Altered Histone Acetylation Is Associated with Age-Dependent Memory Impairment in Mice

Shahaf Peleg *et al.*

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Altered Histone Acetylation Is Associated with Age-Dependent Memory Impairment in Mice

Shahaf Peleg,[1*] Farahnaz Sananbenesi,[1*] Athanasios Zovoilis,[1*] Susanne Burkhardt,[1] Sanaz Bahari-Javan,[1] Roberto Carlos Agis-Balboa,[1] Perla Cota,[1] Jessica Lee Wittnam,[1†] Andreas Gogol-Doering,[2] Lennart Opitz,[3] Gabriella Salinas-Riester,[3] Markus Dettenhofer,[4] Hui Kang,[2] Laurent Farinelli,[5] Wei Chen,[2] André Fischer[1‡]

As the human life span increases, the number of people suffering from cognitive decline is rising dramatically. The mechanisms underlying age-associated memory impairment are, however, not understood. Here we show that memory disturbances in the aging brain of the mouse are associated with altered hippocampal chromatin plasticity. During learning, aged mice display a specific deregulation of histone H4 lysine 12 (H4K12) acetylation and fail to initiate a hippocampal gene expression program associated with memory consolidation. Restoration of physiological H4K12 acetylation reinstates the expression of learning-induced genes and leads to the recovery of cognitive abilities. Our data suggest that deregulated H4K12 acetylation may represent an early biomarker of an impaired genome-environment interaction in the aging mouse brain.

A number of studies indicate that aging correlates with brain region–specific changes of gene expression (*1*–*4*). It is, however, not well understood how aging affects gene expression and if those changes are causally linked to memory impairment. Remodeling of chromatin via histone acetylation, a key mechanism to control gene expression (*5*), has recently been implicated with the formation of long-term memories (*6*–*10*). Therefore, we hypothesized that altered histone acetylation might contribute to age-associated changes in gene expression and cognitive decline.

To detect an age at which cognitive impairment is first manifested, we subjected 3-, 8-, and 16-month-old C57BL/6 mice, which have a mean life span of 26 to 28 months (*11*, *12*), to contextual fear conditioning (*10*), a commonly used test for hippocampus-dependent associative learning. Notably, the hippocampal formation is intimately involved in cognitive function in rodents and humans and is among the first to be affected during dementia (*13*). Whereas all groups were successfully able to learn this task, 16-month-old mice showed significantly less freezing behavior during the memory test, which indicated impaired associative learning (fig. S1A). Additional groups of mice were trained in the Morris water-maze protocol (*10*), a well-established test for hippocampus-dependent spatial memory. All groups improved in their ability to find the hidden platform throughout the training trials, but the escape latency was significantly impaired in 16-month-old mice when compared with the 3- or 8-month-old groups (fig. S1B). Consistently, 16-month-old mice spent less time in the target quadrant during a subsequent probe test (fig. S1C). Groups did not differ in finding a visible platform (fig. S1B), explorative behavior, or the response to a foot shock (fig. S1, D and E). Moreover, hippocampal levels of various markers for neuronal plasticity and integrity such as microtubule-associated protein 2, synaptoporin, postsynaptic density–95, synaptophysin (Svp), and glutamate receptor 1 were similar among 3- and 16-month-old mice (fig. S1, F and G). These findings suggest that the impairments in hippocampus-dependent memory formation displayed by 16-month-old mice cannot be explained by major structural changes, altered exploratory behavior, or impaired response to foot shock.

To test whether memory impairment correlates with altered chromatin plasticity, we first investigated whether hippocampal histone acetylation differs between 3- and 16-month-old naïve mice. Quantitative immunoblot analysis did not reveal significant changes of histone H3 acetylation on lysine residues (K) 9 and 14 or H4 acetylation on K5, 8, 12, or 16, which showed that the basal hippocampal histone acetylation profile is similar among 3- and 16-month-old naïve mice (fig. S2). In line with these data, the levels and activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs) were similar in 3- and 16-month-old mice (fig. S3).

