ready for immunohistochemistry use (10µm thickness sample fixed on a slide) by the French national brain bank neuro-CEB. The tissue sections were fixed by paraformaldehyde 4% for 5 min and then were permeabilized in 1XPBS/2% Triton for 15 min. Nonspecific labeling was blocked by 1XPBS/0.1% Triton/5% horse serum for 30 min, and sections were then incubated overnight with Phospho-Tau (Ser404) antibody (Thermo-Fischer reference: 44-758G). After three washes, they were incubated with Alexa Fluo 594 donkey anti-rabbit (1 h at room temperature) followed by 3 washes with 1xPBS/0.1%Triton X-100. After two PBS washes, the sections were mounted with Mowiol.

Statistical Analysis

Statistical analyses were performed using Student's t-test (PRISM version 5; GraphPad, San Diego, CA, USA). Data are expressed as the mean \pm SEM. Differences at $p < 0.05$ were considered significant. In cases of variance inequality, a Welch's t test was performed. In addition, when the test for normal distribution (Shapiro-Wilk test) was not respected, we used the Mann-Whitney non-parametric test.

Power analysis

Due to the small number of patients in the study, we determined for each result a post hoc or retrospective power analysis. This analysis takes into account the N and the standard deviation for each group and gives a minimum expected difference between the group means according to the power chosen. It is generally considered that the power must be at least 0.80 to be satisfactory. For this purpose, each analysis was taken over with a statistical software (GraphPad –StatMate 2.00) to determine if for each of them the power is sufficient. When the power analysis exceeds 80%, we left the result as statistically significant, but when the power analysis shows that the difference observed between the two groups is less than 80%, we will report that there is only a tendency (despite statistically significant with the t-test). For these analyses, this will indicate the need to have more samples to conclude.

Results

Creb-binding-protein (CBP) levels are strongly decreased in frontal cortex of AD brain.

Post-mortem human brain samples were obtained from control (n=5) and AD patients (n=8 for frontal cortex and n=6 for hippocampus), on which we controlled the specific hallmarks of

AD. All AD samples from the frontal cortex showed hyper-phosphorylation of several Tau epitopes, increased amounts of amyloid β peptides, neuronal loss (NeuN) and increased neuroinflammation (GFAP) (Figure 1, see materials and methods).

CBP acetyltransferase levels were evaluated by western blot analyses in *post-mortem* brain extracts from AD and aged-matched controls. Interestingly, CBP was dramatically decreased in AD patients F2 area (73% lower compared to controls, Welch's t test $p < 0.05$, figure 2A) and only a trend in the hippocampus (61% lower in AD patients compared to controls, figure 2B). PCAF, another acetyltransferase involved in memory processes (Maurice et al., 2008), tends to decrease in frontal cortex of AD patients, (25% decrease in AD patients compared to controls, $p < 0.05$ but power analyses $< 80\%$, figure 2A). In the hippocampus, no significant differences were observed for PCAF levels (figure 2B).

HDAC1 and HDAC2 are decreased in frontal cortex while only HDAC1 is decreased in hippocampal region of AD patients

The Class I family of HDACs plays a significant role in learning and memory processes (Guan et al., 2009; Nott et al., 2008; McQuown et al., 2011; Shu et al., 2018) as well as in extinction processes (Bahari-Javan et al., 2012) and the levels of HDAC1, 2 and 3 were investigated. We found that HDAC1 levels tend to decrease in frontal cortex and were decreased in hippocampus samples of AD patients (45% lower compared to controls, $p < 0.05$ but power analyses $< 80\%$ for frontal cortex; 72% lower compared to controls, $p < 0.05$, for hippocampus, figure 3B). We also found a strong reduction of HDAC2 in AD patients F2 area compared to the controls (more than 85%, Mann-Whitney test $p < 0.01$, figure 3A), but HDAC2 levels were unchanged in the hippocampus samples (figure 3B). HDAC3 was not found affected in the AD samples, neither in the frontal cortex nor in the hippocampus (figure 3A and 3B).

Total histone levels are increased in whole tissue extracts but decreased in nuclear extracts of AD patient's frontal cortex

Analyses of the AD frontal cortex F2 area surprisingly revealed that total H3 and H2B histone levels were both increased when measured in total protein extracts but significantly only for H3tot (H3tot, 51% higher, $p < 0.0001$, H2Btot, 46% higher, $p < 0.05$ but power analyses $< 80\%$, figure 4A and 4B). Then, whereas the bulk acetylation levels of H3 (H3K9K14, H3Ac) and of H2B (tetra-acetylation, H2BAc) were significantly increased in AD tissues, the ratio of H3Ac/H3tot and H2BAc/H2Btot were not significantly different in the F2 area of AD compared to controls (figure 4C). By contrast, in the hippocampus, total histone levels were similar between AD patients and controls (figure 4D and 4E), and the acetylation status of H3 and H2B showed opposite regulation. In all, the H3Ac/H3tot ratio was significantly decreased in AD patients (60% lower compared to the controls, Welch's t test $p < 0.05$, figure 4F) whereas

the H2B_{Ac}/H2B_{tot} ratio tend to increase in pathological samples (71% higher compared to the controls, $p < 0.05$ but power analyses $< 80\%$, figure 4F).

Taking into consideration that histones are produced and degraded in the cytoplasm, we investigated if their levels in the nucleus were comparable to that found in whole cells. For this, we prepared nuclear protein extracts from frontal cortex and hippocampal region of AD and control cases. To validate the nuclear extraction, we analyzed the presence of the post-synaptic protein neurogranin by Western blot in the total and nuclear protein-extracts. We confirmed the absence of this cytoplasmic protein in the nuclear protein-extracts, neurogranin being only found in the total protein-extracts (of the control patients) (figure 1H for frontal cortex, data not shown for hippocampus).

Interestingly, in the frontal cortex, nuclear-extracted total histone levels presented a different regulation as compared to whole tissue extracts: total H3 histone levels tend to decrease in AD patients compared to controls (H3_{tot}, 12% lower compared to controls, $p < 0.05$ but power analyses $< 80\%$, figure 5A and 5B) and H2B histone levels showed also a decrease tendency (H2B_{tot}, 16% lower compared to controls, $p = 0.06$, figure 5A and 5B). As the net nuclear levels of H3 acetylation were unchanged, then the H3_{ac}/H3_{total} ratio was significantly increased (14% higher, Mann-Whitney test $p < 0.01$, figure 5C). The H2B_{Ac}/H2B_{tot} ratio in the nuclear extracts remained unchanged in both regions as also observed in whole tissue extracts.

In the hippocampal region, nuclear-extracted total H3 or H2B histone levels remained unchanged. However, we noted that H3 acetylation levels were very heterogeneous between nuclear extracts from the different AD patients and the H3_{Ac}/H3_{total} ratio tend to increase but this was not significant (100% upper compared to controls, $p = 0.07$) (Figure 5D). Globally, acetylation of histones seemed much less impacted when nuclear extracts were analyzed compared to whole tissue samples (Figure 6).

Discussion

Epigenetic studies in the human brain of AD patients are poorly documented and with conflicting results, but the clear identification of specific epigenetic dysregulations could help defining new therapeutic targets. Notably, identifying which alterations and how different brain regions are affected are important aspects to design new therapeutic strategies. In this study, we assessed a series of acetylation modifying enzymes and histone levels in two brain regions, the F2 area of the frontal cortex and the hippocampus within the same cohort of patients, and provide evidence of differential dysregulations in each brain region. A major result is that the frontal cortex displayed the main epigenetic impairments (epigenetic enzyme levels) compared to the hippocampus in end-stage AD patients. Additionally, the frontal cortex also displayed dysregulations of total histone levels that were not observed in the

hippocampus (figure 6). Lastly, we found that the net levels of acetylated-histones H3 measured in nuclear extracts were increased mainly in frontal cortex.

Epigenetic impairments are more severe in the frontal cortex than in the hippocampus of AD patients.

AD brains exhibit significant atrophy, predominantly in the temporal and parietal lobes, with deposition of senile plaques and neurofibrillary degeneration initially in the entorhinal cortex and the hippocampus, that further affect associative regions of the neocortex with the disease progression (Braak and Braak, 1995). Thus, in end-stage brains, one could expect stronger dysregulations in regions that are primarily affected. Herein, when assessing hippocampal and frontal area from the same patients, we showed that most of the epigenetic enzymes, i.e. CBP, PCAF, HDAC1 and HDAC2 levels were significantly decreased in the frontal area of AD patients compared to controls, when only HDAC1 levels were significantly affected and CBP also showed a trend to decrease in their hippocampal area. Regarding histone levels, we found an increase in their total amounts only in the frontal cortex. These results suggest that neurons from the frontal cortex area are more vulnerable to epigenetic changes and/or that (remaining) hippocampal neurons are able to better compensate epigenetic dysregulations than neurons from the frontal cortex area. These dramatic changes observed in the frontal cortex are likely associated to the severe loss in neuronal plasticity and synaptic functions in this area. Such epigenetic damage in the frontal cortex may impair functions requiring hippocampo-cortical interactions such as systemic consolidation for long term memory formation, an early symptoms of AD (Braak and Braak, 1995).

CBP loss in brain structures involved in memory processes in AD patients

An interesting finding is that CBP levels were strongly decreased in the F2 area of the frontal cortex and showed a strong downward trend in the hippocampus of AD patients. This confirms previous observations that CBP (and its homolog p300) expression present a significant reduction in the prefrontal cortex of AD patients (Bartolotti et al., 2016), and extend this result to the hippocampus. Taking into consideration the role of CBP in cognitive processes (Alarcon et al., 2004; Korzus et al., 2004; Wood et al., 2005; Chen et al., 2010; Valor et al., 2011; Barrett et al., 2011; Bousiges et al., 2010), this dramatic CBP decrease may play a role in the clinical AD symptoms. In this line, our group has shown recently that CBP levels were decreased in the dorsal hippocampus of 12-month-old mice presenting a tauopathy (THY-Tau22 mice) displaying memory and plasticity dysfunctions (Chatterjee et al., 2018). Similarly, the amyloid pathway appears to be involved in the CBP decline, as an early CBP downregulation in the hippocampus of APP^{swe}/PS1 Δ E9 mice was associated with their memory loss (Bartolotti et al., 2016; Ettcheto et al., 2017). CBP is known to play a role in neuronal survival, both during development and in the adult brain (Del Blanco et al., 2019; Lipinski et al., 2019). In neurons CBP protein loss (Rouaux et al., 2003) or inactivation of its enzymatic activity (Saha and Pahan, 2006) has been associated with neurodegenerative conditions. Interestingly, the

bromodomain of CBP promotes cell survival by blocking A β 42 mediated neurodegeneration in the retinal neurons of a transgenic fruit fly model with a phenotype resembling Alzheimer's like neuropathology (Cutler et al., 2015). Thus, it is likely that CBP loss observed in the AD human brain might potentiate A β and/or Tau-mediated neurodegeneration. In addition, it has been shown that A β induces post-translational degradation of CBP (Song et al., 2015), an event which could underlie CBP loss in AD brains. Thus, we suggest that CBP loss in AD patients is functionally important and might be a potential target for therapeutic interventions. This hypothesis is supported by the study of Caccamo and colleagues, who showed that CBP gene transfer in the brain of an animal model of AD, restored CREB function and improved learning and memory (Caccamo et al., 2010). Furthermore, we recently showed that deficient memory formation and plasticity processes could be fully restored in tauopathic mice (THY-Tau22 mice) after a treatment with a CBP/p300 HAT activator molecule (Chatterjee et al., 2018), emphasizing the importance of CBP in such processes and demonstrating the feasibility of a pharmacological approach targeting this enzyme at symptomatic stages in mice. Lastly, as CBP is a circadian clock regulator via the formation of complex with CLOCK (Gustafson and Partch, 2015), severe CBP loss could also account for the disturbances in the circadian rhythms as observed in AD patients (Leng et al., 2019). Notably, A β -mediated CBP degradation in AD mice was shown in the context of circadian rhythm disruption (Song et al., 2015).

