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Histone H2A.Z subunit exchange controls consolidation of recent and remote memory

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Abstract

Memory formation is a multi-stage process that initially requires cellular consolidation in the hippocampus, after which memories are downloaded to the cortex for maintenance, in a process termed systems consolidation¹. Epigenetic mechanisms regulate both types of consolidation^{2–7}, but histone variant exchange, in which canonical histones are replaced with their variant counterparts, is an entire branch of epigenetics that has received limited attention in the brain^{8–12} and has never, to our knowledge, been studied in relation to cognitive function. Here we show that histone H2A.Z, a variant of histone H2A, is actively exchanged in response to fear conditioning in the hippocampus and the cortex, where it mediates gene expression and restrains the formation of recent and remote memory. Our data provide evidence for H2A.Z involvement in cognitive function and specifically implicate H2A.Z as a negative regulator of hippocampal consolidation and systems consolidation, probably through downstream effects on gene expression. Moreover, alterations in H2A.Z binding at later stages of systems consolidation suggest that this histone has the capacity to mediate stable molecular modifications required for memory retention. Overall, our data introduce histone variant exchange as a novel mechanism contributing to the molecular basis of cognitive function and implicate H2A.Z as a potential therapeutic target for memory disorders.

As a first step in exploring the role of H2A.Z in cognitive function, we used immunohistochemistry to confirm its expression throughout the hippocampus (Extended Data Fig. 1a–c). Next, we showed that *H2afz*, a gene encoding H2A.Z, was inhibited at 30 min ($F_{3,14} = 6.38$, $P = 0.006$) and returned to baseline levels 2 h after contextual fear conditioning in mice (Extended Data Fig. 1d). In addition, H2A.Z levels ($F_{3,9} = 5.34$, $P = 0.02$) were reduced and promoter methylation was increased ($F_{3,21} = 12.34$, $P < 0.001$) 30

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Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Supplementary Information is available in the online version of the paper.

Author Contributions J.D.S. and I.B.Z. conceived the experiments. I.B.Z. conducted the experiments and B.S.P. and D.M.E. assisted in performing the experiments. J.J.D. analysed the next-generation sequencing data.

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min after training (Extended Data Fig. 1e, f). Although promoter methylation negatively affects transcription^{4,6,7}, this role is complex¹³ and may not be the direct cause of H2A.Z inhibition in our study.

H2A.Z positioning around the transcriptional start site (TSS) is strongly associated with transcription^{11,14,15}. Using chromatin immunoprecipitation (ChIP), we investigated H2A.Z exchange at the -1 (first nucleosome upstream of the TSS) and +1 (first nucleosome downstream of the TSS) nucleosomes of memory-associated genes during consolidation (Fig. 1). At 30 min after training, H2A.Z binding was reduced at the +1 nucleosome of memory-promoting genes (*Npas4*: Welch's $F_{3,4.98} = 67.10$, $P < 0.001$; *Arc*: Welch's $F_{3,6.8} = 153.95$, $P < 0.001$; *Egr1*: Welch's $F_{3,7.86} = 282.71$, $P < 0.001$; *Egr2*: $F_{3,18} = 3.50$, $P = 0.04$; *Fos*: $F_{3,9} = 39.61$, $P < 0.0001$), and the expression of corresponding genes was increased during this time (*Npas4*: $F_{3,15} = 22.38$, $P < 0.001$; *Arc*: $F_{3,15} = 16.34$, $P < 0.001$; *Egr1*: $F_{3,15} = 12.55$, $P < 0.001$; *Egr2*: $F_{3,15} = 9.72$, $P = 0.001$; *Fos*: $F_{3,6} = 60.71$, $P < 0.001$). In contrast, H2A.Z incorporation for the memory suppressor *Ppp3ca* increased at the +1 nucleosome ($F_{3,9} = 5.83$, $P = 0.02$) when gene expression was reduced ($F_{3,17} = 4.07$, $P = 0.03$) (Fig. 1 and Extended Data Fig. 2), suggesting that H2A.Z at the +1 nucleosome restricts transcription. These findings are consistent with reports of stimulus-induced H2A.Z eviction^{16–20} and evidence for the +1 nucleosome acting as a transcriptional barrier^{15,21}. Given that our data are normalized to histone H3 to correct for potential changes in nucleosome occupancy, we conclude that H2A.Z eviction in particular is associated with activity-induced gene expression.

