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Sodium Butyrate Improves Memory Function in an Alzheimer's Disease Mouse Model When Administered at an Advanced Stage of Disease Progression

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Abstract. Dysregulation of histone acetylation has been implicated in the onset of age-associated memory impairment and the pathogenesis of neurodegenerative diseases. Elevation of histone acetylation via administration of histone deacetylase (HDAC) inhibitors is currently being pursued as a novel therapeutic avenue to treat memory impairment linked to Alzheimer's disease (AD). Here we show that severe amyloid pathology correlates with a pronounced dysregulation of histone acetylation in the forebrain of APPPS1-21 mice. Importantly, prolonged treatment with the pan-HDAC inhibitor sodium butyrate improved associative memory in APPPS1-21 mice even when administered at a very advanced stage of pathology. The recovery of memory function correlated with elevated hippocampal histone acetylation and increased expression of genes implicated in associative learning. These data advance our understanding of the potential applicability of HDAC inhibitors for the treatment of AD and suggest that HDAC inhibitors may have beneficial effects even when administered long after the onset of disease-associated symptoms.

Keywords: Alzheimer's disease, gene expression, histone acetylation, histone deacetylases, memory impairment

INTRODUCTION

Alzheimer's disease (AD) is a debilitating age-related disorder that develops on the pathological background of amyloid-β (Aβ) plaques and neurofibrillary tangles [1, 2]. Disease progression eventually causes severe dementia and cognitive impairment resulting in a drastic decline in the quality of life. Apart from family history, one of the greatest risk factors for AD is age [3]. The continued extension of human lifespan in modern society has led to a progressive increase in the incidence of AD making it the most common neurodegenerative disorder today. This poses a mounting challenge to develop effective therapies to combat the pathogenesis of dementia and AD in the coming decades. The precise molecular mechanisms underlying the majority of sporadic forms of AD still remain elusive. The current therapeutic arsenal has achieved limited success in ameliorating the cognitive impairment observed in AD patients. Thus, the incidence and pathology of AD present a formidable

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socio-economic burden to our population making it necessary to develop novel therapeutic strategies.

Recent studies have suggested that the dysregulation of epigenetic gene expression might play an important role during the onset of age-associated memory impairment and AD pathogenesis [4–9]. Epigenetic mechanisms, such as DNA methylation and covalent histone modifications, are key processes that regulate genome-environment interaction [10]. The acetylation of histones at distinct lysine residues is regulated by the antagonistic activities of histone acetlytransferases (HATs) and histone deacetylases (HDACs). These enzymes collectively maintain a balanced yet dynamic state of histone acetylation [11]. It is known that histone acetylation renders the DNA accessible to the transcription machinery and enhances gene expression. Conversely, HDAC activity represses gene expression by compressing the chromatin structure. Notably, distinct patterns of histone acetylation are associated with specific modes of transcription, a phenomenon commonly referred to as the "histone code" [12]. While dynamic changes in histone acetylation have been linked to the genetic program required for memory formation [4, 9, 13, 14], a number of recent studies suggest that targeting histone acetylation using HDAC inhibitors leads to neuroprotective and neuro-regenerative effects in animal models for neurodegenerative diseases [10, 15–17]. To this end, treatment with pan-HDAC inhibitors such as sodium butyrate (SB), trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA), or sodium phenylbutyrate was found to improve memory function in mouse models for aging, brain injury, p25-mediated neuronal loss, and amyloid pathology [4–9, 18]. These findings suggest HDAC inhibitors as potential therapeutic agents to attenuate cognitive impairment observed in AD.

The currently used methods for diagnosis of AD often reveal an advanced stage of pathology in the patients. To further evaluate the potential of HDAC inhibitors as therapeutic strategies, it is therefore important to test their therapeutic efficacy in advanced stages of pathology in AD mouse models. Moreover, it is also necessary to test HDAC inhibitors in as many animal models of AD as possible before proceeding with clinical trials.

In this study, we employed the APPPS1-21 double-transgenic mice that co-express KM670/671NL mutated amyloid-β protein precursor (AβPP) and L166 P mutated presenilin 1 (PS1) under the neuron-specific Thy1 promoter element [19]. These mice exhibit very aggressive AD pathology in the form of Aβ plaques as early as 2 months of age. This is accompanied by dystrophic synaptic boutons, inflammatory responses, and robust impairment of cognitive function and synaptic plasticity at 8 months of age [19, 20]. Here we show that 15-month-old APPPS1-21 mice exhibit severe amyloid pathology, memory impairments and decreased hippocampal and cortical histone acetylation and thus comprise a suitable model to test the effect of HDAC inhibitors at an advanced stage of disease progression. We found that a 6-week administration of the HDAC inhibitor sodium butyrate enhanced histone acetylation and significantly facilitated associative memory function in 15-month-old APPPS1-21 mice. This effect correlated with the elevated expression of genes associated with learning and memory processes. These data provide further evidence that targeting histone acetylation using HDAC inhibitors could be a suitable therapeutic strategy to treat memory impairment associated with AD pathogenesis.

MATERIALS AND METHODS

Animals

Double transgenic Thy1-APP (KM670/671NL) and Thy1-PS1 (L166 P) mice [19] were housed under standard conditions with access with food and water ad libitum. All experiments were approved by the Animal Ethics Committee of the state of Lower Saxony, Germany.