We also observed a 25% decrease of PCAF levels in frontal cortex, however the levels were less impacted than those of CBP, and PCAF levels were not changed in the hippocampus. CBP is specifically targeted by caspases and calpains at the onset of neuronal apoptosis, and CBP was identified as a caspase-6 substrate whereas PCAF was not (Rouaux et al., 2003). Caspase 6 activation is an early event in AD (LeBlanc, 2013) which may preferentially trigger CBP degradation (over PCAF) in the AD patients' samples.

Class I HDACs alterations in AD patients

In AD compared to control brains, we found that HDAC1 was strongly decreased in both structures, whereas HDAC2 levels were only found decreased in the frontal cortex and HDAC3 levels remained unchanged. In the frontal cortex of AD patients, our findings are in agreement with those of Anderson (Anderson et al., 2015) who reported a decrease in both HDAC1 and 2. The decrease of HDAC2 was further confirmed in this structure by Mastroeni and colleagues (Mastroeni et al., 2011). Other studies reported increased levels of these HDACs in AD brains (Graff et al., 2012; Mahady et al., 2018). However, the study led by Graff et al. investigated hippocampal area of Braak stage I-II AD patients (Graff et al., 2012). Mahady and colleagues assessed HDAC levels in the frontal cortex of patients with no, mild, moderate or severe AD status and found an increase of HDAC1 and HDAC3 levels in mild and moderate AD, while the level of both HDACs strongly decreased in severe AD patient's brains (Mahady et al., 2018). Apparently conflicting, these studies actually focused on different regions of the brain independently and at different braak stages. Thus, our study led at the end stage of the disease in the frontal cortex and hippocampal region of the same AD patients reconcile most of the

literature by showing that indeed, a differential effect is observed in these regions: some epigenetic alterations can be measured in the frontal area while these regulations seem preserved in the hippocampus at the same stage.

HDAC1 levels were severely impaired in both structures evaluated. This contrasts with the fact that HDAC1 levels are found increased in the prefrontal cortex of schizophrenia compared with control subjects (Sharma et al., 2008 ; Jakovceski et al., 2013) and suggests that HDAC1 decrease is targeted by neurodegenerative conditions. Notably, A β toxicity may account for HDAC1 degradation as it was shown to target HDAC1 SUMOylation which has a neuroprotective role (Tao et al., 2017). HDAC1 plays a prominent role in DNA repair in postmitotic neurons (Dobbin et al., 2013) through interactions with FUS (Wang et al., 2013) and the strong decrease of HDAC1 may be relevant to excessive neuronal DNA damage observed in AD patients (Brasnjevic et al., 2008). Lastly, HDAC1 also has a role in memory extinction processes (Bahari-Javan et al., 2012).

HDAC2, clearly involved in memory formation and synaptic plasticity (Guan et al., 2009; Graff et al., 2012; Nott et al., 2008), is strongly decreased in frontal cortex but seems less impacted in the hippocampal region in our study. As described in Graff et al (2012), HDAC2 is upregulated in the hippocampus at early stages of AD and this was suggested to participate to AD-induced synaptic loss (Yamakawa et al., 2017) The c-Abl tyrosine kinase phosphorylation - a kinase otherwise reported to be increased in the hippocampal CA1 area of AD patients - prevents HDAC2 proteasomal degradation thereby increasing its stabilization (Gonzalez-Zuniga et al., 2014). Thus, HDAC2 levels may be stabilized in the hippocampus at early stage and stay somewhat elevated in the hippocampus of end-stage patients, when compared to HDAC1 levels for example. Such regulation may not occur in the frontal area. Further studies will be needed in order to determine whether these alterations are a result of the pathological progression or if they are associated with cell protective mechanisms.

HDAC3 levels were surprisingly not affected in AD brains. As observed for HDAC2, they may also have been increased at earlier stages of the disease as found in rodent AD models (Zhu et al., 2017; Janczura et al., 2018). HDAC3 is also involved in memory processes with participation of memory formation and extinction (McQuown et al., 2011; Alaghband et al., 2017).

Thus, at late stage of the disease, specific HDAC isoforms -such as HDAC1- seem to be preferentially targeted for degradation, but their degree of alteration is region specific and some compensatory mechanisms may take place. However, it is becoming clearer that HDAC levels are changing upon the pathological course of the disease, some of them being induced at early stages of AD but most studies agree that there is a significant decrease at later stages of the pathology for the majority of HDACs. This is also emphasized by recent *in vivo* PET studies showing an overall loss of class I HDAC activities in different cortices of AD patients (Pascoal et al., 2018) and our result point to HDAC1 as one of the mainly altered HDAC isoform. These observations call for caution in the use of HDAC inhibitors as therapeutics in late stage

(symptomatic) AD patients, especially when used as systemic treatment (Narayan and Dragunow, 2010).

Different histones subcellular distribution in AD patient's brain.

We found that the total level of both H3 and H2B histones was increased in the F2 area of the frontal cortex cells of AD patients. Narayan and colleagues previously showed a global histone (H3tot and H4tot) increase in inferior and middle temporal gyrus of AD patients (Narayan et al., 2015). In our study, after nuclear extraction, the levels of total histones were no longer increased in AD patients compared to controls (figure 5A), suggesting that the increase histone levels were associated with the cytoplasmic fraction (figure 5C) and pointing to a dysregulation in histone trafficking/catabolism in this brain region. Interestingly, Narayan and colleagues also showed an increase of H3 and H4 histones in the cytoplasm of inferior and middle temporal gyri of AD patients. They hypothesized that this increase could be a consequence of compromised degradation pathways during the disease more particularly due to the ubiquitin-proteasome system impairment that potentially leads to the accumulation of non-degraded histones (Narayan et al., 2015). However, we cannot exclude that increased histone levels might be nuclear and that the nuclear envelop may show increased leakage in AD patients than in controls after long *post-mortem* delays. An old study showed that dinucleosomes and polymeric nucleosome structures were increased in isolated nuclei of neuronal fractions obtained from AD *post-mortem* brain tissues compared with a predominantly mononucleosome load in neurologically normal controls (Lewis et al., 1981). They suggested that mononucleosomes are structurally more susceptible to internal degradation and, therefore, are rapidly digested into smaller entities in control brain. In the AD brain, the reduced clearance of these mononucleosome structures may lead to the aberrant accumulation of polymeric nucleosomes as a consequence of reduced/compromised protein degradation pathways. Furthermore, anti-histones antibodies were found increased in serum of AD patients and a significant correlation was found between anti-histone serum titers and degree of dementia in the AD group, suggesting a global increase of histones in AD. Anti-histone antibodies increase can be due to the alteration of cell membrane fluidity and integrity which facilitates the leakage of the histones to the serum (Mecocci et al., 1993). This histone leakage is known to be toxic to the surrounding cells and interestingly, it was found that histones bind with high affinity and specificity to the secreted amyloid protein precursor (sAPP) decreasing the histone cytotoxicity (Potempska et al., 1993; Currie et al., 1997).

However, H3tot may be decreased in the nucleus of frontal cortex as we found when analyzing nuclear extracts. Lithner and colleagues also reported a total histone decrease in Tg2576 mice (Lithner et al., 2013) and suggested it may reflect a disruption of nucleosome turnover, which could affect nucleosome occupancy of chromatin and influence gene transcription (Deal et al., 2010; Wang et al., 2011). Altogether, these modifications of total histones in AD, whether nuclear or cytoplasmic, could have a strong impact on the 3D chromatin structure, gene transcription and pathology progression. Thus, future treatments should focus on a way to

improve the turnover of histones in order to ensure their replacement and thus maintain the physiological transcriptional activity.

In conclusion, we report a strong disruption of some HAT / HDAC enzymes in brain of AD patients, particularly of the CBP HAT and the class I HDAC1. This is likely to modify the brain histone acetylation homeostasis at rest but also the ability of the brain to respond to learning. However, histone acetylation was shown to be highly variable upon the post-mortem delays (Barrachina et al., 2012; Jarmasz et al., 2019). Interestingly, Jarmasz et al (2019) showed that after death, histone acetylation loss occurred primarily in the nuclei of large neurons, while immunoreactivity in glial cell nuclei was relatively unchanged and increased H3 acetylation levels observed in the F2 frontal area of AD patients (Fig. 5C) may be associated with glial cells. This also emphasizes the difficulty of interpreting studies obtained in whole (aged) tissues with some post-mortem delays.

To summarize, we showed a decrease of epigenetic enzymes (HDAC1, CBP) occurring concomitantly in two key structures of AD patients, the hippocampus and the frontal cortex. In these patients, the frontal cortex seemed more vulnerable as it was more severely altered than the hippocampus: PCAF and HDAC2 levels were further significantly decreased, as the total amount of nuclear histones. These changes may thus occur early in the pathology and drive neuronal plasticity and synaptic loss at very early stages of the disease. Moreover, these modifications may vary during the progression of the pathology (Mahady et al., 2018) and as we have shown according to the structures impacted. Our observations suggest that neuropsychological tests assessing complex cognitive functions relying on hippocampo-cortical interactions (such as long-term memory formation) could be routinely run in the aging population for more efficient diagnosis of AD.

Author Disclosures

All other authors declare that they have no conflicts of interest.