At the -1 nucleosome, H2A.Z binding increased for both memory-promoting and memory-suppressing genes (*Npas4*: $F_{3,8} = 12.89$, $P = 0.002$; *Egr1*: Welch's $F_{3,3.39} = 9.18$, $P = 0.04$; *Egr2*: $F_{3,8} = 7.47$, $P = 0.01$; *Fos*: $F_{3,8} = 8.23$, $P = 0.008$; *Ppp3ca*: $F_{3,8} = 9.20$, $P = 0.004$) at 30 min, irrespective of changes in gene expression (Fig. 1 and Extended Data Fig. 2). Various studies have associated H2A.Z binding in the -1 nucleosome with steady-state gene activity^{11,12}, but our data suggest that stimulus-induced changes in H2A.Z binding do not correlate with transcription at this time point.

H2A.Z binding returned to baseline levels within 2 h, except for a delayed increase in *Bdnf* exon IV expression ($F_{3,17} = 15.09$, $P < 0.001$) and a concomitant reduction in H2A.Z binding at the +1 nucleosome ($F_{3,18} = 3.72$, $P = 0.03$) (Extended Data Fig. 2). Of note, H2A.Z was evicted in context-only mice, even though gene expression increased only with context and shock pairing. This may reflect the 2 h time point, since *Bdnf IV* expression is typically elevated 1 h after training⁴.

Indeed, the association between gene expression and H2A.Z binding was no longer evident 2 h after training. Whereas H2A.Z binding returned to baseline, gene expression remained elevated (*Arc*: Welch's $F_{3,9.27} = 12.16$, $P = 0.001$; *Egr1*: Welch's $F_{3,8.25} = 6.68$, $P = 0.01$; *Egr2*: Welch's $F_{3,4.77} = 11.13$, $P = 0.01$; *Fos*: $F_{3,17} = 5.54$, $P = 0.008$). For *Ppp3ca*, H2A.Z binding increased 2 h after training at -1 ($F_{3,10} = 28.35$, $P < 0.001$) and +1 ($F_{3,10} = 4.10$, $P = 0.04$) nucleosomes, even though gene expression returned to baseline. Thus, H2A.Z exchange is uncoupled from gene expression during the late stages of transcription,

consistent with evidence that H2A.Z exchange is primarily involved in transcription initiation¹⁸.

H2A.Z has been associated with both positive and negative effects on transcription^{11,12,22}, with acetylation having a positive effect^{16,17,23}. Using acetylation as an indirect index of the transcriptional impact of H2A.Z, we found that 30 min after training, when H2A.Z exchange is most pronounced, acetylated H2A.Z (H2A.Zac) binding increased at the -1 nucleosome of *Egr1* ($F_{3,8} = 11.07$, $P = 0.03$) and *Fos* ($F_{3,8} = 3.92$, $P = 0.05$). Consistent with H2A.Z eviction from the +1 nucleosome at 30 min, a subset of genes also exhibited reduced acetylation at the +1 nucleosome at this time point (*Egr2*: $F_{3,8} = 4.03$, $P = 0.05$; *Egr1*: $F_{3,8} = 14.13$, $P = 0.001$) (Extended Data Fig. 3).

To directly investigate the involvement of H2A.Z in memory, we conducted adeno-associated virus (AAV)-mediated H2A.Z depletion in the pyramidal cell layer in the dorsal CA1 region of the hippocampus (Fig. 2a, b). The construct effectively reduced H2A.Z messenger RNA ($t_{17} = -4.76$, $P < 0.0001$) and produced a 55.8% reduction in H2A.Z protein levels (Fig. 2c). H2A.Z depletion was associated with improved fear memory, as evidenced by increased freezing 24 h ($t_{30} = 2.28$, $P = 0.04$) and 30 days ($t_9 = -2.31$, $P = 0.05$) after training, compared to mice injected with a scramble control (Fig. 2d, e).