Drug administration

Sodium butyrate (SB, Sigma-Aldrich, Germany) was administered as described before [21]. In brief, SB was dissolved in 0.01 M PBS and administered daily at a final concentration of 1.2 g/kg body weight for 6 weeks. Drug administration was continued during the behavioral analysis and mice were sacrificed for molecular analysis 24 h after the last administration.

Behavioral analysis

All behavioral tests were conducted after 6 weeks of SB/vehicle treatment and have been described in detail previously [9, 22]. In brief, exploratory behavior was tested using the Open Field. Mice were exposed to a square open arena (80 cm) with an opaque base and transparent walls (20 cm high). Each mouse was allowed to explore the area for 3 min and its activity recorded using the VIDEOMot2 (version 5.72) video tracking system (TSE, Berlin, Germany). The surface

was cleaned with 70% ethanol after testing each mouse. To measure associative memory, we employed the Fear Conditioning paradigm (TSE, Berlin, Germany). The training consisted of a single exposure to a novel context for 3 min, followed by a tone for 30 s (200 ms) and a single electric foot shock (0.7 mA, constant current, 2 s). Freezing was scored upon the lack of movement in any direction for more than 1 s. The context and tone associated memory tests were performed successively 24 h later. Motor function was assessed using the Rotarod test. Each mouse was subjected to four habituation sessions (10 rpm constant) and four testing sessions (5–40 rpm uniform acceleration for 3 min, 40 rpm constant for 1 min) on the rotating rod. The performance was measured by the length of time each mouse spent on the rod.

Quantitative real-time PCR (qPCR)

Total RNA was isolated using the TRI reagent (Sigma-Aldrich, Steinheim Germany) according to the manufacturer's instructions. RNA was dissolved in 30 µl ddH_2O. qPCR was performed using a Roche 480 Light Cycler (Roche, Mannheim, Germany). cDNA was synthesized from 1 µg of total RNA using the iScript cDNA Synthesis Kit (BIO-RAD, Hercules, USA) according to the manufacturer's instructions. Expression of individual genes was analyzed using the Roche Universal Probe Library (UPL). The housekeeping gene hypoxanthine phosphoribosyltransferase 1 (*Hprt1*) was chosen as an internal reference for normalization of gene expression. Table 1 shows the primers used for qPCR analysis along with the corresponding molecular probes from the UPL.

Immunoblot analysis

Proteins were isolated using the TRI reagent (Sigma-Aldrich, Steinheim Germany) according to the manufacturer's instructions. Proteins were dissolved in 3 M urea in 0.01 M PBS using an ultrasonic homogenizer for 10 s, at 8 cycles and 85% power (Bandelin Electronic, Berlin, Germany). For immunoblot analysis protein lysates were mixed with Laemmli loading buffer, heated at 98°C for 3 min, centrifuged shortly and stored at 4°C. Individual proteins were resolved on a 12% SDS-polyacrylamide gel by electrophoresis (Bio-Rad, Germany) at 120 V. The PageRuler Prestained Protein Ladder (Fermentas, Germany) was taken as molecular weight standard. After resolution, the proteins were transferred to a nitrocellulose membrane at 60 V for 16 h on ice at 4°C. The membrane

Table 1
Primers and UPL molecular probes used for qPCR

Primer	Sequence (5′ – 3′)	Universal Probe
Shank3 L	AGGACGTCCGCAATTACAAC	#97
Shank3 R	AAGCTCAAAGTTCCCTGCAA	#97
Prkca L	ACAGACTTCAACTTCCTCATGGT	#60
Prkca R	CTGTCAGCAAGCATCACCTT	#60
SNAP25 L	GCTCCTCCACTCTTGCTACC	#88
SNAP25 R	CAGCAAGTCAGTGGTGCTTC	#88
Myst4 L	GCAACAAAGGGCAGCAAG	#19
Myst4 R	AGACATCTTTAGGAAACCAAGACC	#19
Acly L	GCCCTGGAAGTGGAGAAGAT	#10
Acly R	CCGTCCACATTCAGGATAAGA	#10
Ncdn L	GCTCCTTAGCACCTCTCCAG	#75
Ncdn R	GCAGCTGCGAAGAAACCT	#75
Fmn2 L	AACAGCAGAAGCCTTTTGTCA	#89
Fmn2 R	TTCTGCCAGTGGGAAGACA	#89
Marcksl1 L	GGCAGCCAGAGCTCTAAGG	#19
Marcksl1 R	TCACGTGGCCATTCTCCT	#19
Gsk3 a L	GAGCCACAGATTACACCTCGT	#76
Gsk3 a R	CTGGCCGAGAAGTAGCTCAG	#76
GluR1 L	GCCCAATGCAGAGCTCAC	#100
GluR1 R	GTCACTCCACTCGAGGTAAC	#100
Hprt1 L	TCCTCCTCAGACCGCTTTT	#95
Hprt1 R	CCTGGTTCATCATCGCTAATC	#95
hAPP L	CCTGGTGATCCATGTCAGAA	#11
hAPP R	AAACACTGCCAAGGTGTCAA	#11

was washed in 0.01 M PBS at room temperature (RT) and incubated in 5% milk prepared in 0.01 M PBS at RT for 1 h to block non-specific sites. Primary antibody dissolved in 0.5% milk in 0.01 M PBS was used to probe the membrane at 4°C overnight on a shaker. The antibodies used were: anti-acetyl-H4 K12 (1:5000, Abcam), anti-acetyl-H4 K5 (1:2000, Millipore), anti-acetyl-H4 K16 (1:2000, Millipore), anti-acetyl-H3 K9 (1:5000, Millipore), anti-acetyl-H3 K14 (1:2500, Millipore), anti-SNAP-25 (Company) and anti-β-Actin (1:1000, Santa Cruz). IRDye 800CW or 680CW conjugated polyclonal anti-mouse or anti-rabbit IgG cross-adsorbed secondary antibodies (1:15000, LI-COR) were dissolved in 0.5% milk in 0.01 M PBS, added on the membrane and incubated for 30 min at RT on a shaker in the dark. Unspecific binding was washed using 0.01 M PBS thrice for 10 min. Detection was performed using the Odyssey IR Scanner.