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References

- Alagband, Y., Kwapis, J.L., Lopez, A.J., White, A.O., Aimiwu, O.V., Al-Kachak, A., Bodinayake, K.K., Oparaugo, N.C., Dang, R., Astarabadi, M., Matheos, D.P., Wood, M.A., 2017. Distinct roles for the deacetylase domain of HDAC3 in the hippocampus and medial prefrontal cortex in the formation and extinction of memory. *Neurobiol Learn Mem* 145, 94-104.
- Alarcon, J.M., Malleret, G., Touzani, K., Vronskaya, S., Ishii, S., Kandel, E.R., Barco, A., 2004. Chromatin acetylation, memory, and LTP are impaired in CBP[±] mice: a model for the cognitive deficit in Rubinstein-Taybi syndrome and its amelioration. *Neuron* 42, 947-959.
- Alberini, C.M., Kandel, E.R., 2014. The regulation of transcription in memory consolidation. *Cold Spring Harb Perspect Biol* 7, a021741.
- Anderson, K.W., Mast, N., Pikuleva, I.A., Turko, I.V., 2015. Histone H3 Ser57 and Thr58 phosphorylation in the brain of 5XFAD mice. *FEBS Open Bio* 5, 550-556.
- Aubry, S., Shin, W., Crary, J.F., Lefort, R., Qureshi, Y.H., Lefebvre, C., Califano, A., Shelanski, M.L., 2015. Assembly and interrogation of Alzheimer's disease genetic networks reveal novel regulators of progression. *PLoS One* 10, e0120352.
- Bahari-Javan, S., Maddalena, A., Kerimoglu, C., Wittnam, J., Held, T., Bahr, M., Burkhardt, S., Delalle, I., Kugler, S., Fischer, A., Sananbenesi, F., 2012. HDAC1 regulates fear extinction in mice. *J Neurosci* 32, 5062-5073.
- Barrachina, M., Moreno, J., Villar-Menendez, I., Juves, S., Ferrer, I., 2012. Histone tail acetylation in brain occurs in an unpredictable fashion after death. *Cell Tissue Res* 13, 597-606.
- Barrett, R.M., Malvaez, M., Kramar, E., Matheos, D.P., Arrizon, A., Cabrera, S.M., Lynch, G., Greene, R.W., Wood, M.A., 2011. Hippocampal focal knockout of CBP affects specific histone modifications, long-term potentiation, and long-term memory. *Neuropsychopharmacology* 36, 1545-1556.
- Bartolotti, N., Segura, L., Lazarov, O., 2016. Diminished CRE-Induced Plasticity is Linked to Memory Deficits in Familial Alzheimer's Disease Mice. *J Alzheimers Dis* 50, 477-89.
- Brasnjevic, I., Hof, P. R., Steinbusch, H. W. & Schmitz, C., 2008. Accumulation of nuclear DNA damage or neuron loss: molecular basis for a new approach to understanding selective neuronal vulnerability in neurodegenerative diseases. *DNA Repair* 7, 1087–1097.
- Benito, E., Urbanke, H., Ramachandran, B., Barth, J., Halder, R., Awasthi, A., Jain, G., Capece, V., Burkhardt, S., Navarro-Sala, M., Nagarajan, S., Schutz, A.L., Johnsen, S.A., Bonn, S., Luhrmann, R., Dean, C., Fischer, A., 2015. HDAC inhibitor-dependent transcriptome and memory reinstatement in cognitive decline models. *J Clin Invest* 125, 3572-3584.
- Bie, B., Wu, J., Yang, H., Xu, J.J., Brown, D.L., Naguib, M., 2014. Epigenetic suppression of neuroligin 1 underlies amyloid-induced memory deficiency. *Nat Neurosci* 17, 223-231.
- Bousiges, O., Neidl, R., Majchrzak, M., Muller, M.A., Barbelivien, A., Pereira de Vasconcelos, A., Schneider, A., Loeffler, J.P., Cassel, J.C., Boutillier, A.L., 2013. Detection of histone acetylation levels in the dorsal hippocampus reveals early tagging on specific residues of H2B and H4 histones in response to learning. *PLoS One* 8, e57816.

Bousiges, O., Vasconcelos, A.P., Neidl, R., Cosquer, B., Herbeaux, K., Panteleeva, I., Loeffler, J.P., Cassel, J.C., Boutillier, A.L., 2010. Spatial Memory Consolidation is Associated with Induction of Several Lysine-Acetyltransferase (Histone Acetyltransferase) Expression Levels and H2B/H4 Acetylation-Dependent Transcriptional Events in the Rat Hippocampus. *Neuropsychopharmacology* 35, 2521-2537.

Braak, H., Braak, E., 1995. Staging of Alzheimer's Disease-Related Neurofibrillary Changes. *Neurobiol Aging* 16, 271-8

Cacabelos, R., Carril, J.C., Cacabelos, N., Kazantsev, A.G., Vostrov, A.V., Corzo, L., Cacabelos, P., Goldgaber, D., 2019. Sirtuins in Alzheimer's Disease: SIRT2-Related GenoPhenotypes and Implications for Pharmacogenetics. *Int J Mol Sci* Mar 12, 20-5.

Caccamo, A., Maldonado, M.A., Bokov, A.F., Majumder, S., Oddo, S., 2010. CBP gene transfer increases BDNF levels and ameliorates learning and memory deficits in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* 107, 22687-22692.

Cadena-del-Castillo, C., Valdes-Quezada, C., Carmona-Aldana, F., Arias, C., Bermudez-Rattoni, F., Recillas-Targa, F., 2014. Age-dependent increment of hydroxymethylation in the brain cortex in the triple-transgenic mouse model of Alzheimer's disease. *J Alzheimers Dis* 41, 845-854.

Campbell, R.R., Wood, M.A., 2019. How the epigenome integrates information and reshapes the synapse. *Nat Rev Neurosci* 20, 133-147.

Chatterjee, S., Cassel, R., Schneider-Anthony, A., Merienne, K., Cosquer, B., Tzeplaeff, L., Halder Sinha, S., Kumar, M., Chaturbedy, P., Eswaramoorthy, M., Le Gras, S., Keime, C., Bousiges, O., Dutar, P., Petsophonsakul, P., Rampon, C., Cassel, J.C., Buee, L., Blum, D., Kundu, T.K., Boutillier, A.L., 2018. Reinstating plasticity and memory in a tauopathy mouse model with an acetyltransferase activator. *EMBO Mol Med* 10.

Chen, G., Zou, X., Watanabe, H., van Deursen, J.M., Shen, J., 2010. CREB binding protein is required for both short-term and long-term memory formation. *J Neurosci* 30, 13066-13077.

Currie, J.R., Chen-Hwang, M.C., Denman, R., Smedman, M., Potempska, A., Ramakrishna, N., Rubenstein, R., Wisniewski, H.M., Miller, D.L., 1997. Reduction of histone cytotoxicity by the Alzheimer beta-amyloid peptide precursor. *Biochim Biophys Acta* 1355, 248-258.

Cutler, T., Sarkar, A., Moran, M., Steffensmeier, A., Puli, O.R., Mancini, G., Tare, M., Gogia, N., Singh, A., 2015. Drosophila Eye Model to Study Neuroprotective Role of CREB Binding Protein (CBP) in Alzheimer's Disease. *PLoS One* 10, e0137691.

De Jager, P.L., Yang, H.S., Bennett, D.A., 2018. Deconstructing and targeting the genomic architecture of human neurodegeneration. *Nat Neurosci* 21, 1310-1317.

Deal, R.B., Henikoff, J.G., Henikoff, S., 2010. Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones. *Science* 328, 1161-1164.

Del Blanco, B., Guiretti, D., Tomasoni, R., Lopez-Cascales, M.T., Munoz-Viana, R., Lipinski, M., Scandaglia, M., Coca, Y., Olivares, R., Valor, L.M., Herrera, E., Barco, A., 2019. CBP and SRF co-regulate dendritic growth and synaptic maturation. *Cell Death Differ*.

Dignam, J.D., Lebovitz, R.M., Roeder, R.G., 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res* 11, 1475-1489.

Dobbin, M.M., Madabhushi, R., Pan, L., Chen, Y., Kim, D., Gao, J., Ahanonu, B., Pao, P.C., Qiu, Y., Zhao, Y., Tsai, L.H., 2013. SIRT1 collaborates with ATM and HDAC1 to maintain genomic stability in neurons. *Nat Neurosci* 16, 1008-1015.

Ettcheto, M., Abad, S., Petrov, D., Pedrós, I., Busquets, O., Sánchez-López, E., Casadesús, G., Beas-Zarate, C., Carro, E., Auladell, C., Olloquequi, J., Pallàs, M., Folch, J., Camins, A., 2017. Early Preclinical Changes in Hippocampal CREB-Binding Protein Expression in a Mouse Model of Familial Alzheimer's Disease. *Mol Neurobiol* 55, 4885-4895

Francis, Y.I., Fa, M., Ashraf, H., Zhang, H., Staniszewski, A., Latchman, D.S., Arancio, O., 2009. Dysregulation of histone acetylation in the APP/PS1 mouse model of Alzheimer's disease. *J Alzheimers Dis* 18, 131-139.

Gjoneska, E., Pfenning, A.R., Mathys, H., Quon, G., Kundaje, A., Tsai, L.H., Kellis, M., 2015. Conserved epigenomic signals in mice and humans reveal immune basis of Alzheimer's disease. *Nature* 518, 365-369.

Gonzalez-Zuniga, M., Contreras, P.S., Estrada, L.D., Chamorro, D., Villagra, A., Zanlungo, S., Seto, E., Alvarez, A.R., 2014. c-Abl stabilizes HDAC2 levels by tyrosine phosphorylation repressing neuronal gene expression in Alzheimer's disease. *Mol Cell* 56, 163-173.

Graff, J., Mansuy, I.M., 2008. Epigenetic codes in cognition and behaviour. *Behav Brain Res* 192, 70-87.

Graff, J., Mansuy, I.M., 2009. Epigenetic dysregulation in cognitive disorders. *Eur J Neurosci* 30, 1-8.

Graff, J., Rei, D., Guan, J.S., Wang, W.Y., Seo, J., Hennig, K.M., Nieland, T.J., Fass, D.M., Kao, P.F., Kahn, M., Su, S.C., Samiei, A., Joseph, N., Haggarty, S.J., Delalle, I., Tsai, L.H., 2012. An epigenetic blockade of cognitive functions in the neurodegenerating brain. *Nature* 483, 222-226.

Graff, J., Tsai, L.H., 2013. Histone acetylation: molecular mnemonics on the chromatin. *Nat Rev Neurosci* 14, 97-111.

Guan, J.S., Haggarty, S.J., Giacometti, E., Dannenberg, J.H., Joseph, N., Gao, J., Nieland, T.J., Zhou, Y., Wang, X., Mazitschek, R., Bradner, J.E., DePinho, R.A., Jaenisch, R., Tsai, L.H., 2009. HDAC2 negatively regulates memory formation and synaptic plasticity. *Nature* 459, 55-60.

Gustafson, C.L., Partch, C.L., 2015. Emerging models for the molecular basis of mammalian circadian timing. *Biochemistry* 54, 134-149.

Jakovcevski M, Bharadwaj R, Straubhaar J, Gao G, Gavin DP, Jakovcevski I, Mitchell AC, Akbarian S. Prefrontal cortical dysfunction after overexpression of histone deacetylase 1. *Biol Psychiatry*. 2013 Nov 1;74(9):696-705. doi:10.1016/j.biopsych.2013.03.020.

Janczura, K.J., Volmar, C.H., Sartor, G.C., Rao, S.J., Ricciardi, N.R., Lambert, G., Brothers, S.P., Wahlestedt, C., 2018. Inhibition of HDAC3 reverses Alzheimer's disease-related pathologies in vitro and in the 3xTg-AD mouse model. *Proc Natl Acad Sci U S A* 115, 11148-11157.

Jarmasz, J.S., Stirton, H., Davie, J.R., Del Bigio, M.R., 2019. DNA methylation and histone post-translational modification stability in post-mortem brain tissue. *Clin Epigenetics* 11, 5.

Klein, H.U., McCabe, C., Gjonaska, E., Sullivan, S.E., Kaskow, B.J., Tang, A., Smith, R.V., Xu, J., Pfening, A.R., Bernstein, B.E., Meissner, A., Schneider, J.A., Mostafavi, S., Tsai, L.H., Young-Pearse, T.L., Bennett, D.A., De Jager, P.L., 2019. Epigenome-wide study uncovers large-scale changes in histone acetylation driven by tau pathology in aging and Alzheimer's human brains. *Nat Neurosci* 22, 37-46.

Korzus, E., Rosenfeld, M.G., Mayford, M., 2004. CBP histone acetyltransferase activity is a critical component of memory consolidation. *Neuron* 42, 961-972.

Kouzarides, T., 2007. Chromatin modifications and their function. *Cell* 128, 693-705.

Kozlov, S., Afonin, A., Evsyukov, I., Bondarenko, A., 2017. Alzheimer's disease: as it was in the beginning. *Rev Neurosci* 28, 825-843.

Kwapis, J.L., Alaghband, Y., Lopez, A.J., White, A.O., Campbell, R.R., Dang, R.T., Rhee, D., Tran, A.V., Carl, A.E., Matheos, D.P., Wood, M.A., 2017. Context and Auditory Fear are Differentially Regulated by HDAC3 Activity in the Lateral and Basal Subnuclei of the Amygdala. *Neuropsychopharmacology* 42, 1284-1294.