Recent data suggest that histone acetylation might play an important role in orchestrating the

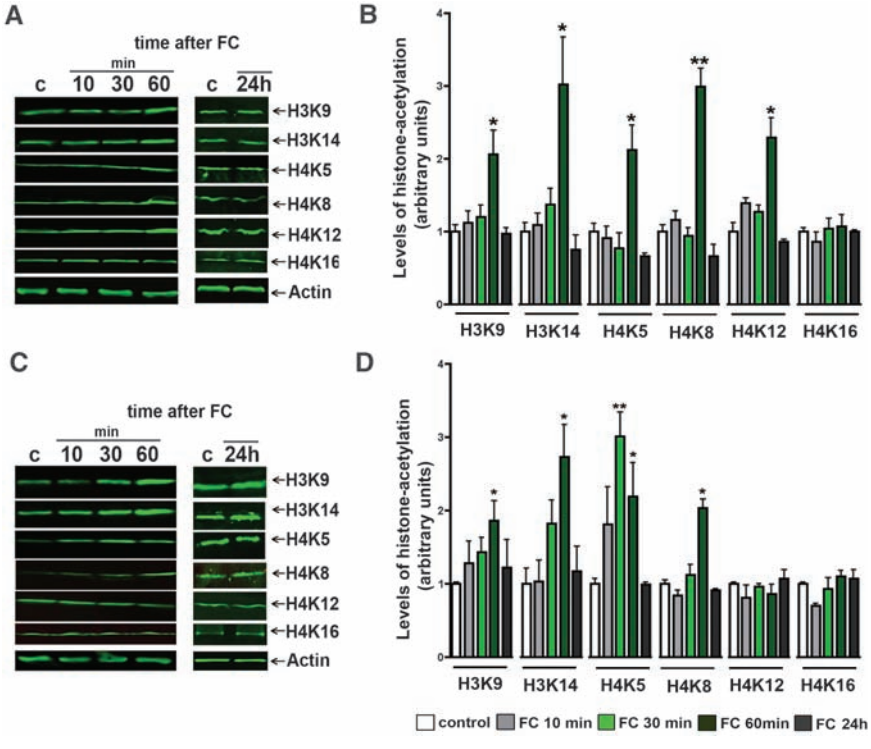

Fig. 1. Impaired learning and memory in 16-month-old mice correlates with deregulated H4K12 acetylation. (**A**) Representative immunoblot showing histone acetylation in 3-month-old mice in response to fear conditioning (FC). Control mice (c) were treated identically but did not receive the foot shock. (**B**) Quantification of (A). (**C**) Analysis similar to that described in (A) was performed in 16-month-old mice. (**D**) Quantification of (C). (**$P < 0.01$, *$P < 0.05$ versus control). n = four or five mice per group. Error bars indicate SEM.

[1]Laboratory for Aging and Cognitive Diseases, European Neuroscience Institute, Grisebach Str. 5, D-37077 Goettingen, Germany. [2]Max Delbrueck Center for Molecular Medicine, Institute for Medical Systems Biology, Robert-Rössle-Strasse 10, D-13125 Berlin-Buch, Germany. [3]DNA Microarray Facility, Georg August University, Humboldtallee 23, D-37073 Goettingen, Germany. [4]Harvard Medical School, Genetics Department, 77 Ave Louis Pasteur, Boston, MA 02115, USA. [5]Fasteris SA, CH-1228 Plan-les-Ouates, Switzerland.

*These authors contributed equally to this work.
†Present address: Department of Psychiatry, Division of Molecular Psychiatry, University Goettingen, von Siebold Str. 7, D-37075 Goettingen, Germany.
‡To whom correspondence should be addressed. E-mail: a.fischer@eni-g.de

gene expression program initiated by memory consolidation (*8*, *14*). Therefore, we used quantitative immunoblotting to analyze hippocampal histone acetylation in 3- and 16-month-old mice at 10, 30, and 60 min and 24 hours after exposure to the fear conditioning training (fig. S4A). When compared with the age-matched control group, 3-month-old mice displayed a transient increase of H3K9 and H3K14 and H4K5, H4K8, and H4K12 acetylation 60 min after fear conditioning (Fig. 1, A and B). A similar transient increase of H3K9, H3K14, H4K5, and H4K8 acetylation was observed in 16-month-old mice. Whereas H4K5 acetylation was up-regulated 60 min after fear conditioning in both age groups, a significant increase of H4K5 acetylation was already detectable 30 min after fear conditioning in 16-month-old mice. These 16-month-old mice failed to up-regulate H4K12 acetylation (Fig. 1, C and D). These data were confirmed by immunohistochemical analysis (fig. S4). Moreover, the levels of total H4 did not change between groups (fig. S4), which showed that memory impairment correlates with a deficit in learning-induced H4K12 acetylation in 16-month-old mice.