Immunohistochemistry

Brain tissue, fixed by immersion in 4% PFA, was embedded in paraffin and cut using a microtome to yield 5 µm thick sections that were mounted on precoated glass slides for all further steps. The sections were deparaffinized and hydrated by the following incubation steps: twice 10 min in xylene, 10 min in

100%, 10 min in 95%, 10 min in 75% and 10 min in 50% ethanol, and twice 5 min in ddH_2O at RT. Antigen retrieval was achieved by boiling the sections in 10 mM sodium citrate for 10 min in a microwave oven. The sections were washed in tap water and immunostaining was performed by washing the sections twice in 0.01 M PBS followed by incubation in blocking buffer (5% goat serum + 0.3% TritonX-100 in 0.01 M PBS) for 90 min at RT to block non-specific sites and incubation in primary antibody diluted in blocking buffer at 4°C overnight on a shaker. Thereafter, the sections were washed thrice in antibody wash buffer (1% goat serum + 0.2% TritonX-100 in 0.01 M PBS) for 10 min at RT. The antibody to detect Aβ 17–24 (4 G8) was purchased from Convance (1:1000). The antibodies against histone acetylation sites and dilutions used were similar to the immunoblot analysis. Secondary antibody incubation was carried out at RT for 2 h with Alexa488- or Cy3-conjugated goat polyclonal anti-rabbit or anti-mouse antibodies dissolved in blocking buffer (1:500, Jackson Immunoresearch Laboratories). The sections were washed thrice in 0.01 M PBS for 10 min, incubated in 4′,6-diamidino-2-phenylindole (DAPI, 10 μg/ml) for 20 min and washed twice in 0.01 M PBS at RT. The sections were dehydrated retrogressively in the alcohol and xylene solutions mentioned above. Sections were analyzed using a Leica SP2 confocal microscope.

Data analysis

The data was analyzed by unpaired student's t-test and one-way or two-way ANOVA (ANalyis Of VAriance) when appropriate. Errors are displayed as Standard Error of Mean (S.E.M).

RESULTS

Cognitive and molecular pathology in APPPS1-21 mice

The APPPS1-21 mouse model for AD is characterized by aggressive, early onset of amyloid pathology at 2 months of age accompanied by robustly impaired memory formation and synaptic plasticity at 8 months of age [19, 20]. Therefore, we reasoned that 15-month-