LeBlanc, A.C., 2013. Caspase-6 as a novel early target in the treatment of Alzheimer's disease. *Eur J Neurosci* 37, 2005-2018.

Leng, Y., Musiek, E.S., Hu, K., Cappuccio, F.P., Yaffe, K., 2019. Association between circadian rhythms and neurodegenerative diseases. *Lancet Neurol* 18, 307-318.

Lewis, P.N., Lukiw, W.J., De Boni, U., McLachlan, D.R., 1981. Changes in chromatin structure associated with Alzheimer's disease. *J Neurochem* 37, 1193-1202.

Lipinski, M., Del Blanco, B., Barco, A., 2019. CBP/p300 in brain development and plasticity: disentangling the KAT's cradle. *Curr Opin Neurobiol* 59, 1-8.

Lithner, C.U., Lacor, P.N., Zhao, W.Q., Mustafiz, T., Klein, W.L., Sweatt, J.D., Hernandez, C.M., 2013. Disruption of neocortical histone H3 homeostasis by soluble A β : implications for Alzheimer's disease. *Neurobiol Aging* 34, 2081-2090.

Mahady, L., Nadeem, M., Malek-Ahmadi, M., Chen, K., Perez, S.E., Mufson, E.J., 2018. Frontal Cortex Epigenetic Dysregulation During the Progression of Alzheimer's Disease. *J Alzheimers Dis* 62, 115-131.

Marques, S.C., Lemos, R., Ferreira, E., Martins, M., de Mendonca, A., Santana, I., Outeiro, T.F., Pereira, C.M., 2012. Epigenetic regulation of BACE1 in Alzheimer's disease patients and in transgenic mice. *Neuroscience* 220, 256-266.

Mastroeni, D., Grover, A., Delvaux, E., Whiteside, C., Coleman, P.D., Rogers, J., 2011. Epigenetic mechanisms in Alzheimer's disease. *Neurobiol Aging* 32, 1161-1180.

Maurice, T., Duclot, F., Meunier, J., Naert, G., Givalois, L., Meffre, J., Celerier, A., Jacquet, C., Copois, V., Mechti, N., Ozato, K., Gongora, C., 2008. Altered memory capacities and response to stress in p300/CBP-associated factor (PCAF) histone acetylase knockout mice. *Neuropsychopharmacology* 33, 1584-1602.

McQuown, S.C., Barrett, R.M., Matheos, D.P., Post, R.J., Rogge, G.A., Alenghat, T., Mullican, S.E., Jones, S., Rusche, J.R., Lazar, M.A., Wood, M.A., 2011. HDAC3 is a critical negative regulator of long-term memory formation. *J Neurosci* 31, 764-774.

Mecocci, P., Ekman, R., Parnetti, L., Senin, U., 1993. Antihistone and anti-dsDNA autoantibodies in Alzheimer's disease and vascular dementia. *Biol Psychiatry* 34, 380-385.

Narayan, P., Dragunow, M., 2010. Pharmacology of epigenetics in brain disorders. *Br J Pharmacol* 159, 285-303.

Narayan, P.J., Lill, C., Faull, R., Curtis, M.A., Dragunow, M., 2015. Increased acetyl and total histone levels in post-mortem Alzheimer's disease brain. *Neurobiol Dis* 74, 281-294.

Nativio, R., Donahue, G., Berson, A., Lan, Y., Amlie-Wolf, A., Tuzer, F., Toledo, J.B., Gosai, S.J., Gregory, B.D., Torres, C., Trojanowski, J.Q., Wang, L.S., Johnson, F.B., Bonini, N.M., Berger, S.L., 2018. Dysregulation of the epigenetic landscape of normal aging in Alzheimer's disease. *Nat Neurosci* 21, 497-505.

Nott, A., Watson, P.M., Robinson, J.D., Crepaldi, L., Riccio, A., 2008. S-Nitrosylation of histone deacetylase 2 induces chromatin remodelling in neurons. *Nature* 455, 411-415.

Oliveira, A.M., Wood, M.A., McDonough, C.B., Abel, T., 2007. Transgenic mice expressing an inhibitory truncated form of p300 exhibit long-term memory deficits. *Learn Mem* 14, 564-572.

Pascoal, T.A., Chamoun, M., Shin, M., Benedet, A.L., Mathotaarachchi, S., Kang, M.S., Theriault, J., Savard, M., Thomas, E., Massarweh, G., Soucy, J.P., Gauthier, S., Rosa-Neto, P., 2018. Imaging epigenetics in the human brain with the novel [¹¹C]MARTINOSTAT PET in preclinical AD, MCI, AD, and frontotemporal dementia individuals. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association* 14, 9-10

Potempska, A., Ramakrishna, N., Wisniewski, H.M., Miller, D.L., 1993. Interaction between the beta-amyloid peptide precursor and histones. *Arch Biochem Biophys* 304, 448-453.

Rao, J.S., Keleshian, V.L., Klein, S., Rapoport, S.I., 2012. Epigenetic modifications in frontal cortex from Alzheimer's disease and bipolar disorder patients. *Transl Psychiatry* 2, e132.

Rouaux, C., Jokic, N., Mbebi, C., Boutillier, S., Loeffler, J.P., Boutillier, A.L., 2003. Critical loss of CBP/p300 histone acetylase activity by caspase-6 during neurodegeneration. *EMBO J* 22, 6537-6549.

Saha, R.N., Pahan, K., 2006. HATs and HDACs in neurodegeneration: a tale of disconcerted acetylation homeostasis. *Cell Death Differ* 13, 539-550.

Schneider, A., Chatterjee, S., Bousiges, O., Selvi, B.R., Swaminathan, A., Cassel, R., Blanc, F., Kundu, T.K., Boutillier, A.L., 2013. Acetyltransferases (HATs) as targets for neurological therapeutics. *Neurotherapeutics* 10, 568-588.

Sharma RP, Grayson DR, Gavin DP. Histone deacetylase 1 expression is increased in the prefrontal cortex of schizophrenia subjects: analysis of the National Brain Databank microarray collection. *Schizophr Res.* 2008 Jan;98(1-3):111-7.

Shu, G., Kramar, E.A., Lopez, A.J., Huynh, G., Wood, M.A., Kwapis, J.L., 2018. Deleting HDAC3 rescues long-term memory impairments induced by disruption of the neuron-specific chromatin remodeling subunit BAF53b. *Learn Mem* 25, 109-114.

Song, H., Moon, M., Choe, H.K., Han, D.H., Jang, C., Kim, A., Cho, S., Kim, K., Mook-Jung, I., 2015. Abeta-induced degradation of BMAL1 and CBP leads to circadian rhythm disruption in Alzheimer's disease. *Mol Neurodegener* 10, 13.

Stilling, R.M., Fischer, A., 2011. The role of histone acetylation in age-associated memory impairment and Alzheimer's disease. *Neurobiol Learn Mem* 96, 19-26.

Tao, C.C., Hsu, W.L., Ma, Y.L., Cheng, S.J., Lee, E.H., 2017. Epigenetic regulation of HDAC1 SUMOylation as an endogenous neuroprotection against A β toxicity in a mouse model of Alzheimer's disease. *Cell Death Differ* 24, 597-614.

Valor, L.M., Pulopulos, M.M., Jimenez-Minchan, M., Olivares, R., Lutz, B., Barco, A., 2011. Ablation of CBP in forebrain principal neurons causes modest memory and transcriptional defects and a dramatic reduction of histone acetylation but does not affect cell viability. *J Neurosci* 31, 1652-1663.

Wang, X., Bryant, G.O., Floer, M., Spagna, D., Ptashne, M., 2011. An effect of DNA sequence on nucleosome occupancy and removal. *Nat Struct Mol Biol* 18, 507-509.

Wang, W.Y., Pan, L., Su, S.C., Quinn, E.J., Sasaki, M., Jimenez, J.C., Mackenzie, I.R., Huang, E.J., Tsai, L.H., 2013. Interaction of FUS and HDAC1 regulates DNA damage response and repair in neurons. *Nat Neurosci* 16, 1383-91.

Wood, M.A., Kaplan, M.P., Park, A., Blanchard, E.J., Oliveira, A.M., Lombardi, T.L., Abel, T., 2005. Transgenic mice expressing a truncated form of CREB-binding protein (CBP) exhibit deficits in hippocampal synaptic plasticity and memory storage. *Learn Mem* 12, 111-119.

Wu, J., Bie, B., Yang, H., Xu, J.J., Brown, D.L., Naguib, M., 2013. Suppression of central chemokine fractalkine receptor signaling alleviates amyloid-induced memory deficiency. *Neurobiol Aging* 34, 2843-2852.

Yamakawa, H., Cheng, J., Penney, J., Gao, F., Rueda, R., Wang, J., Yamakawa, S., Kritskiy, O., Gjonneska, E., Tsai, L.H., 2017. The Transcription Factor Sp3 Cooperates with HDAC2 to Regulate Synaptic Function and Plasticity in Neurons. *Cell Rep.* 20, 1319-1334

Zhang, K., Schrag, M., Crofton, A., Trivedi, R., Vinters, H., Kirsch, W., 2012. Targeted proteomics for quantification of histone acetylation in Alzheimer's disease. *Proteomics* 12, 1261-1268.

Zhu, X., Wang, S., Yu, L., Jin, J., Ye, X., Liu, Y., Xu, Y., 2017. HDAC3 negatively regulates spatial memory in a mouse model of Alzheimer's disease. *Aging Cell* 16, 1073-1082.

Figure 1: Characteristic hallmarks of Alzheimer's disease (AD) in frontal cortex of controls (n=5) and AD patients (n=8). Samples were tested with three different types of Tau phosphorylation antibodies, two by western blot: S396, Thr212/Ser214 (A, B) and one in immunohistochemistry: Ser404 (D). The presence of A β peptides was also evaluated (C). For characteristic hallmarks of AD all the patients are represented. The results show that only the AD patients displayed the pathological markers. Other markers of neurodegeneration (NeuN) (E) or gliosis (GFAP) (F) were also studied in the F2 area of the frontal cortex. Blots are shown in quintuplicate. Quantified results are represented as fold induction of the protein of interest, the result obtained in the controls being arbitrarily set at 100%. Student's t-test: ***p<0.001. (G) To control our nuclear protein-extraction we analyzed the neurogranin (which is a postsynaptic protein) by western blot. Protein amount loaded in the gels and exposition time (1 min exposition) of the membranes to the chemidoc touch were similar between total extraction and nuclear extraction (frontal cortex). Neurogranin is not detectable in nuclear extraction and in total protein extraction from AD patients. AD: Alzheimer's disease patients, Ct: control patients

Figure 2: Decrease of CBP levels in AD patients. (A) HAT (CBP and PCAF) protein expression assessed by western blot and normalized on beta Actin in frontal cortex of controls (n = 5) and AD patients (n=8). (B)HAT (CBP and PCAF) protein expression assessed by western blot and normalized on total proteins in hippocampus of controls (n = 5) and AD patients (n = 6). Blots are shown in quintuplicate. Quantified results are represented as fold induction of the enzyme, the result obtained in the controls being arbitrarily set at 100%. Student's t-test: *p<0.05; # the power analysis is less than 80%, which means more samples would be required to conclude. AD: Alzheimer's disease patients, Ct: control patients