To analyze if deregulated H4K12 acetylation impacts learning-induced gene expression, we performed a high-density oligonucleotide microarray to compare the entire hippocampal gene expression profile of 3- and 16-month-old mice during memory consolidation (fig. S5A). To this end, 3- and 16-month-old mice were subjected to fear conditioning. Explorative behavior during the training and the response to the foot shock were similar among groups (fig. S5B). Animals that were not subjected to fear conditioning but otherwise were treated identically served as controls. Notably, the gene expression profile was nearly identical among 3- and 16-month-old control mice (Fig. 2A and fig. S6). This is consistent with our finding that histone acetylation, HAT, and HDAC activities are similar among those groups (figs. S2 and S3). In 3-month-old mice, 2229 genes (1980 up-regulated versus 449 down-regulated) were differentially expressed 1 hour after fear conditioning as compared with the age-matched control group (Fig. 2A and table S1). However, the hippocampal transcriptome of 16-month-old mice remained almost unchanged in response to fear conditioning. When compared with the age-matched control group, only six genes were differentially expressed among groups (Fig. 2A and fig. S7).

Further analysis revealed that, in 3-month-old mice, 1539 of the differentially expressed genes were specifically linked to associative learning, hereafter called "learning-regulated genes" (fig. S8 and tables S2 and S3). The learning-regulated genes were associated with biological processes such as transcription, protein modification, or intracellular signaling (fig. S5C and table S4). Additional data mining revealed that 3-month-old mice regulate key signaling pathways implicated with memory formation and synaptic remodeling in response to fear conditioning (fig. S9 and table S5). Using quantitative polymerase chain reaction (qPCR) analysis, we confirmed the differential expression of genes selected to represent signaling pathways identified by data