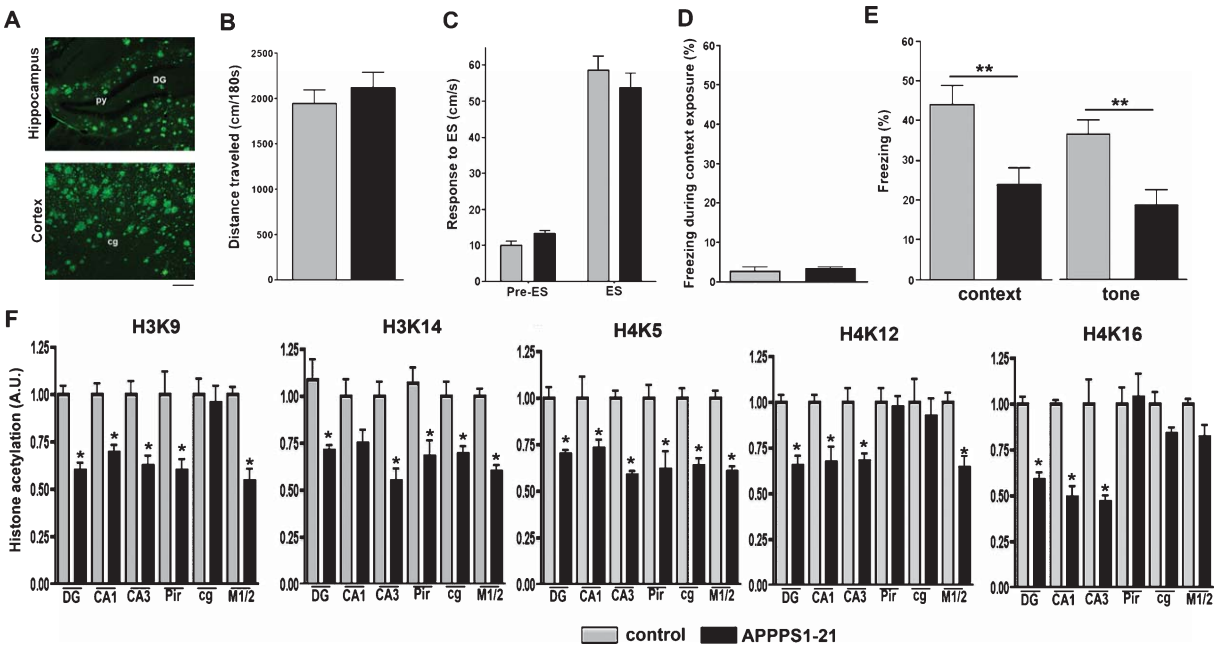

Fig. 1. Amyloid pathology and histone acetylation in APPPS1-21 mice. A) Confocal microscopy images showing Aβ plaques in the hippocampus and cortex of APPPS1-21 mice. B-E) Impaired associative memory function in APPPS1-21 mice compared to WT controls, B) Total distance covered in the novel context, C) Response to electric shock (ES) shown by maximum velocity before (pre-ES) and after the ES, D) Basal freezing behavior shown upon context exposure, E) Freezing levels shown 24 h after training in the contextual and cued memory tests. F) Quantified immunohistochemical analysis showing histone acetylation at specific sites on histones H3 and H4 in APPPS1-21 mice compared to WT controls in different brain regions (DG: dentate gyrus, Pir: piriform cortex, cg: cingulate cortex, M1/2 : motor cortex 1 and 2).

old APPPS1-21 mice might be suitable to investigate an advanced stage of AD pathology. Severe Aβ plaque load was detectable within the hippocampus and cortex of 15-month-old APPPS1-21 mice (Fig. 1A). Next, we subjected a group of 15-month-old APPPS1-21 and age-matched control mice to behavioral testing. No significant difference was observed in the distance traveled when mice were exposed to an open field, indicating that novelty-induced exploratory behavior is normal in 15-month-old APPPS1-21 mice (Fig 1B). We then analyzed associative memory formation, which is impaired in AD patients and mouse models of amyloid pathology [6, 8, 23, 24]. To this end, we employed the Pavlovian fear conditioning paradigm, a well-established test to assess memory function in rodents [21]. Notably, the response to the electric foot-shock (Fig. 1C) and freezing behavior during the training session were similar among the groups (Fig. 1D). However, when memory function was analyzed in a context- and tone-dependent memory test performed 24 h later, the 15-month-old APPPS1-21 mice displayed significantly reduced freezing behavior when compared to the age-matched control group (Fig. 1E). This finding shows that associative memory function is severely impaired in 15-month-old APPPS1-21 mice. Next, we employed immunohistochemical analysis to assess histone modifications in the hippocampus and cortex of 15-month-old APPPS1-21 mice. We tested for a number of specific H3 and H4 acetylations that have been previously linked to memory function in mice [9]. We observed a significant reduction in hippocampal and cortical acetylation at H3K9, H3K14, H4K5, H4K8, H4K12, and H4K16 when compared to wild type controls (Fig. 1F).

These data establish that 15-month-old APPPS1-21 mice exhibit severe deficits in associative memory formation and reduced histone acetylation in hippocampal and cortical brain regions.

Enhanced associative memory in APPPS1-21 mice following SB treatment

Based on the above findings, we decided to test whether administering an HDAC inhibitor would have any effect on memory function in 15-month-old APPPS1 mice that represent a rather advanced stage of disease progression.

A group of 14-month-old APPPS1-21 mice received a daily dose of the HDAC inhibitor sodium butyrate (SB; 1.2 g/kg). SB is a potent HDAC inhibitor that affects neuronal histone acetylation when administered via intraperitoneal injection (IP) [8, 21]. Moreover, SB shows some specificity to class I HDACs and was shown to enhance associative learning in rodents upon IP administration [8, 21]. A corresponding group of APPPS1-21 mice received vehicle treatment and therefore served as control. After 6 weeks of SB treatment, animals were subjected to either molecular or behavioral analysis (Fig. 2A). No difference in hippocampal and cortical Aβ plaque load was observed among groups (Fig. 2B and C). When subjected to the open field test, the exploratory behavior was indistinguishable among the groups (Fig. 2D). The specific assessment of the time spent in the center vs. periphery of the open field indicates basal anxiety levels [22]. No difference was found among the groups suggesting that SB treatment did not affect basal anxiety in APPPS1-21 mice (Fig. 2E). Next, we analyzed associative memory function. SB- and vehicle-treated APPPS1-21 mice were subjected to Pavlovian fear conditioning. The distance traveled during the training period (Fig. 3A) and the response to the electric foot-shock (Fig. 3B) was similar among groups. Associative memory was tested 24 h later. Notably, SB-treated APPPS1-21 mice displayed significantly enhanced freezing behavior during the context- (Fig. 3C; SB: $56.3\% \pm 7.6\%$, Veh: $33.3\% \pm 4.5\%$) and tone-dependent (Fig. 3D; SB: $47.6\% \pm 5.5\%$, Veh: $27.7\% \pm 6.3\%$) memory tests. These results indicate that SB treatment facilitates associative learning in APPPS1-21 mice.

Elevation of histone acetylation in APPPS1-21 mice by SB treatment

To assess the effect of SB treatment on histone acetylation in APPPS1-21 mice, we investigated the changes in hippocampal and cortical histone acetylation at specific lysine residues using immuoblot analysis. We found that hippocampal acetylation at H3K14, H4K5, and H4K12 sites were significantly elevated in APPPS1-21 mice treated with SB (Fig. 4A and B). Interestingly, no differences were observed between the groups for hippocampal H3K9 acetylation (Fig. 4B) or any histone modification analyzed in cortical lysates (Fig. 4C and D).