To investigate the basis for improved memory, we quantified the effect of H2A.Z depletion on training-induced gene expression. H2A.Z depletion increased *Bdnf* exon IV (virus (scramble or *H2afz*)–training (naive or fear conditioned) interaction ($F_{1,17} = 8.31$, $P = 0.01$)) and *Arc* (virus–training interaction; $F_{1,17} = 6.03$, $P = 0.025$) expression 30 min after training, whereas the expression of other memory-promoting genes increased only as a function of fear conditioning, irrespective of H2A.Z manipulation (main effect of training (*Npas4*: $F_{1,12} = 7.12$, $P = 0.02$; *Egr1*: $F_{1,17} = 16.23$, $P = 0.001$; *Egr2*: $F_{1,12} = 38.53$, $P < 0.001$; *Fos*: $F_{1,12} = 93.69$, $P < 0.001$)). The memory suppressor genes *Ppp3ca* and *Ppp1cc* were not altered by training or by H2A.Z manipulation, suggesting that the effects of H2A.Z depletion are gene-specific (Fig. 2g–n). These data are consistent with evidence that H2A.Z depletion increases gene expression^{19,20,22}, but it is not clear which specific genes account for the memory-enhancing effects of H2A.Z depletion.

To examine the genome-wide transcriptional impact of H2A.Z depletion, we performed directional, poly(A)⁺ RNA sequencing. H2A.Z knockdown altered baseline expression of 451 genes (Fig. 3a and Supplementary Table 1). In H2A.Z-depleted mice, fear conditioning altered the expression of 202 genes (Fig. 3b and Supplementary Table 2), including a number of the early learning-related genes identified via quantitative real-time PCR (qPCR; *Arc*, *Fos*, *Egr1* and *Egr2*; Fig. 3c, e). Thus, whereas H2A.Z knockdown altered the baseline expression of 153 genes that are affected by training, our whole-genome sequencing results are consistent with a lack of H2A.Z involvement in baseline expression of known memory-related genes (for example, *Arc*). Gene ontology analysis of training-induced changes identified genes involved in sequence-specific DNA binding and DNA regulation (Fig. 3d), consistent with the rapid transcriptional role of H2A.Z following learning.

Whereas initial memory consolidation is dependent on the hippocampus, epigenetic modifications in the cortex are implicated in systems consolidation and memory maintenance^{2,6}. In contrast to the hippocampus, we did not find differences in cortical H2A.Z expression after fear conditioning (Extended Data Fig. 4). However, H2A.Z binding at the +1 nucleosome was reduced 2 h after training (*Arc*: $F_{3,12} = 4.05$, $P = 0.03$; *Egr1*: $F_{3,12} = 3.53$, $P = 0.049$; *Egr2*: $F_{3,12} = 5.36$, $P = 0.01$), whereas H2A.Z binding increased at the +1 nucleosome of the memory suppressor *Ppp3ca* ($F_{3,12} = 4.06$, $P = 0.03$). Changes in H2A.Z binding at the -1 nucleosome were found only for *Ppp3ca* ($F_{3,12} = 13.84$, $P < 0.001$), where less H2A.Z was present at 2 h (Extended Data Figs 5 and 6). Training-induced H2A.Z eviction at 2 h implicates H2A.Z in the early stages of systems consolidation.

At 7 days, when memory becomes increasingly dependent on the cortex, H2A.Z binding increased at the -1 nucleosome of memory-promoting genes (*Arc*: $F_{3,21} = 3.03$, $P = 0.05$; *Egr1*: $F_{3,12} = 5.46$, $P = 0.01$; *Egr2*: $F_{3,12} = 3.66$, $P = 0.04$; *Bdnf IV*: $F_{3,12} = 4.21$, $P = 0.03$) and the -1 nucleosome of the memory suppressor *Ppp3ca* ($F_{3,21} = 5.98$, $P = 0.004$). These changes were no longer evident at 30 days (Extended Data Figs 5 and 6), indicating that TSS-flanking H2A.Z is associated with systems consolidation, but perhaps not with memory maintenance, consistent with a recent study of cortical histone acetylation².