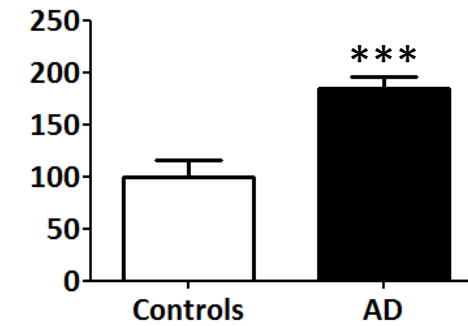
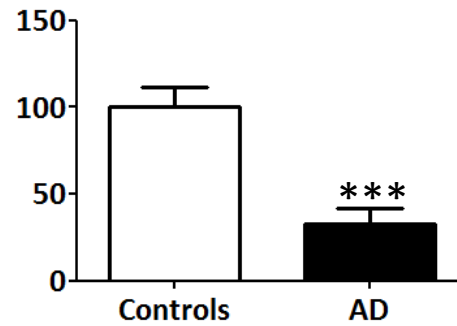
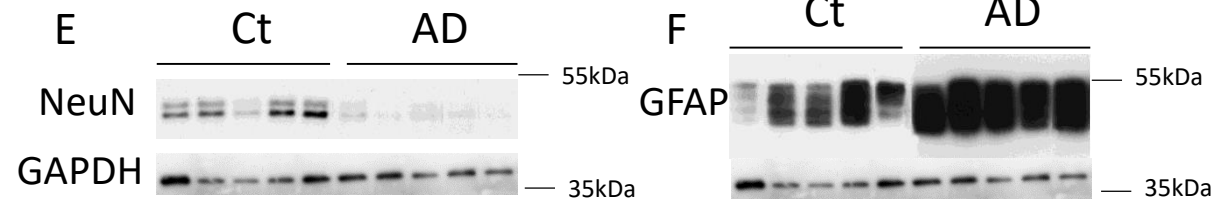
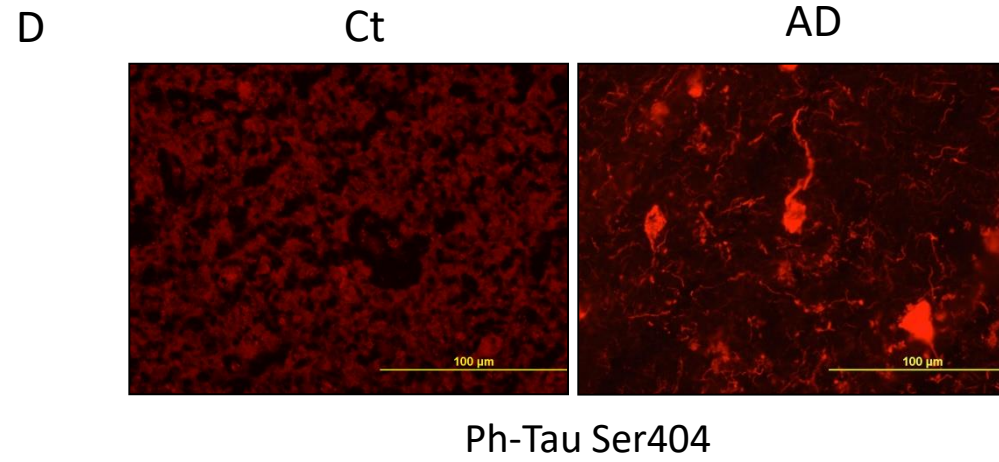
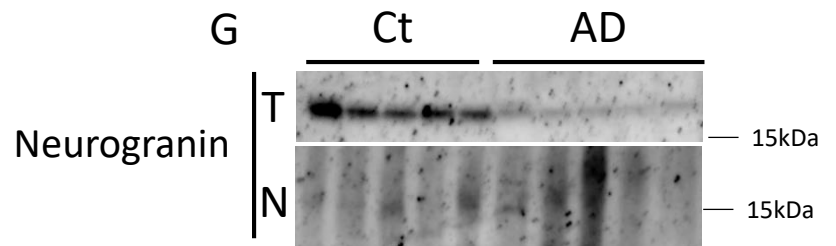
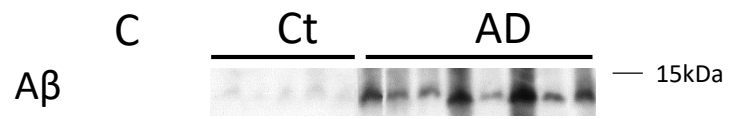
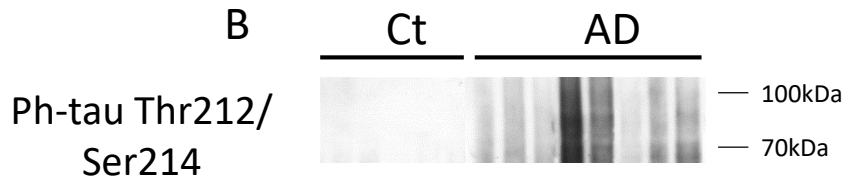
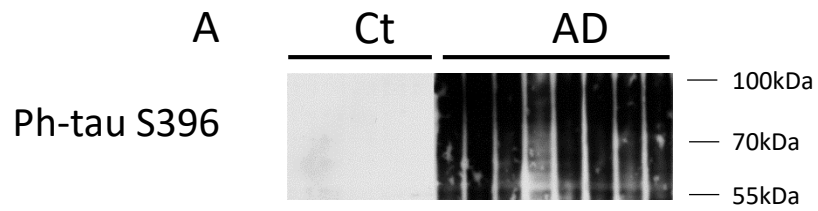
Figure 3: Decrease of HDAC1 and HDAC2 in frontal cortex and HDAC1 decrease in hippocampus of AD patients. (A) HDAC (HDAC1, HDAC2 and HDAC3) protein expression assessed by western blot and normalized on beta Actin in frontal cortex of controls (n = 5) and AD patients (n=8). (B)HDAC (HDAC1, HDAC2 and HDAC3) protein expression assessed by western blot and normalized on total proteins in hippocampus of controls (n = 5) and AD patients (n = 6). Blots are shown in quintuplicate. Quantified results are represented as fold induction of the enzyme, the result obtained in the controls being arbitrarily set at 100%. Student's t-test: **p<0.01, *p<0.05; # the power analysis is less than 80%, which means more samples would be required to conclude. AD: Alzheimer's disease patients, Ct: control patients

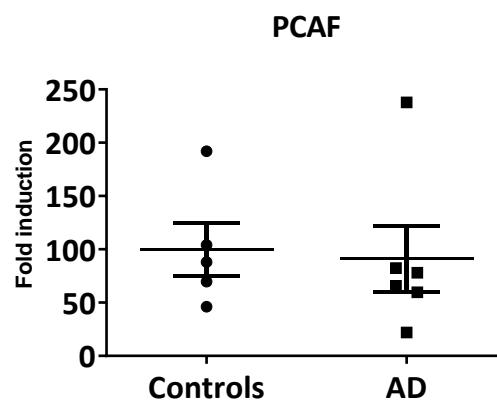
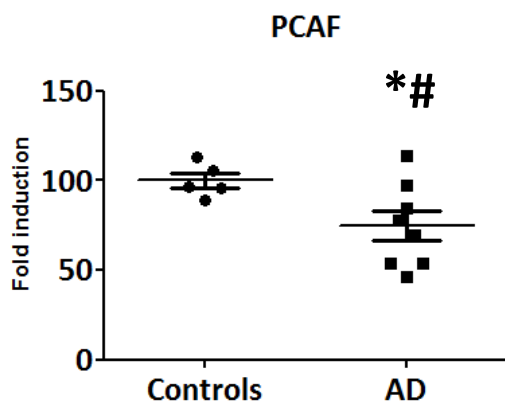
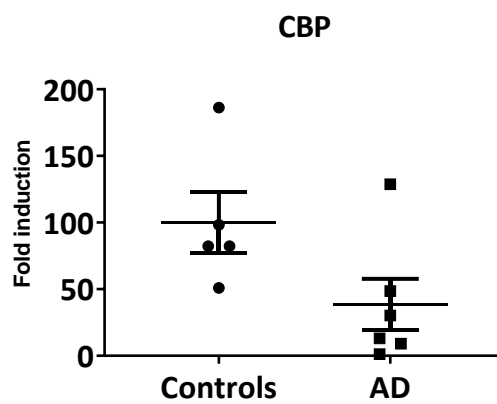
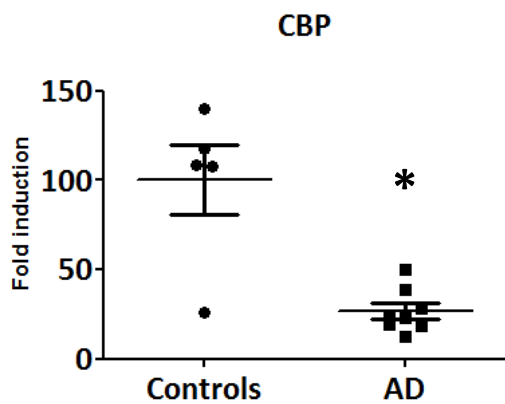
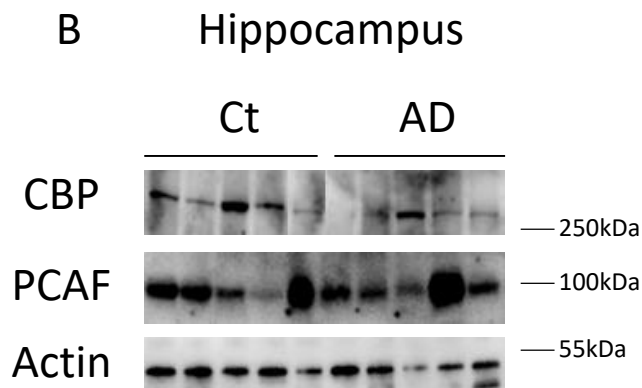
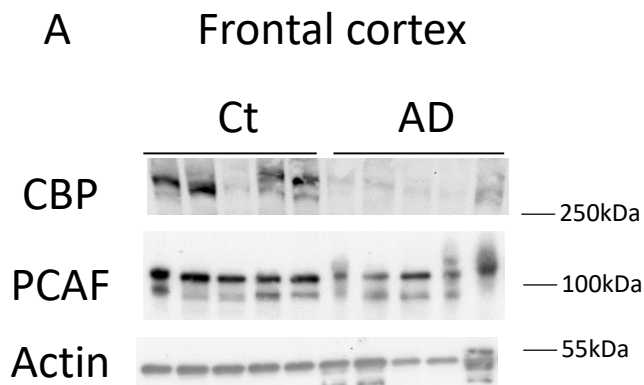
Figure 4: Increase of total (H3 and H2B) histone levels in whole tissue extracts from AD patients F2 cortical area. Acetylated (Ac) and Total (Tot) histone levels were measured by western blot analyses and normalized on beta Actin for histone H3 and H2B in (A)(B) frontal cortex of controls (n = 5) and AD (n = 8) patients and normalized on total proteins in hippocampus (D) (E) of controls (n = 5) and AD patients (n = 6). Lysine acetylation measured here are H3K9K14 and H2BK5K12K15K20. Western blots are represented in quintuplicates. Quantified results are represented as fold induction of the total or acetylated histone or (C) (F) the Ac/Tot ratio for each histone, the result obtained in the controls being arbitrarily set at 100%. Student's t-test: ***p<0.001, **p<0.01; *p<0.05; # the power analysis is less than 80%, which means more samples would be required to conclude. AD: Alzheimer's disease patients, Ct: control patients