Regulation of genes involved in memory consolidation by SB treatment

It was previously shown that elevated levels of hippocampal histone acetylation regulate the expression of genes implicated with the consolidation of associative memories [9]. More specifically, using an unbiased

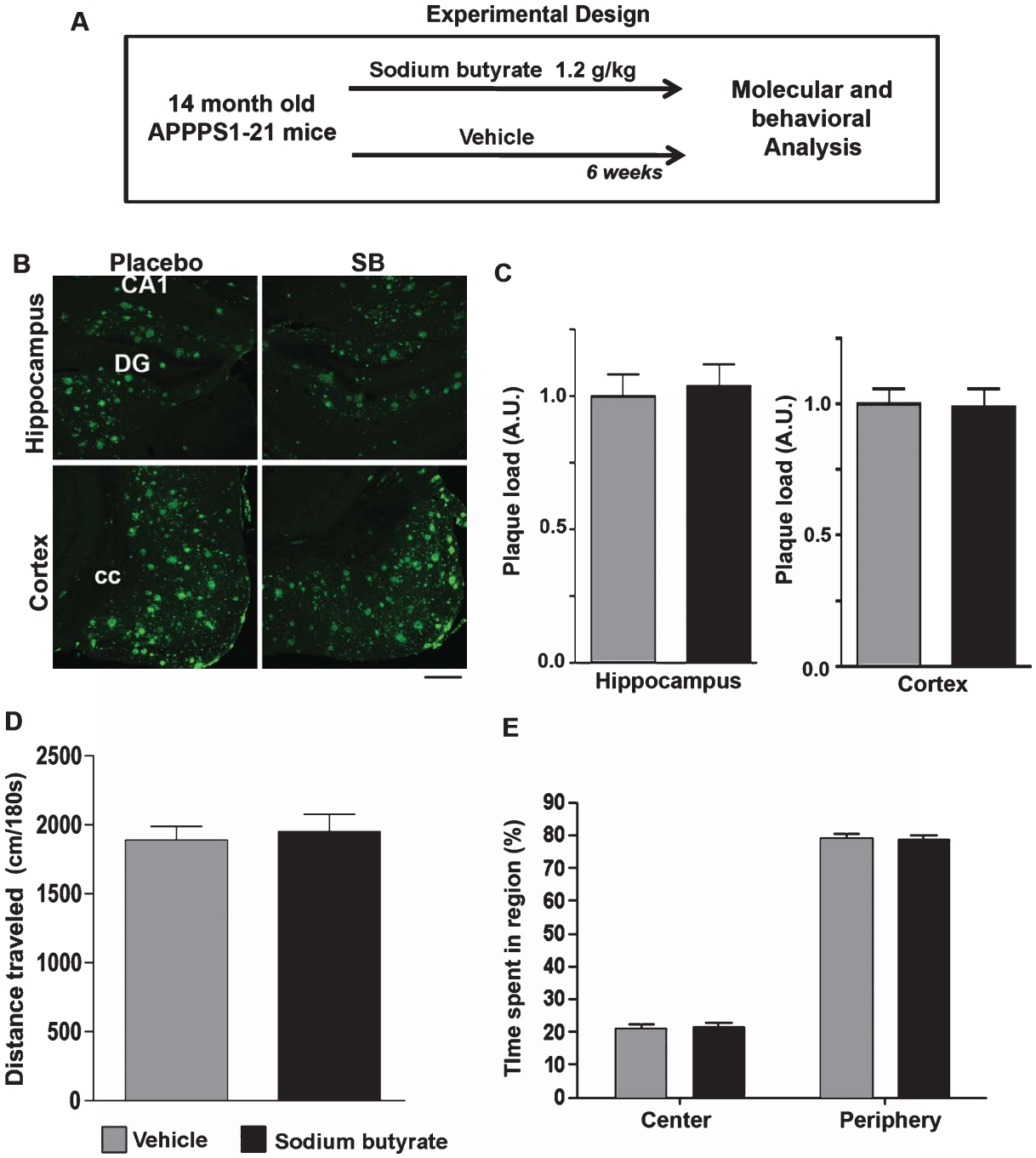

Fig. 2. SB treatment of APPPS1-21 mice and behavioral analysis. A) Schematic representation of the experimental paradigm depicting oral SB/vehicle treatment of APPPS1-21 mice followed by behavioral analysis. B) Confocal microscopy images showing no change in Aβ plaques upon SB treatment in the hippocampus and cortex of APPPS1-21 mice. C) Quantification of data shown in B. D, E) Exploratory behavior shown by total distance covered (D) and time spent in different regions (E) in the open field by SB- and vehicle-treated APPPS1-21 mice.

gene expression analysis, a number of genes were found to increase in response to a learning stimulus. For the selected genes that were chosen to represent signaling pathways involved in neuronal plasticity, differential expression upon SB treatment was confirmed via qPCR analysis [9]. Therefore, we compared the