In contrast to observations of cortical H2A.Z exchange at 2 h, we did not find differences in H2A.Zac binding at this time (Extended Data Fig. 7). At 7 days, H2A.Zac binding was reduced at a subset of -1 (*Egr1*: $F_{3,11} = 4.96$, $P = 0.02$; *Egr2*: $F_{3,11} = 3.58$, $P = 0.05$; *Bdnf IV*: $F_{3,11} = 6.83$, $P = 0.007$; *Ppp3ca*: $F_{3,11} = 4.05$, $P = 0.03$; *Ppp1cc*: $F_{3,11} = 3.57$, $P = 0.05$) and +1 (*Egr1*: $F_{3,11} = 3.59$, $P = 0.046$; *Bdnf IV*: $F_{3,11} = 4.17$, $P = 0.03$; *Ppp1cc*: $F_{3,11} = 3.56$, $P = 0.05$) nucleosomes (Extended Data Fig. 8). Although we cannot conclude with certainty that H2A.Z binding at these loci is repressive, reduced H2A.Zac binding suggests that an activity-associated modification^{14,16,23} is removed during systems consolidation in the cortex.

Next, we infused H2A.Z AAV into the medial pre-frontal cortex (mPFC; Fig. 4a) and confirmed a reduction in *H2afz* mRNA, as well as a 68.34% reduction in protein levels (Fig. 4b, c). H2A.Z depletion did not affect fear memory at the hippocampus-dependent 24 h time point, whereas significantly higher freezing was observed in H2A.Z-depleted mice at the two remote time points (30 days: $t_{14} = -5.28$, $P < 0.0001$; and 7 days: $t_8 = -3.07$, $P = 0.02$) (Fig. 4d–f). In separate mice, H2A.Z knockdown enhanced *Fos* expression irrespective of training 30 min after fear conditioning (main effect of virus: $F_{1,12} = 4.77$, $P = 0.049$) and reduced the expression of *Ppp3ca* (training–virus interaction: $F_{1,12} = 16.28$, $P < 0.002$) in untrained H2A.Z knockdown compared to untrained scrambled mice. The expression of remaining genes increased only as a function of fear conditioning (*Npas4*: $F_{1,12} = 108.65$, $P < 0.001$; *Arc*: $F_{1,12} = 156.00$, $P < 0.001$; *Egr1*: $F_{1,12} = 10.73$, $P = 0.007$; *Egr2*: $F_{1,12} = 115.03$, $P < 0.001$, *Fos*: $F_{1,12} = 63.77$, $P < 0.001$) (Fig. 4h–o). Although the virus was present throughout the consolidation and maintenance stages, the emergence of memory enhancement at 7 days, and altered *Fos* and *Ppp3ca* expression at 30 min, are consistent with an early role in systems consolidation.

Overall, H2A.Z has a restrictive effect on recent and remote memory. However, consistent with the wide genomic distribution of H2A.Z¹¹, our sequencing data demonstrate that its depletion both up- and downregulates 451 different genes, making it difficult to ascertain specific targets through which H2A.Z regulates memory.

While our data clearly indicate that H2A.Z is dynamically regulated during learning and memory, the basis for H2A.Z regulation in the central nervous system is not known and indeed, its regulation is not fully understood in any biological system. Recent studies identified DNA methylation, sirtuin 1 and H3 acetylation at lysine 56^{24–26} as negative regulators of H2A.Z. These factors have a known role in memory^{3,7,27} and represent potential regulators of H2A.Z in fear conditioning. Notably, many changes in H2A.Z binding were not specific to associative learning, although H2A.Z exchange specificity was evident at numerous genes in the cortex and a subset of genes in CA1. Thus, although H2A.Z has the capacity for specific regulation of associative learning, its exchange is also sensitive to broader environmental stimuli.

Overall, we show that H2A.Z is a novel regulator of memory and introduce histone variant exchange as an additional epigenetic contributor to the complex coordination of gene expression in memory. Further, our data suggest that H2A.Z antagonists may provide a novel therapeutic target for memory disorders.

METHODS

Animals

Male C57BL/6J mice (Jackson Laboratories) of approximately 9–12 weeks of age were used for the experiments. Mice were pair housed upon arrival and food and water were available *ad libitum*. The mice were given at least one week to habituate to the colony before cage mates were randomly assigned to the behavioural treatment group, such that mice in the same cage always belonged to the same test group. All protocols complied with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Alabama Animal Care Committee. All animals were handled for 4 days before fear conditioning.