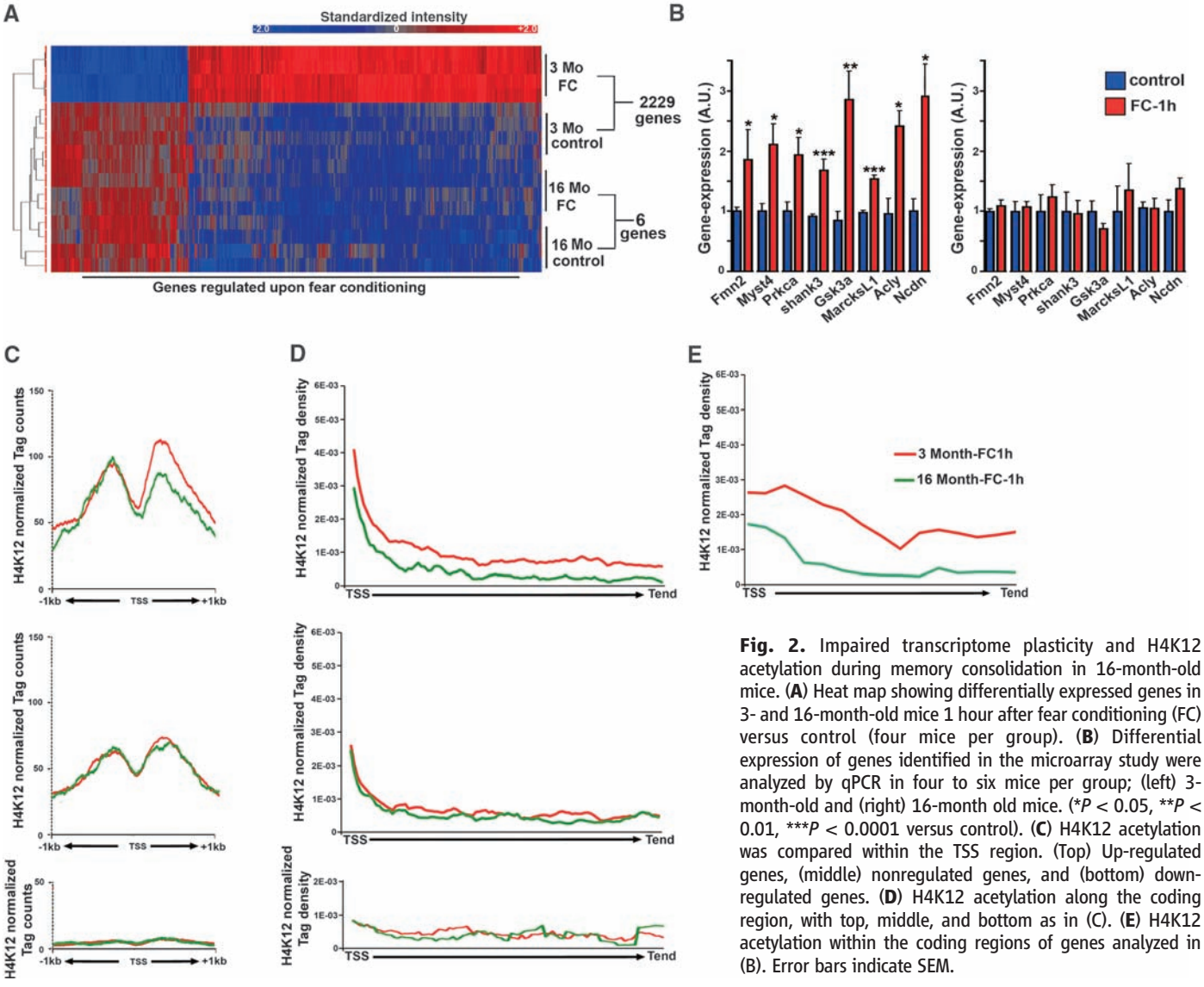

Fig. 2. Impaired transcriptome plasticity and H4K12 acetylation during memory consolidation in 16-month-old mice. (**A**) Heat map showing differentially expressed genes in 3- and 16-month-old mice 1 hour after fear conditioning (FC) versus control (four mice per group). (**B**) Differential expression of genes identified in the microarray study were analyzed by qPCR in four to six mice per group; (left) 3-month-old and (right) 16-month old mice. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$ versus control). (**C**) H4K12 acetylation was compared within the TSS region. (Top) Up-regulated genes, (middle) nonregulated genes, and (bottom) down-regulated genes. (**D**) H4K12 acetylation along the coding region, with top, middle, and bottom as in (C). (**E**) H4K12 acetylation within the coding regions of genes analyzed in (B). Error bars indicate SEM.

mining (Fig. 2B and table S6). Our data reveal that 16-month-old mice show severe impairment in regulating gene expression on exposure to relevant environmental stimuli that initiate learning behavior. As a result, key signaling pathways initiated in 3-month-old mice during associative learning are not properly regulated in 16-month-old mice.

Next, we investigated how altered H4K12 acetylation contributes to the lack of learning-induced gene expression in 16-month-old mice. We decided to take a genome-wide approach to analyze H4K12 acetylation of learning-regulated genes in 3- and 16-month-old mice after fear-conditioning. To this end, we used the recently developed ChIP-seq technology (*15*) that depends on the cross-linking of proteins to specific DNA elements, followed by immunoprecipitation of the protein-DNA complex and high-throughput sequencing of the recovered DNA (*16*, *17*). Hippocampal tissue isolated from 3- and 16-month-old mice 1 hour after fear conditioning was subjected to H4K12 ChIP-seq (fig. S10). To generate a genome-wide map of H4K12 acetylation in the hippocampus of young and old mice during memory formation, the resulting reads were mapped to a reference mouse genome (fig. S10).

We first examined H4K12 acetylation in the region spanning the transcription start site (TSS), which is essential for transcriptional initiation (*16*). To this end, we compared enrichment in H4K12 acetylation in 3- and 16-month-old mice at regions extending 1 kb upstream and 1 kb downstream of the TSS (hereafter, referred to as the TSS region) of (1) up-regulated genes, (2) randomly chosen genes that were not regulated upon fear conditioning, and (3) learning-regulated genes that were down-regulated (Fig. 2C, top, middle, and bottom, respectively). H4K12 acetylation of down- or nonregulated genes did not differ among groups (Fig. 2C). However, H4K12 acetylation of up-regulated genes was altered in 16-month-old mice (Fig. 2C, top). Impaired H4K12 acetylation was specifically observed in the genomic region 1 kb downstream of the TSS (Fig. 2C), which marks the beginning of the gene-coding region. H4K12 acetylation 1 kb upstream of the TSS, a region that marks the gene promoter, was similar among groups. Therefore, we analyzed the distribution of H4K12 acetylation within the coding regions of up-, non-, and down-regulated genes, as well as the genes individually analyzed in Fig. 2B (Fig. 2, D and E, and fig. S11). The levels of H4K12 acetylation in up-regulated genes were lower in 16-month-old mice than in the 3-month-old group. No difference was observed for non- and down-regulated genes (Fig. 2D).