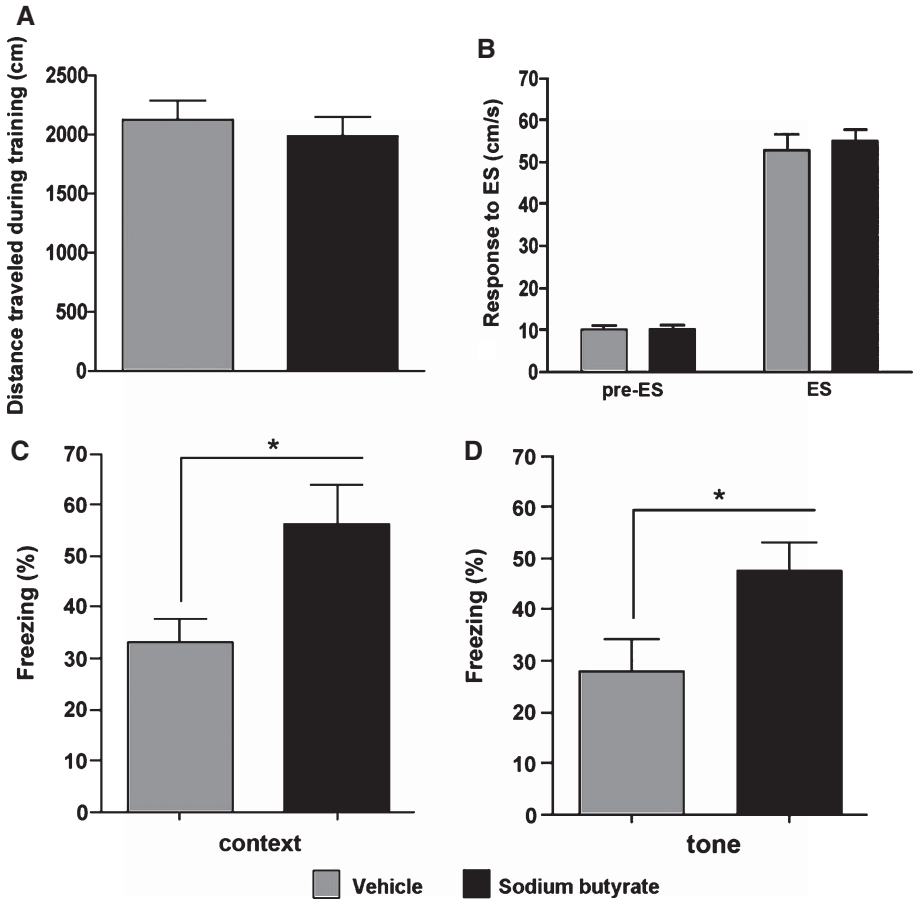

Fig. 3. Improved associative memory in APPPS1-21 mice upon SB treatment. A) Basal activity in the novel context shown by total distance covered. B) Response to electric shock. C, D) Freezing levels in contextual (C) and cued (D) memory test 24 h after training.

expression of ten of those representative genes in SB- and vehicle-treated APPPS1-21 mice. We observed that within the hippocampus of APPPS1-21 mice, SB treatment resulted in the significant upregulation of 8 out of 10 investigated genes, including *Myst4*, *Fmn2*, *Marcksl1*, *Gsk3*α, *GluR1*, *Snap25, Prkca, and Shank3* (Fig. 5A). Notably, the expression of the human *App* transgene, which is under the control of the Thy1 promoter, did not change upon SB treatment (Fig. 5A). In line with our data showing that SB treatment did not affect cortical levels of histone acetylation, we failed to detect significant changes in the expression of the selected genes in the cortices of SB-treated APPPS1-21 mice (Fig. 5B). To investigate whether increased gene expression in SB treated mice would result in altered levels of the corresponding proteins, we decided to analyze, as a representative example, hippocampal levels of SNAP25. Consistent with the gene expression data, we found that SNAP25 levels were significantly increased in SB treated APPPS1-21 mice (Fig. 5C).

DISCUSSION

Epigenetic regulation of gene expression in the mammalian brain is known to play an important role in cognitive function [14] and its dysregulation has been linked to the pathogenesis of neurodegenerative diseases [17]. Enhancing histone acetylation by inhibiting HDACs is being recognized as a potential therapeutic strategy to treat memory decline during AD [10, 16, 17].

Ours is the first study to employ APPPS1-21 mice to test the effect of HDAC inhibition on memory function. When compared to other models of amyloid pathology, the APPPS1-21 mice are characterized by early