Fear conditioning

For ChIP and gene expression experiments, mice were placed into the training chamber and given 2 min to explore the novel context. After 2 min, mice in the context plus shock (CS) group received 3 electric foot-shocks (0.7 mA, 2 s duration) administered 1 min apart, with an additional minute allowed for exploration before removal from the chamber. Mice in the context-only (C) group were exposed to the same procedure without foot-shock delivery, whereas shock-only (S) controls were given foot-shocks in rapid succession in the dark to avoid contextual learning. Naive (N) mice were used as untrained controls. Freezing behaviour was scored by unbiased automated software (Video Freeze, Med Associates Inc.) and confirmed manually by an experimenter blind to group assignment. Brains were collected 30 min, 2 h, 7 days, or 30 days after training and processed for ChIP.

For AAV-injected mice, a milder fear conditioning paradigm was used. For mice with AAV injections in area CA1, the procedure consisted of 3 min of exploration and a single foot-shock (0.5 mA, 2 s duration), with an additional 30 s for exploration. This protocol was increased to include 3 shocks for gene expression experiments. To ensure the development of a persistent memory trace over 30 days, mice with AAV injections in the mPFC were trained using a moderate protocol consisting of two foot-shocks (0.5 mA, 2 s duration). For behavioural testing, mice were returned to the testing chamber for 4 min and freezing behaviour was measured as an index of fear memory 24 h or 30 days after training. Mice that received intra-cortical AAV injections and were tested 24 h after training were re-tested 7 days after training for the assessment of memory at the transition between recent and remote memory.

We confirmed that H2A.Z depletion did not produce non-specific effects on behaviour, as demonstrated by normal locomotor activity, movement velocity, vertical activity and time spent in the centre of an open field, a commonly used index of anxiety (Extended Data Fig. 9).

Stereotaxic surgery for viral delivery

Viral knockdown of H2A.Z was achieved using a commercially available and validated *H2afz* short hairpin RNA (shRNA) packaged in AAV2/9 (3.7×10^{13} genome copies per ml^{-1}) fused to the U6 promoter and labelled with enhanced green fluorescent protein (eGFP; driven by a CMV promoter; Vector Biolabs). *H2afz* is the primary of two genes coding for H2A.Z and both produce protein products indistinguishable with our antibodies. The following shRNA sequence was employed:

CCGGCGACACCTGAAATCTAGGACACTCGAGTGTCTAGATTTCAGGTGTCGTT
TTTG. A commercially available scramble shRNA expressing eGFP was used as a control (Vector Biolabs).

Mice were anaesthetized with isoflurane and secured in a Kopf stereotaxic apparatus. Viral particles were bilaterally delivered into the CA1 (anterior/posterior (AP) -1 ; medial/lateral (ML) ± 1.5 ; dorsal/ventral (DV) -1.6 ; $1.5 \mu\text{l}$ per hemisphere) or the anterior cingulate cortex (AP $+1.9$; ML ± 0.4 ; DV -1.8 ; $1 \mu\text{l}$ per hemisphere) at a rate of 225 nl min^{-1} , with 2 weeks allowed for recovery. These parameters were selected to avoid an altered behavioural profile observed with delivery of larger volumes. Only mice with correctly targeted injections were included in the analysis. Stereotaxic coordinates were based on Paxinos and Franklin²⁸ and are depicted in Figs 1a and 4a.

Immunohistochemistry

Mice were perfused with 10% formalin and embedded in paraffin. Paraffin-embedded slides were dried at $55-60^\circ\text{C}$ for one hour in the oven and put through sequential 5 min washes with xylene, 100%, 95%, 70%, 50% and 30% ethanol, water and PBS. For antigen retrieval, slides were immersed in 10 mM sodium citrate (pH6.0) in a plastic copland jar for 11 min in the microwave at 95°C (550 W). After cooling to room temperature in a running cold water bath, sections were put through a sequential rinse in PBS with 1% H_2O_2 , and again in PBS before a 1 h blocking step (10% normal goat serum, 0.3% TX-PBS, 1% bovine serum

albumin). Sections were incubated in 1:200 concentration of H2A.Z antibody (Cell Signaling Technology catalogue no. 2718S) overnight at room temperature in humidified chamber with 5% normal goat serum with 0.3% TX-PBS and 1% bovine serum albumin. The next day, sections were washed with PBS before incubating with secondary anti-rabbit antibody (1:500). For DAB (3,3'-Diaminobenzidine) staining, sections were rinsed with PBS, then incubated in ABC (avidin–biotin–peroxidase complex) for 1 h at room temperature, rinsed with PBS, immersed in DAB and rinsed with PBS. The stained sections were dehydrated with ethanol, then placed in xylene and coverslipped with DPX (di-*N*-butyle phthalate in xylene; neutral mounting medium). For fluorescent staining, sections were incubated in AlexaFluor 594 for 2 h at room temperature, rinsed in PBS and coverslipped with mounting media.