ChIP-seq analysis of H3K9 acetylation did not reveal higher enrichment in 3-month-old compared with 16-month-old mice (fig. S12), which is in line with our findings that H4K12, but not H3K9, acetylation is deregulated in 16-month-old mice on fear conditioning (see also Fig. 1). A recent study showed that in blood cells H4K12 is mainly enriched within gene bodies and therefore associated with transcriptional elongation, whereas other sites, such as H3K9, peak in the TSS region (*16*). In line with this, we found that high levels of gene expression in the hippocampus also correlate with high levels of H4K12 acetylation along the coding regions of genes, whereas no such correlation was observed for H3K9 (fig. S13). These data suggest that the severe lack of learning-induced gene expression in 16-month-old mice is linked, at least in part, to deregulated H4K12 acetylation associated with impaired transcriptional elongation of up-regulated genes.

To analyze this in greater detail, we investigated the Formin 2 gene, *Fmn2*, as an example. Formin 2 is an actin nucleator highly expressed in the adult brain (*18*), and actin dynamics are of great importance for synaptic plasticity and memory formation (*19*, *20*). Moreover, *Fmn2* was induced in 3-month-old, but not in 16-month-old, mice on fear conditioning (Fig. 2B), which correlated with impaired H4K12 acetyla-

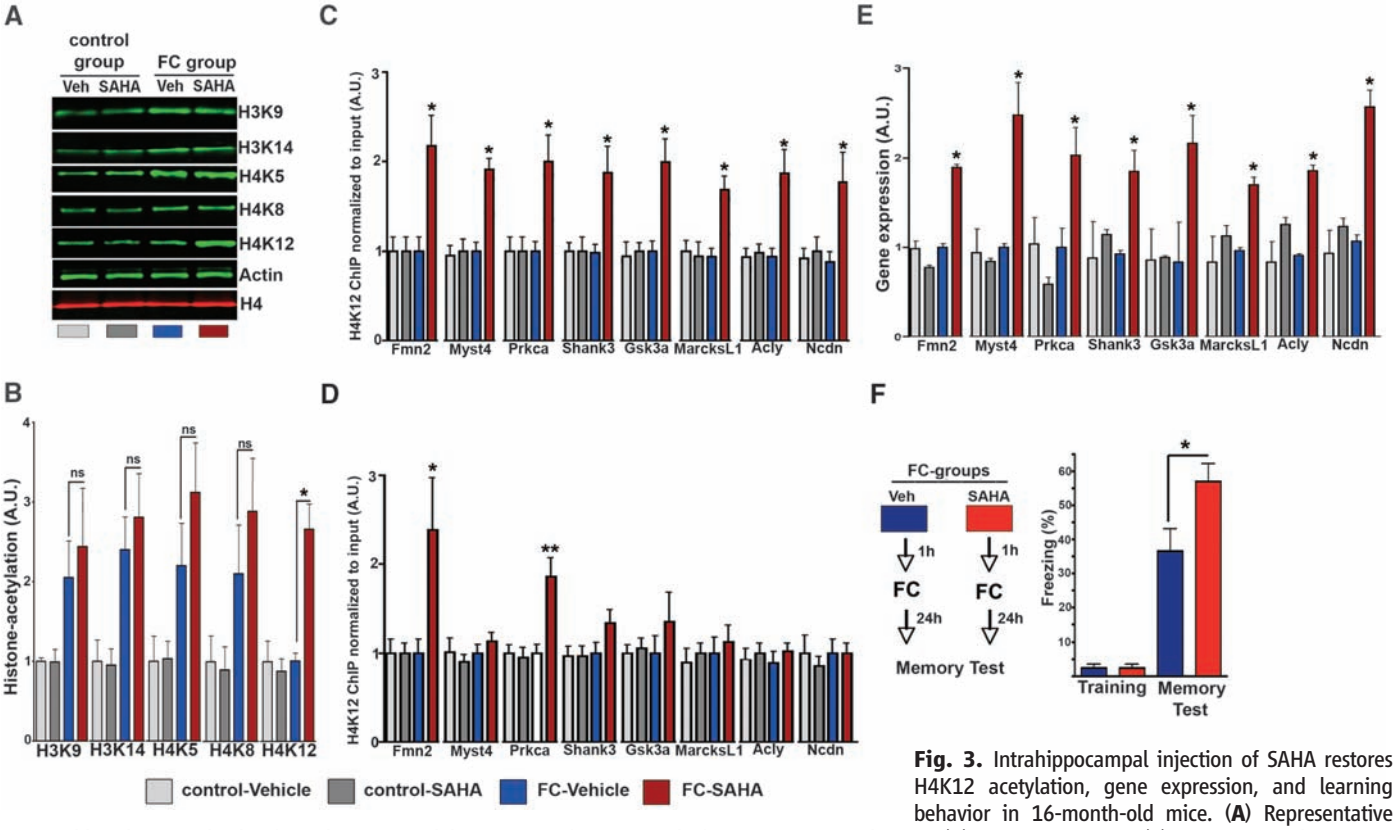

Fig. 3. Intrahippocampal injection of SAHA restores H4K12 acetylation, gene expression, and learning behavior in 16-month-old mice. (**A**) Representative immunoblot showing the levels of hippocampal histone acetylation in vehicle or SAHA-treated mice. (**B**) Quantification of (A). H4K12 acetylation was significantly increased in the SAHA-treated mice after fear conditioning (*$P < 0.05$, four mice per group). A.U., arbitrary units. (**C**) H4K12 ChIP of coding region. SAHA-treatment increased learning-induced H4K12 acetylation in the coding regions of all investigated genes (*$P < 0.05$, four mice per group). (**D**) H4K12 ChIP of promoter region. SAHA-treatment increased