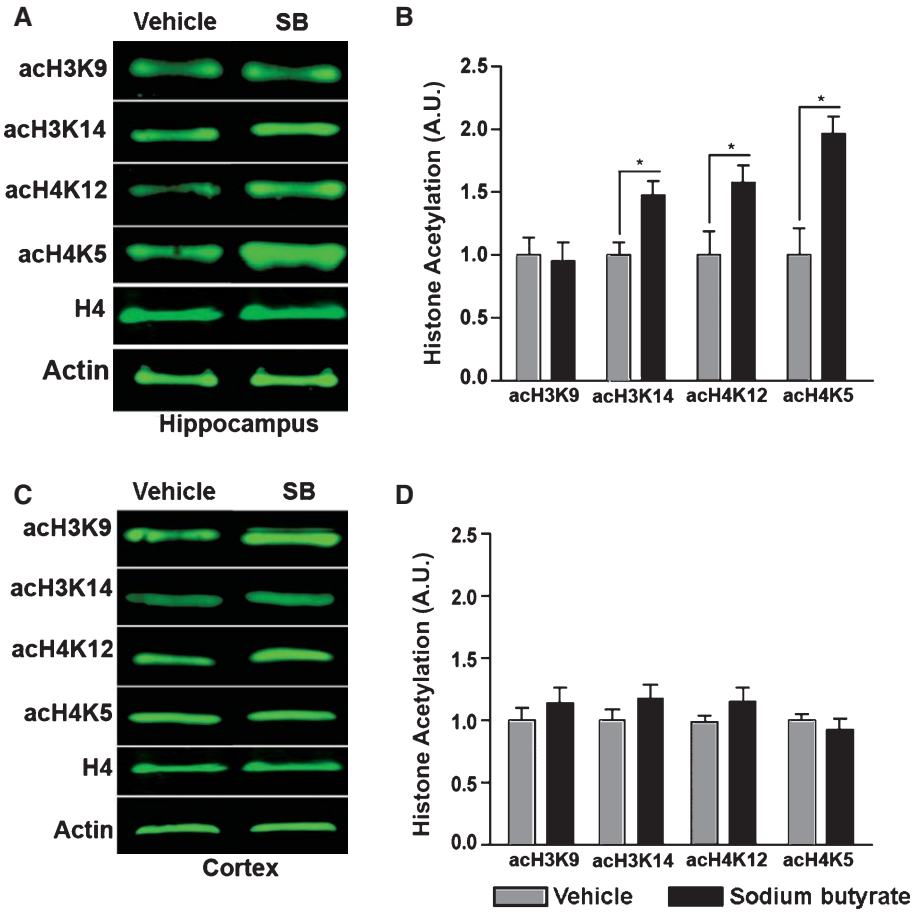

Fig. 4. Histone acetylation in APPPS1-21 mice upon SB treatment. A–D) Western blot showing increased histone acetylation at specific lysine residues on histones H3 and H4 in the hippocampus (A) and the cortex (C) of SB-treated APPPS1-21 mice compared to the vehicle-treated group. Panels B and D show quantification for A and C respectively. $n = 4$ per group.

onset of amyloid pathology already at 2 months of age [19]. Therefore, we found this model suitable to further investigate the effect of HDAC inhibitor treatment in an advanced disease state, which is of utmost importance since this situation is commonly seen in AD patients upon diagnosis. We found that 15-month-old APPPS1-21 mice exhibited severe impairment of contextual and cued associative memory function that was accompanied by massive hippocampal and cortical amyloid pathology and decreased histone acetylation. Although in this study we focused on the hippocampus and cortex, the fact that cued conditioning was impaired in APPPS1-21 mice and rescued by SB treatment suggests that other brain regions, such as the amygdala, may also be affected.

While the precise mechanism by which amyloid pathology impairs histone acetylation remains to be elucidated, it is interesting to note that transient changes in histone acetylation are linked to neuronal plasticity [9, 13, 25–27]. Thus, it can be speculated that reduced histone acetylation in APPPS1-21 mice could result from Aβ-mediated impairment of synaptic function [28]. Importantly, 6 weeks of daily SB administration was able to rescue impairment in associative memory without affecting hippocampal and cortical Aβ plaque load. These data concur with a recent study showing that 3 weeks of SB treatment was able to reinstate associative memory in 6-month-old APPswe/PS1 dE9 mice, a time point that marks the onset of memory disturbances in this mouse model [8].

HDAC inhibitors affect gene expression by increasing histone acetylation [29]. In line with this, we observed that SB treatment led to a significant increase in hippocampal histone acetylation at lysine residues H3 K14, H4 K5, and H4 K12 when analyzed via immunoblot analysis. These sites are among those