Chromatin immunoprecipitation (ChIP)

Brains were collected 30 min or 2 h (CA1), or 2 h, 7 days or 30 days (mPFC) after training and kept at -80°C until processing. Samples were cross-linked with 1% formaldehyde, washed six times with ice-cold PBS and a cocktail of protease inhibitors (Roche). Samples were then homogenized in 500 μl of SDS lysis buffer (50 mM Tris, pH 8.1, 10 mM EDTA, 1% SDS and protease inhibitor tablets (Roche)) and chromatin was sheared by sonication at 40% power (6 times for 10 s, 50 s rest between sonications). Lysate was centrifuged at 5,500 relative centrifugal force for 5 min at 4°C , aliquoted, and diluted to 1:10 with ChIP dilution buffer (EMD Millipore). The aliquots were treated with 25 μl of Magna ChIP Protein G magnetic beads (EMD Millipore) and 4 μl of H2A.Z (Cell Signaling Technology catalogue no. 2718S), H2A.Zac (Abcam catalogue no. ab18262) or H3 (Cell Signaling Technology catalogue no. 2650S) antibody and incubated overnight at 4°C . The next day, beads were pelleted with a magnetic separator and washed sequentially with low-salt, high-salt, LiCL immune complex and TE buffers (EMD Millipore). Immune complexes were extracted using $1 \times$ TE buffer and proteinase K (EMD Millipore ChIP kit) and heating at 65°C for 2 h, followed by 95°C for 10 min. Samples were purified with the Qiagen PCR cleanup kit and quantified using PCR.

The PCR primers were designed to span the -1 nucleosome (approximately -80 to -240 base pairs from the TSS) or the $+1$ (approximately $+25$ to $+171$ base pairs from TSS), based on published descriptions of nucleosome positioning in the liver and the brain¹¹. The list of primer sequences can be found in Extended Data Table 1. ChIP data for H2A.Z and H2A.Zac were each normalized to H3 to control for potential changes in nucleosome occupancy.

mRNA expression and real-time PCR

RNA was extracted using the RNeasy Plus Mini Kit (Qiagen) and complementary DNA was synthesized using iScript (BioRad). *H2afz*, *Ppp3ca*, *Ppp1cc* and *Egr2* mRNA was quantified using pre-designed probes available from Applied Biosystems, using β -actin and HPRT as a control. *Arc*, *Egr1*, *Bdnf IV*, *Fos*, *Npas4*, as well as *HPRT* and *Rpl13a* (control genes) were designed in-lab and ordered from IDT. The list of primer sequences can be found in Extended Data Table 1.

Methylated DNA immunoprecipitation

Brains were collected 30 min after training and kept at -80°C until processing. DNA was extracted using the QIAamp DNA Micro Kit (Qiagen). Two μg of DNA was diluted in 800 μl of buffer EB (Qiagen PCR cleanup kit) and sonicated at 40% power (8 times for 10 s, 30 s rest between sonications). Sonicated DNA was incubated at 95°C for 15 min and used as input control. Three-hundred μl of DNA was diluted in 300 μl of IP dilution buffer (EMD Millipore), incubated with 4 μl of 5mC antibody (Epigentek catalogue no. A1014) and 25 μl of Protein A beads (Invitrogen) at 4°C for 1 h. The beads were pelleted with a magnetic separator and washed with $1 \times$ bind/wash buffer 2 times for 3 min each. After the final wash, beads were re-suspended in 100 μl $1 \times$ TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0 with 1% SDS and Proteinase K) and heated at 65°C for 2 h, then at 95°C for 10 min. The beads were again pelleted with a magnetic separator and the DNA was purified using the Qiagen PCR cleanup kit and quantified using PCR.