learning-induced H4K12 acetylation in the formin 2 and Prkca promoter (*$P < 0.05$, four mice per group). (**E**) Gene expression. Increased learning-induced gene expression in 16-month-old mice treated with SAHA ($P < 0.05$, five or six mice per group). (**F**) Associative learning was enhanced in SAHA-treated mice (*$P < 0.05$, nine mice per group). Error bars indicate SEM.

tion throughout the coding region (Fig. 2E and fig. S11). We confirmed this finding using ChIP followed by qPCR analysis to show that H4K12 acetylation of *Fmn2* increases 1 hour after fear conditioning in 3-month-old, but not in 16-month-old, mice (fig. S14, A and B). The observed changes in H4K12 acetylation and mRNA expression translated into differential protein production. Formin 2 protein levels transiently increased in response to fear-conditioning in 3-month-old, but not in 16-month-old, mice (fig. S14C).

Next, we used mice lacking *Fmn2* (*21*) to investigate its role in memory formation. These mice are viable and show normal brain anatomy (fig. S14D). Associative learning was similar among 3-month-old *Fmn2*$^{-/-}$ mice and wild-type littermates. However, 8-month-old *Fmn2*$^{-/-}$ mice showed impaired associative learning ability when compared with an age-matched control group (fig. S14E). Pain sensation, explorative behavior, and basal anxiety were similar among groups (fig. S14, F to H). These data suggest that reduced formin 2 levels contribute to age-related memory impairment.

Nevertheless, the age-associated memory impairment in *Fmn2*$^{-/-}$ mice cannot be directly compared with memory impairment observed in wild-type mice. In 16-month-old wild-type mice expression of *Fmn2*, as well as those of the 1538 other learning-regulated genes, is normal under basal conditions. However, these mice fail to increase the levels of learning-induced genes during associative learning. Therefore, we propose that rather than targeting the expression levels of a single candidate gene, elevation of H4K12 acetylation during aging might be a more efficient approach to restore learning abilities.