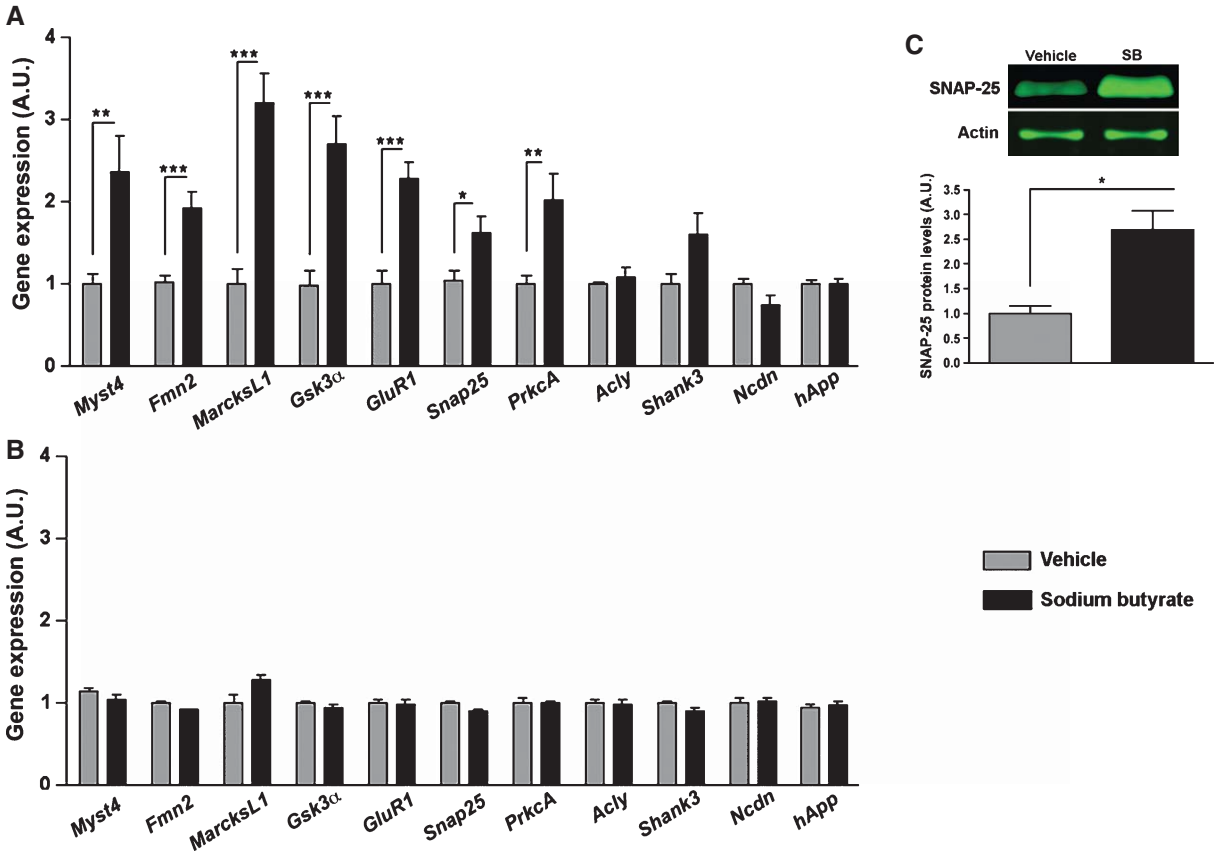

Fig. 5. Regulation of gene expression by SB treatment. A, B) Quantitative Real-Time PCR analysis showing expression levels of genes involved in memory consolidation and the human *App* transgene in the hippocampus (A) and cortex (B) of APPPS1-21 mice upon chronic SB treatment. C) Western blot images (top) showing SNAP-25 protein levels in the hippocampus in SB- and vehicle-treated APPPS1-21 mice along with quantification (bottom). $n = 4$ per group.

hypoacetylated in the APPPS1-21 mice as shown by immunohistochemical analysis. Although, these data should not be compared directly, our findings suggest that SB treatment, at least in part, rescues decreased histone acetylation in APPPS1-21 mice. Interestingly, H3 K9 acetylation was not affected upon SB treatment. This is in agreement with recent findings suggesting that particular patters of histone acetylation correlate with the gene expression profile initiated during memory formation [30]. In this regard, especially H4 K12 acetylation was found to be a prerequisite for proper associative memory formation [9, 17, 31]. As such, HDAC inhibitors that were able to elevate hippocampal H4 K12 acetylation could reinstate memory formation in aged mice while drugs that elevated H3 K9 but not H4 K12 acetylation failed to do so [9]. While this finding excludes a role for H3 K9 acetylation in memory function, our data suggest that acetylation at H4 K12 might more crucial than at H3 K9 in rescuing cognitive deficits.

Previous work has shown that the process of memory consolidation involves epigenetic regulation of specific genes in the hippocampus that in turn could be regulated by SB treatment [9]. In line with increased hippocampal histone acetylation, SB treated APPPS1-21 mice showed elevated expression of representative genes that were selected on the basis of this study [9]. Furthermore, we show that in the case of SNAP-25, a protein with well-documented function in synaptic plasticity [32], increased gene expression translated into elevated protein levels. These data suggest that the improved memory function in APPPS1-21 could, at least in part, be a result of increased expression of genes linked to synaptic plasticity. However, HDACs also target non-histone proteins [17] and it cannot be excluded that modifications on proteins other than histones could

contribute to the observed effect. While our data show that SB treatment improves histone acetylation, gene expression and memory function in APPPS1-21 mice when administered at an advanced stage of pathology, it remains to be investigated if and how altered histone acetylation contributes to gene expression changes during AD pathogenesis.

Interestingly, SB treatment failed to increase histone acetylation and gene expression in the cerebral cortices of APPPS1-21 mice. While the precise reason for this effect remains to be elucidated, this result suggests that the hippocampus is more susceptible to HDAC inhibitor treatment. This concurs with several studies showing that memory consolidation results in dynamic changes in histone acetylation within the hippocampus [4, 9, 13] while to our knowledge, no such data are available for cortical regions. These data may also in part reflect the difference in the expression pattern of HDAC proteins within different brain regions. For example, HDAC2 is a likely candidate to be responsible for the memory enhancing effect of HDAC inhibitors [31] and is expressed at much higher levels within the hippocampus, when compared to cortical regions [33]. Therefore, it is possible that our treatment paradigm preferentially affects the hippocampus. However, since total cortex was analyzed, a more detailed analysis on specific cortical sub-regions would be required to rule out any effect of SB treatment on cortical histone acetylation and gene expression.

Our findings that SB improves memory function when administered to APPPS1-21 mice at an advanced pathological stage are also concordant with recent data showing that another pan-HDAC inhibitor, phenylbutrate, was able to reinstate associative memory function in 6- and 16-month-old tg2576 mice [6, 7], another mouse model for amyloid pathology. Similarly, administration of the HDAC inhibitor TSA to 4-month-old APP/PS1 mice reinstated associative memory function in this mouse model [5]. Although SB is has been generally used as a pan-HDAC inhibitor, recent data suggest that it shows selectivity to class I HDACs [8]. Taken together, these data strongly support the idea that targeting histone acetylation via HDAC inhibitors might be a suitable therapeutic strategy to treat memory impairment even at advanced stages of AD pathology. In fact, the use of HDAC inhibitors is tempting because some of the compounds have already been approved for treatment in humans, although for cancer and not AD [34]. Nevertheless, currently available HDAC inhibitors are often characterized by adverse side effects [35, 36]. This may partly be explained by the fact that the currently used HDAC inhibitors such as SB are not selective and affect many of the 11 HDACs that are expressed in the adult brain [33]. Hence, it has been proposed that more selective compounds may have an even greater therapeutic impact [17].

In conclusion, our study shows that the HDAC inhibitor sodium butyrate leads to cognitive improvement in APPPS1-21 mice even when administered at an advanced stage of disease progression. This provides further evidence that the use of appropriate HDAC inhibitors could become a suitable therapeutic strategy to treat memory impairment during AD pathogenesis.

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