Western blots

Histones were extracted from snap-frozen tissue using the EpiQuick Total Histone extraction kit (Epigentek) and the 8 μg of the extracts were resolved on a 15% gel at 75 V and transferred to a PVDF membrane at 75 V for 2 h. The membrane was blocked in Li-Cor Odyssey blocking buffer for 1 h at room temperature and incubated in H2A.Z primary antibody (1:1,000, Cell Signaling) at 4°C overnight. The next day, membranes were washed 3 times in 0.1% TBS-T and incubated for 1 h in goat anti-rabbit AlexaFluor 800 (1:15,000, Li-Cor), washed 3 times in 0.1% TBS-T and imaged on the Li-Cor Odyssey fluorescent imaging system. The membranes were then incubated in actin primary antibody (1:2,000, Abcam) for 1 h at room temperature, then in goat anti-mouse AlexaFluor 800 for 1 h before washing in 0.1% TBS-T and imaging with the Licor Odyssey.

RNA-sequencing

RNA-sequencing experiments were carried out in collaboration with the Hudson Alpha Genome Services Laboratory. RNA from three biological replicates per condition was extracted, DNase-treated, and purified (RNeasy, Qiagen). One μg of total RNA underwent quality control (Bioanalyzer; all RIN values >7.9), and was prepared for directional poly(A)⁺ sequencing at Hudson Alpha using NEBNext reagents (New England Biolabs) according to the manufacturer's recommendations with minor modifications (including the use of custom library adapters and indexes). RNA libraries were quantified (Kapa Library Quant Kit, Kapa Biosystems) and underwent sequencing (25 million total 50-base-pair paired-end reads) on an Illumina sequencing platform (HiSeq2000).

RNA-seq Data Analyses—Raw paired-end sequenced reads were quality controlled, filtered for read quality (FASTX toolkit, Galaxy) and aligned to the mouse genome (mm 10 assembly) in Galaxy using TopHat v1.4.0 (with custom settings $-p\ 8 -r\ 175$). Genome-aligned sequenced reads were examined using Cufflinks and CuffDiff modules in Galaxy, using RefSeq gene identification from the UCSC table browser. For each sample, gene/transcript expression levels were determined by computing the fragments per kilobase of exon per million mapped reads (FPKM) independently for each sample using Cufflinks. All

values were quartile-normalized to improve expression estimates of low-abundance transcripts. Gene expression differences between groups (that is, scrambled vs H2a.Z knockdown) were calculated in CuffDiff using all replicates for each group and per-condition dispersion modelling. Significance testing was conducted on genes with a minimum of 100 aligned reads in a locus, and statistical significance was assessed using Student's *t*-tests and a false-discovery rate of 0.05. *H2afz* knockdown was confirmed with qPCR ($t_7 = -2.43$, $P = 0.045$). Gene ontology analysis of significantly altered genes was conducted using DAVID v6.7, via the WebGestalt portal, with Benjamini–Hochberg false-discovery rate applied to correct for multiple comparisons. The raw data can be accessed through the GEO database, accession number GSE58797.

Statistics

Sample sizes were determined using freely available online power analysis software (<http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html>), assuming a moderate effect size of 0.5.

Analyses were conducted using one-way and two-way analyses of variance (ANOVA). Follow-up analyses were conducted using Fisher's least significant difference and independent samples *t*-tests, where appropriate. The *P* value for all cases was set to 0.05 and follow-up analyses for specific comparisons were only conducted when the omnibus ANOVA was significant, given that ANOVA is robust to potential violations of normality. Homogeneity of variance was confirmed using Levene's test for equality of variances. When the assumption was violated, comparisons were conducted using Welch ANOVA and significant results were followed up using Games-Howell post hoc tests, which is appropriate for unequal variances, unequal group sizes and small sample sizes. The use of Welch ANOVA is specified next to the *F* value for the appropriate comparison in the text. All of the comparisons were conducted using two-tailed tests of significance.