To test this hypothesis, we implanted microcannulae into the hippocampi of 16-month-old mice. One group of mice was injected with the potent HDAC inhibitor suberoylanilide hydroxamic acid (SAHA, 10 μg per hippocampus); the other group received vehicle solution. One hour after injection, mice were subjected to contextual fear conditioning and killed 1 hour later for molecular analysis. Mice that were injected with SAHA or vehicle and used for molecular analysis 2 hours later without being subjected to fear conditioning served as additional control groups (fig. S15A). Notably, SAHA-treated mice showed significantly increased hippocampal H4K12 acetylation after fear conditioning (Fig. 3, A and B). In line with this finding, we observed that, after fear conditioning, SAHA-treated mice displayed elevated H4K12 acetylation in the coding regions of learning-regulated genes (Fig. 3C). The effect of SAHA on H4K12 acetylation in the promoter regions was less pronounced, which further supported a predominant role of H4K12 in transcriptional elongation (Fig. 3D). The expression of learning-regulated genes (table S5) was significantly higher in 16-month-old mice that were treated with SAHA before fear conditioning (Fig. 3E).

These data suggest that increasing H4K12 acetylation restores learning-induced gene expression. This work may show relevance therapeutically for the treatment of age-associated memory impairment via the restoration of physiological expression levels for genes contributing to memory consolidation. Indeed, 16-month-old mice treated with SAHA showed facilitated associative learning when compared with the vehicle group (Fig. 3F). The response to the foot shock, explorative behavior, and tone-dependent fear conditioning that is hippocampus independent were not affected (fig. S15, B to D), which showed that the SAHA-mediated increase of H4K12 acetylation is sufficient to restore associative learning in 16-month-old mice. Similar data were obtained using the pan-HDAC inhibitor sodium butyrate (fig. S16), whereas administration of an HDAC inhibitor that failed to increase H4K12 acetylation in 16-month-old mice also failed to reinstate learning ability (fig. S17).

In summary, our data suggest that deregulated H4K12 acetylation is causally involved in age-associated memory impairment. Although we cannot exclude that other histone modifications also contribute to this effect, recent studies support a unique role of H4K12 in the orchestration of gene expression (*16*). As such, H4K12 acetylation seems to be of particular importance for transcriptional elongation that is characterized by high levels of histone modifications along gene bodies (*16*, *22*). Consistently, we show that deregulation of H4K12 acetylation in 16-month-old mice is mainly found along gene bodies of up-regulated genes. This may also explain why a deficit in H4K12 acetylation could mediate the observed profound effect on learning-regulated gene expression. As such, even in the presence of proper transcriptional initiation, further up-regulation of learning-regulated genes above baseline levels would be impaired when transcriptional elongation is affected. The precise mechanisms that underlie the selective deregulation of H4K12 acetylation in 16-month-old mice remain to be elucidated. It is likely that during aging a combination of multiple factors contributes to deregulated histone acetylation. Although we could not detect significant differences between changes in hippocampal HAT or HDAC activity in 3-month-old versus 16-month-old mice (figs. S3 and S18), this does not exclude the possibility that specific HATs or HDACs contribute to deregulated gene expression and learning impairment in 16-month-old mice. In fact, the HATs Myst4 and Gcn5l2, as well as HDAC2 and HDAC4, were differentially expressed in 3-month-old, but not in 16-month-old, mice during memory formation (fig. S18 and table S3). Recent data showed that HDAC2 regulates memory formation (*23*). Moreover, small changes in the metabolic state of the cell may favor the dysfunction of mechanisms that are engaged with H4K12 acetylation. For example, a recent study demonstrated that histone acetylation critically depends on citrate levels (*24*), which are reduced in the aging brain (*25*) (fig. S19).

In conclusion, we found that the administration of HDAC inhibitors that shift the balance of H4K12 acetylation is able to reinstate learning-induced gene expression and memory function in 16-month-old mice. Our data also suggest that H4K12 acetylation–dependent changes in gene expression may serve as an early biomarker for an impaired genome-environment interaction in the aging brain.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/328/5979/753/DC1
Materials and Methods
Figs. S1 to S19
Tables S1 to S7
References

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ERRATUM

Post date 25 June 2010

Reports: "Altered histone acetylation is associated with age-dependent memory impairment in mice" by S. Peleg *et al.* (7 May, p. 753). There were 2229 genes, but the text misstates the numbers that were up-regulated and down-regulated. It should have read, "In 3-month-old-mice, 2229 genes (1977 up-regulated versus 252 down-regulated) were differentially expressed." The body of table S1 is correct, but the title contains the same mistake.