Figure 5: Total and acetylated histone levels in nuclear protein extracts from AD patients brain. Acetylated (Ac) and Total (Tot) histone levels were measured by western blot analyses and normalized on beta Actin for histone H3 and H2B in frontal cortex (A) of controls (n = 5) and AD (n = 8) patients

and normalized on total proteins in hippocampus (D) of controls (n = 5) and AD patients (n = 6). Lysine acetylation measured here are H3K9K14 and H2BK5K12K15K20. Western blots are represented in quintuplicates. Quantified results (B, C for frontal cortex and E, F for hippocampus) are represented as fold induction of the total histone or the Ac/Tot ratio for each histone, the result obtained in the controls being arbitrarily set at 100%. Student's t-test: **p<0.01; *p<0.05; # the power analysis is less than 80%, which means more samples would be required to conclude. AD: Alzheimer's disease patients, Ct: control patients

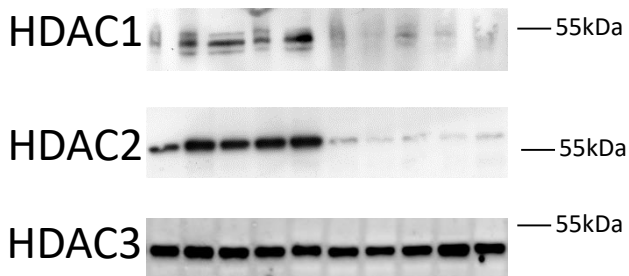
Figure 6: Schematization of the data. The figure shows the results obtained during total tissue extraction and nuclear extraction of hippocampal and frontal cortex structures for enzymes regulating histone acetylation (CBP, PCAF, HDAC1, HDAC2, HDAC3) as well as for total and acetylated histone modifications. The increases and decreases are represented by green arrows pointing upwards and red arrows pointing downwards respectively. The level of variation is represented by the thickness of the arrow, a thick arrow represents a large variation while a thin arrow represents a small variation. * p = 0.06 for H2Btot in the nuclear frontal cortex and p=0.075 for CBP hipp, not significant but suggests a trend.





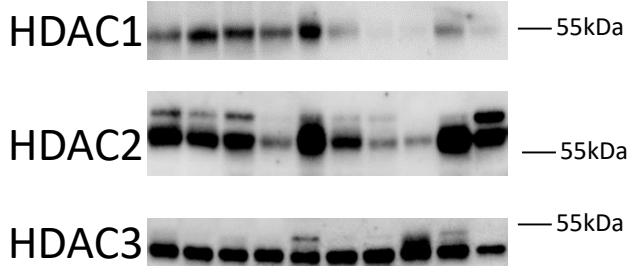
A Frontal cortex

Ct AD

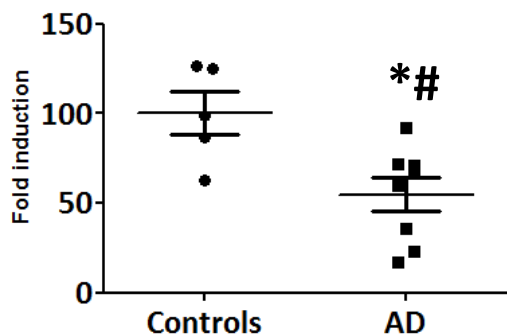


B Hippocampus

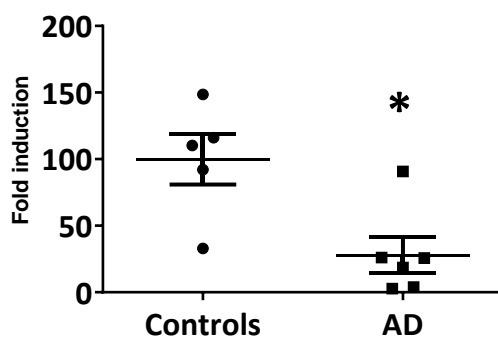
Ct AD



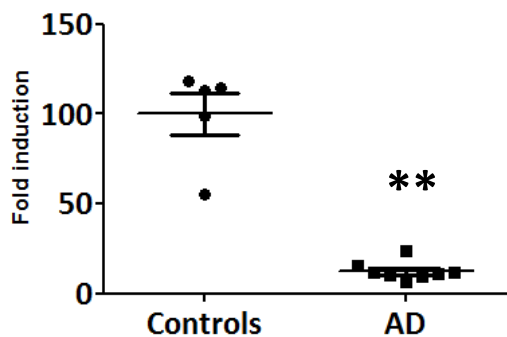
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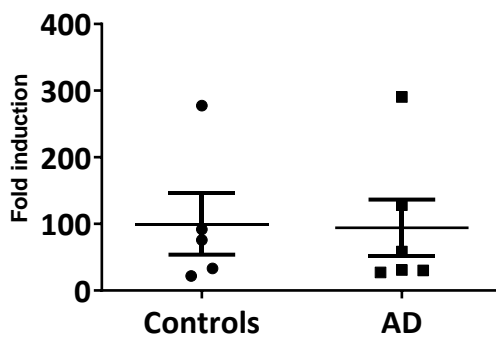
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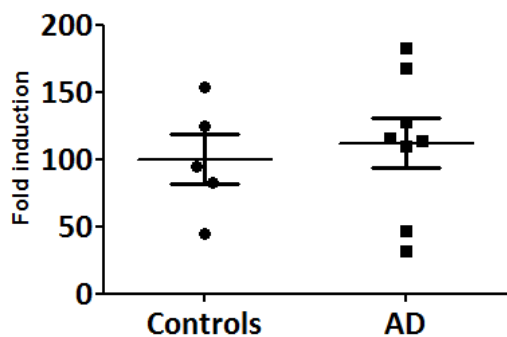
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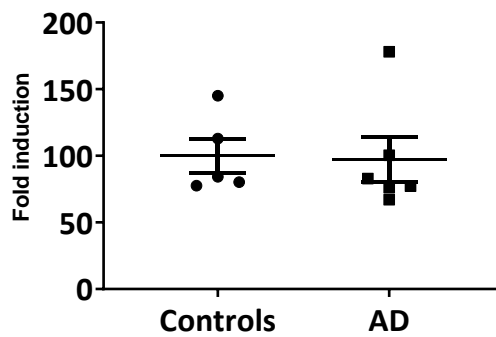
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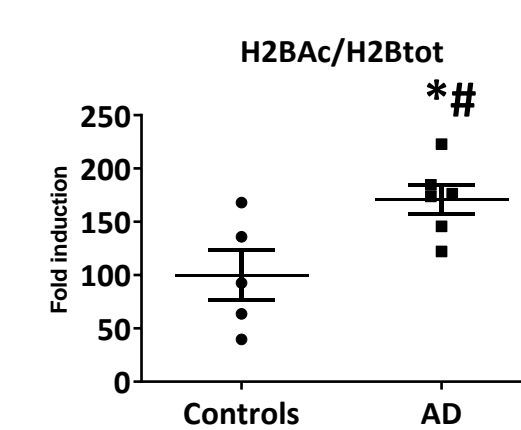
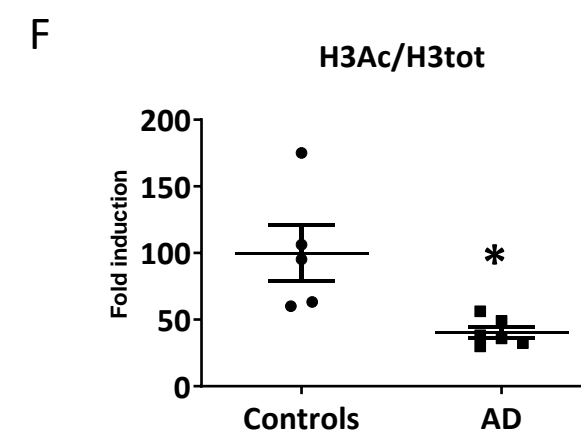
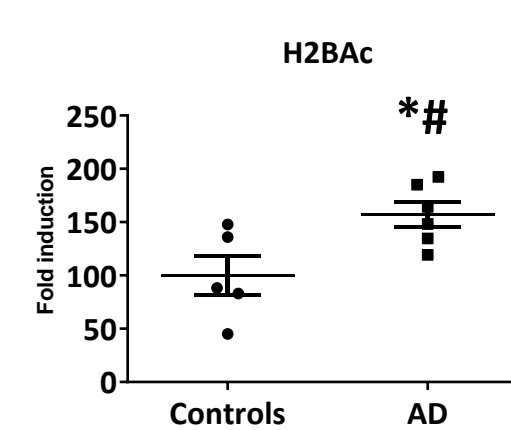
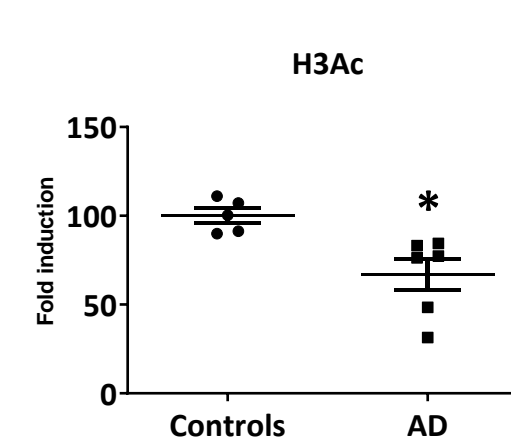
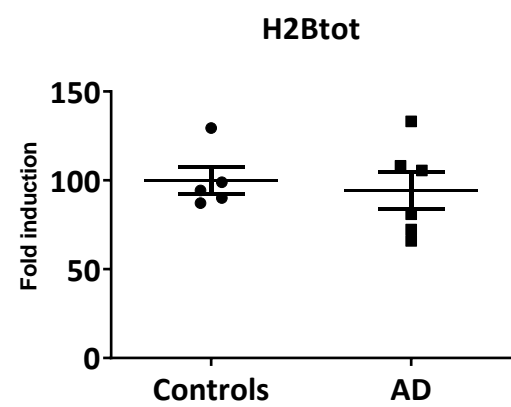
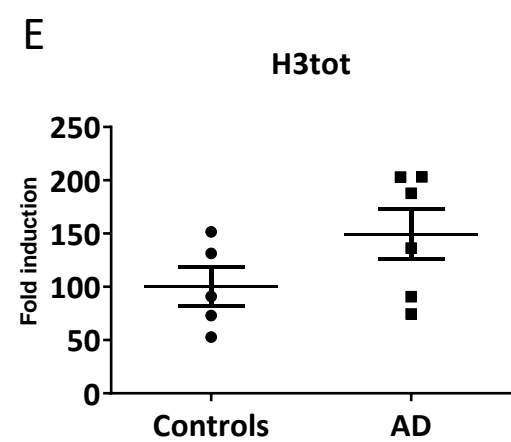
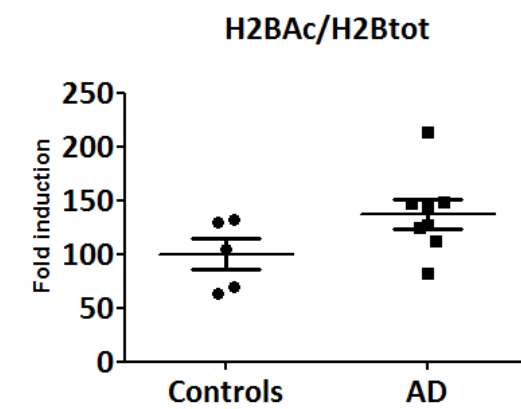
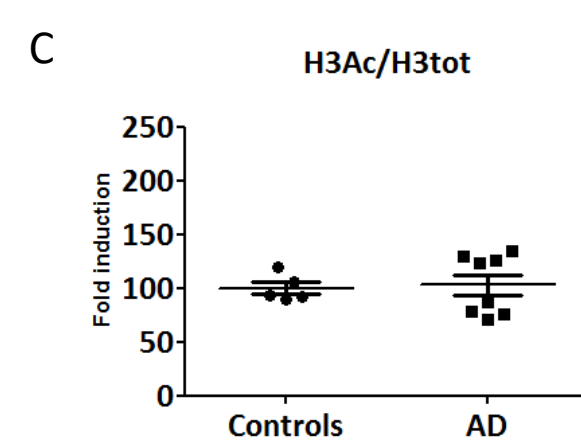
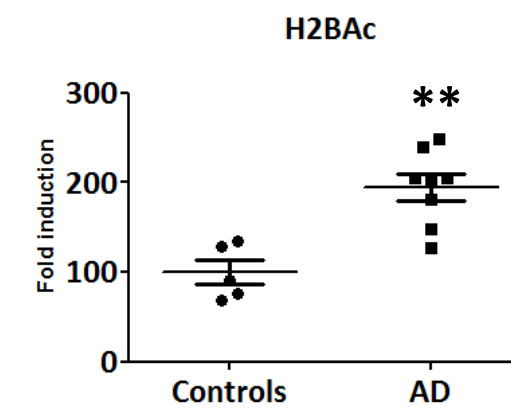
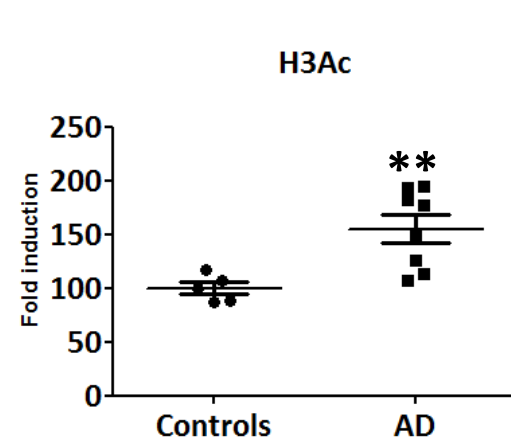
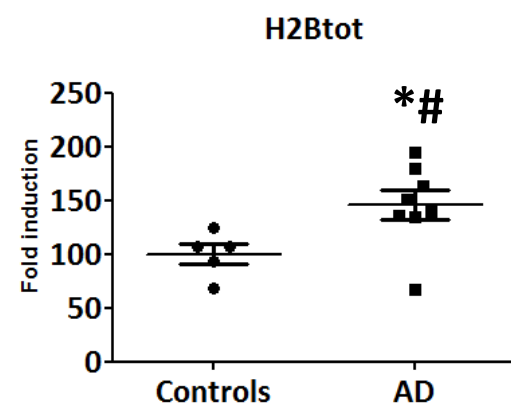
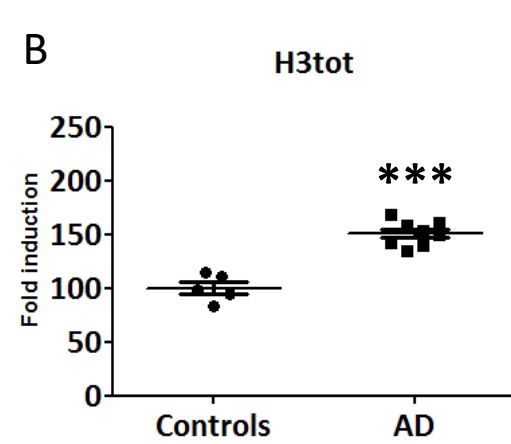
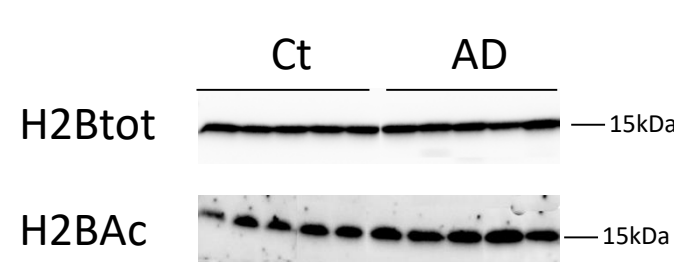
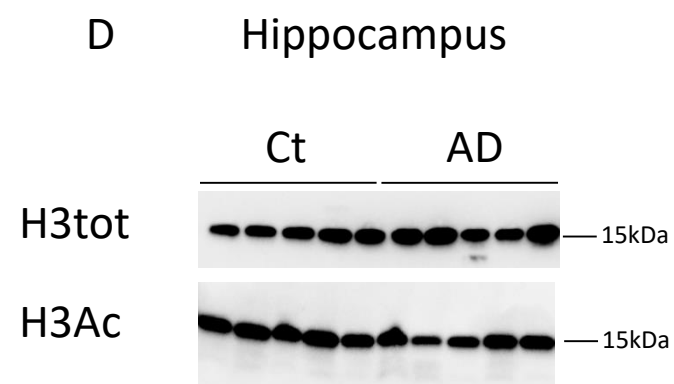
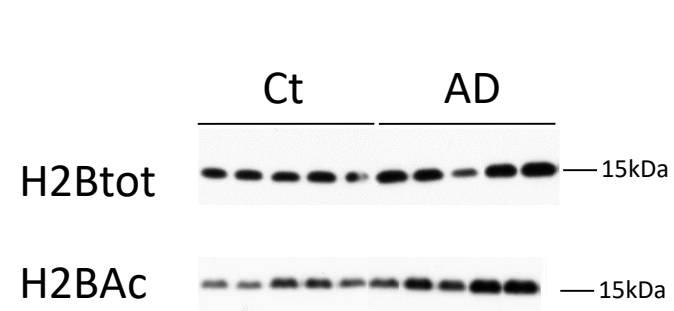
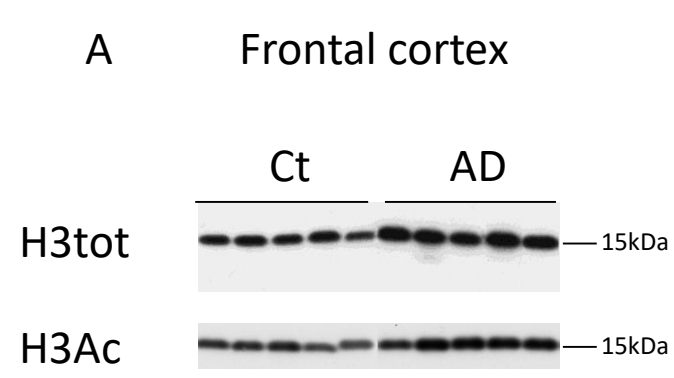


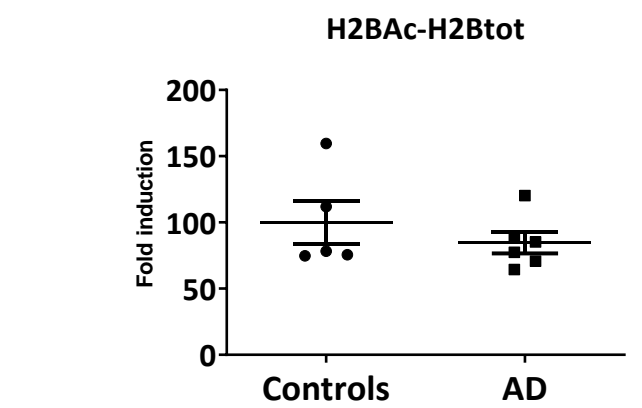
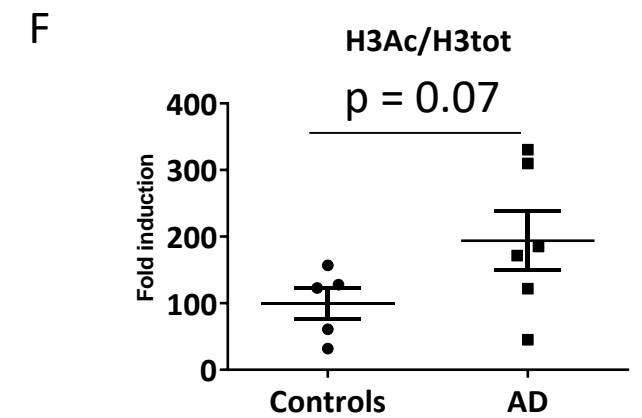
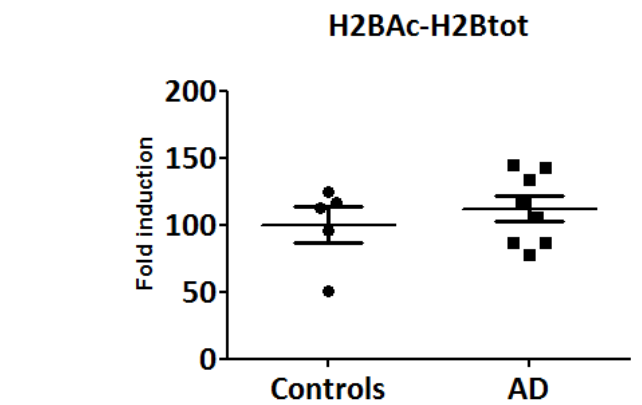
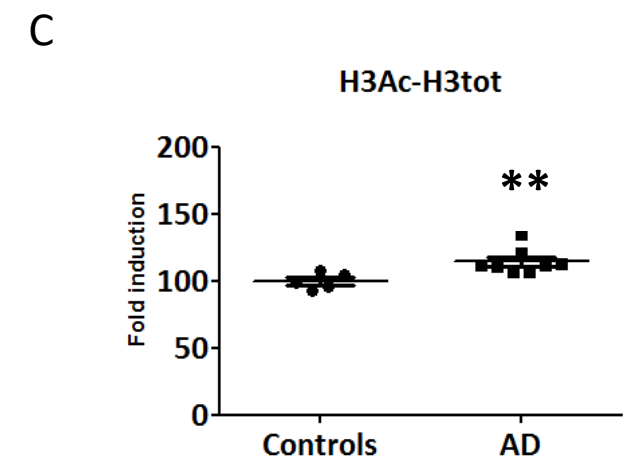
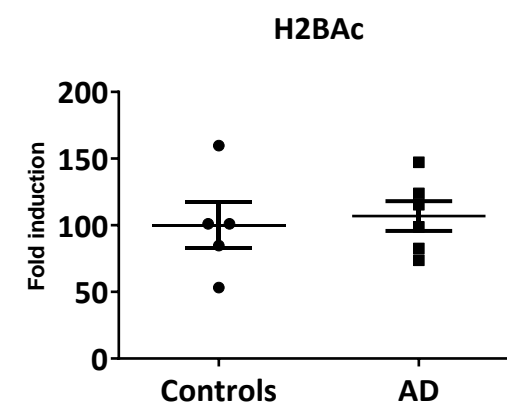
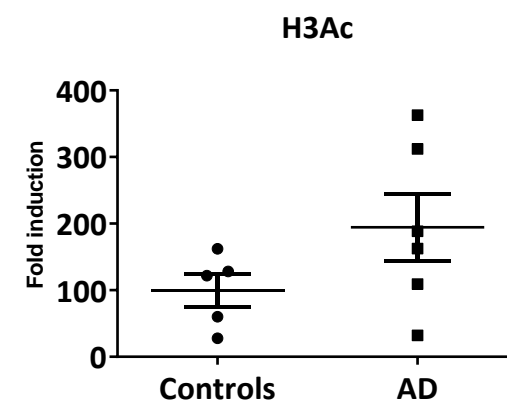
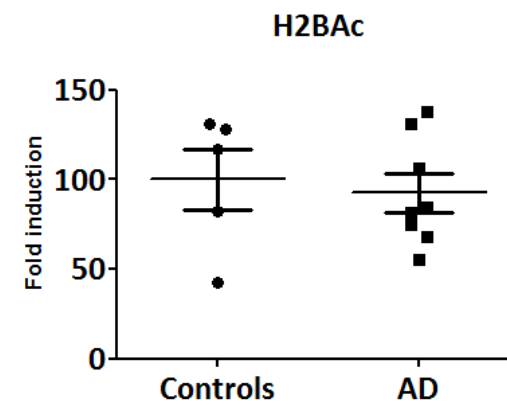
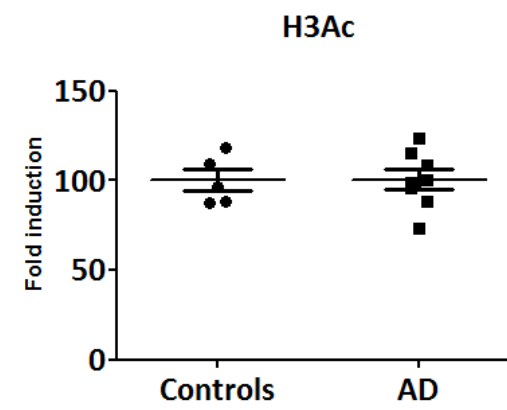
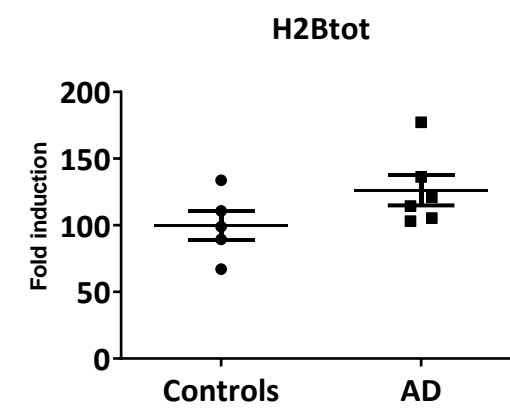
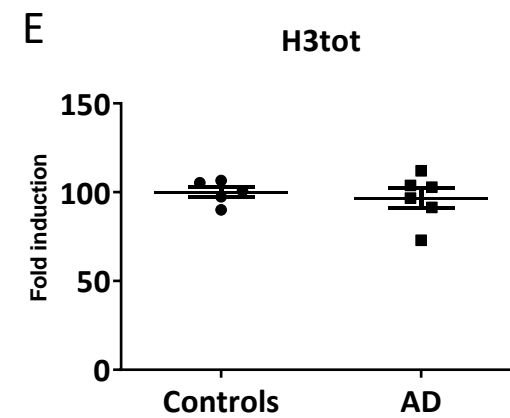
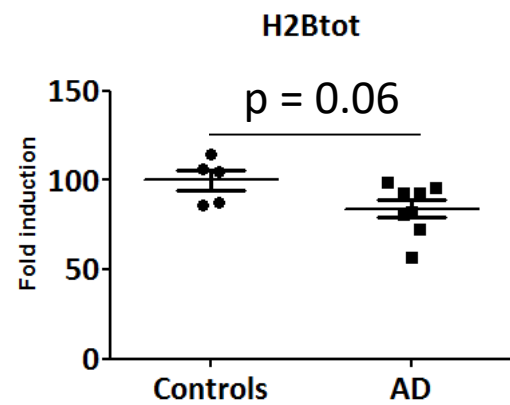
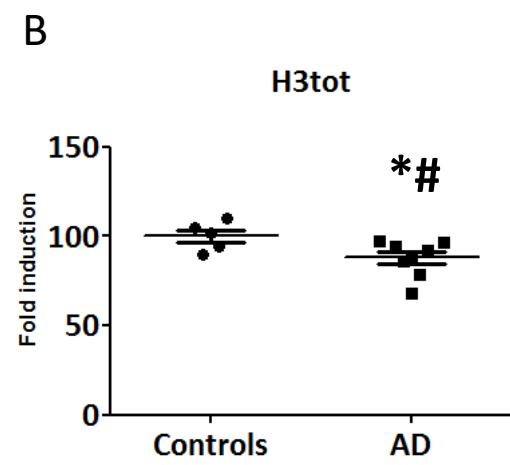
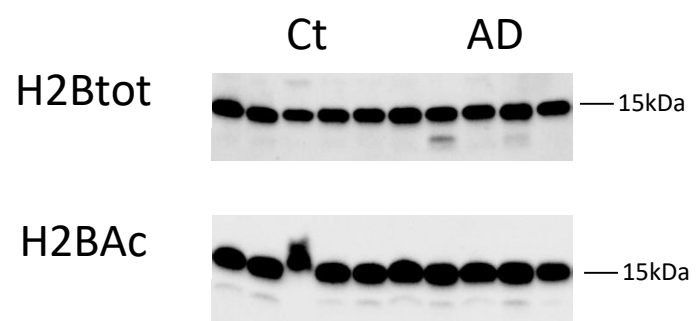
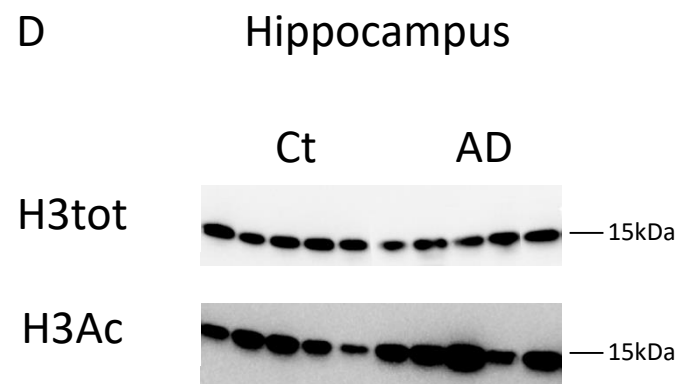
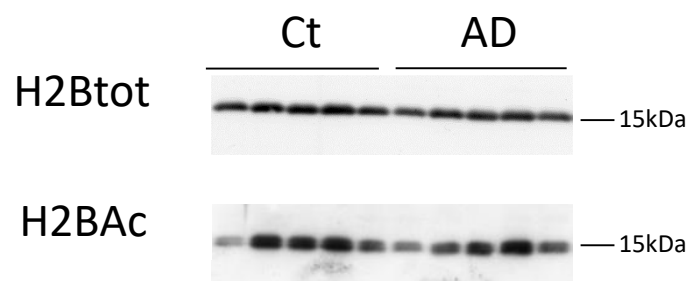
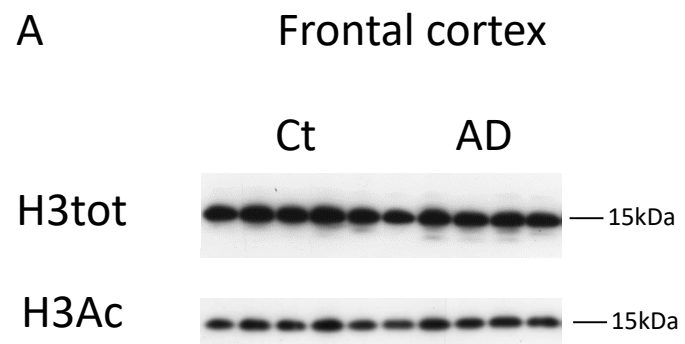
HDAC3



HDAC3



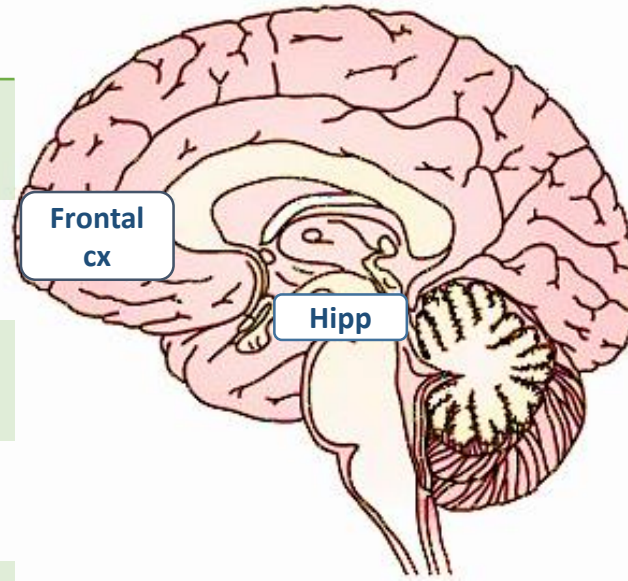




Frontal Cortex

Whole tissue extracts

CBP	↓
PCAF	↓
HDAC1	↓
HDAC2	↓
HDAC3	-



	Whole tissue extracts	Nuclear extracts
--	-----------------------	------------------

H3 total	↑	↓
H3 ac/tot	-	↑
H2B total	↑	↓*
H2B ac/tot	-	-

↑ ± 25%
 ↑ ± 50%
 ↑ ± 75%

Hippocampus

Whole tissue extracts

CBP	↓*
PCAF	-
HDAC1	↓
HDAC2	-
HDAC3	-

	Whole tissue extracts	Nuclear extracts
--	-----------------------	------------------

H3 total	-	-
H3 ac/tot	↓	-
H2B total	-	-
H2B ac/tot	↑	-

Table 1: Patients characteristics

ANONIMITY N°	SEX	AGE	FROZEN HEMISPHERE	Brain weight (g)	Post Mortem Delay	Profession	NEUROPATHOLOGICAL DIAGNOSIS	ASSOCIATED PATHOLOGY	Frozen samples at -80°C		Histological sections
Alzheimers disease (Braak stage V-VI)									Hippocampus	Frontal cortex	Frontal cortex
7853	F	86	R	1180	30h	nd	Alz Braak stage: VI; Thal stage: V	-	OK	OK	NO
8629	M	81	R	1230	17h30	nd	Alz Braak stage: VI; Thal stage: V	-	OK	OK	OK
7654	M	78	R	1250	8h30	draughtsman	Alz Braak stage: VI; Thal stage: II	-	OK	OK	OK
6563	M	82	R	1238	25h	nd	Alz Braak stage: VI; Thal stage: V	-	OK	OK	NO
7689	M	72	R	1458	44h	engineer	Alz Braak stage: VI; Thal stage: V	-	OK	OK	NO
6742	M	76	L	1215	10h	nd	Alz Braak stage: VI; Thal stage: V	electrode stimulation carrier	NO	OK	OK
7106	M	83	L	1070	21h	nd	Alz Braak stage: VI; Thal stage: IV		NO	OK	NO
7857	F	85	L	1195	21h	librarian	Alz Braak stage: VI; Thal stage: V	Dementia with Lewy body	OK	OK	NO
CONTROLS (Braak stage 0-I)											
4078	M	73	R	508 (right hemisphere)	10h	nd	Control	-	OK	OK	NO
8866	M	84	R	1355	15h30	nd	Control	-	OK	OK	NO
5991	M	66	R	1500	7h	rail agent	Control	vasculopathy	OK	OK	NO
3356	F	86	R	1226	nd	nd	Control	Cerebrovascular accident	OK	OK	NO
8251	M	79	R	1273	nd	nd	Control	mild atrophy and discrete spongiosis	OK	OK	NO
6203	M	78	L	1280	23h	nd	Control	dramatic amyloid angiopathy	NO	NO	OK
3549	M	69	L	1254	6h	engineer	Control	Epileptic	NO	NO	OK