Extended Data

Extended Data Figure 1. Hippocampal H2A.Z is expressed throughout the hippocampus and is inhibited 30 min after fear conditioning

a, b, Chromogenic staining of H2A.Z (**a**) and negative control (**b**). **c**, Fluorescent staining of H2A.Z (red) and DAPI (blue) shows H2A.Z distribution in CA1 and dentate gyrus (DG). **d**, **e**, *H2afz* mRNA expression (**d**; n mice per group: N = 4; C = 7; S = 2; CS = 5) and H2A.Z protein expression (**e**; n mice per group: N = 3; C = 3; S = 4; CS = 3) 30 min after training. **f**, DNA methylation at the *H2afz* promoter 30 min after fear conditioning (n mice per group: N = 7; C = 9; S = 5; CS = 4). N, naive; C, context; S, shock; CS, context plus shock. Data expressed as mean $\pm$ s.e.m. *Follow-up comparisons with $P < 0.05$.

Extended Data Figure 2. H2A.Z exchange in CA1

H2A.Z binding at -1 nucleosome (first column for each time point) and +1 nucleosome (second column for each time point) of *Egr1*, *Fos*, *Bdnf IV* and *Ppp1cc* 30 min (left; *n* mice per group for *Egr1* and *Bdnf IV*: *N* = 7; *C* = 5; *S* = 4; *CS* = 6; *Ppp3ca*: *N* = 4, *C* = 3, *S* = 3, *CS* = 3; *n* mice per group for *Fos* and *Ppp1cc*: *N* = 4, *C* = 3, *S* = 3, *CS* = 3) or 2 h (right; *n* mice per group: *N* = 10; *C* = 2; *CS* = 4; *S* = 6) after training. Gene expression is shown in the third column for each time point (*n* mice per group: *N* = 5, *C* = 6; *S* = 2; *CS* = 6; for *Fos* and *Ppp1cc*: *N* = 3; *C* = 3; *S* = 2; *CS* = 2). Data are expressed as mean $\pm$ s.e.m. *Follow-up comparisons with *P* < 0.05.

Extended Data Figure 3. Acetylated H2A.Z binding at the -1 and +1 nucleosomes 30 min after fear conditioning in CA1

H2A.Zac binding was investigated at the -1 nucleosome (displayed in the first column for each set of genes) and the +1 nucleosome (displayed in the second column for each set of genes) of *Npas4*, *Egr2*, *Arc* and *Ppp3ca* (left) and *Egr1*, *Fos*, *Bdnf IV* and *Ppp1cc* genes (right) 30 min after fear conditioning. *n* mice per group: N = 3, C = 2; S = 4; CS = 3. N, naive; C, context; S, shock; CS, context plus shock. Data are expressed as mean $\pm$ s.e.m.

*Follow-up comparisons with $P < 0.05$.

Extended Data Figure 4. H2A.Z expression in the mPFC after training

a, b, *H2afz* expression was investigated in the mPFC 30 min (**a**; *n* mice per group: N = 2; C = 3; S = 3; CS = 3) or 2 h (**b**; *n* mice per group: N = 8; C = 5; S = 4; CS = 8) after fear conditioning. N, naive; C, context; S, shock; CS, context plus shock. Data are expressed as mean $\pm$ s.e.m.

Extended Data Figure 5. H2A.Z exchange in the mPFC
H2A.Z binding was investigated at the –1 nucleosome (displayed in the first column for each time point) and the +1 nucleosome (displayed in the second column for each time point) of *Egr1*, *Egr2*, *Arc* and *Ppp3ca* genes 2 h (left; *n* mice per group: N = 4; C = 4; S = 3; CS = 5), 7 days (middle; *n* = 4 mice per group; *n* for –1 *Arc* and +1 *Ppp3ca*: N = 7; C = 6; S = 4; CS = 8) or 30 days (right; *n* mice per group: N = 2; C = 3; S = 3; CS = 3) after fear conditioning. N, naive; C, context; S, shock; CS, context plus shock. Data are expressed as mean ± s.e.m. *Follow-up comparisons with *P* < 0.05.

Extended Data Figure 7. Acetylated H2A.Z binding at the -1 and +1 nucleosomes 2 h after fear conditioning in the mPFC
H2A.Zac binding was investigated at the -1 nucleosome (displayed in the first column for each set of genes) and the +1 nucleosome (displayed in the second column for each set of genes) of *Egr1*, *Egr2*, *Arc* and *Ppp3ca* (left) and *Npas4*, *Fos*, *Bdnf IV* and *Ppp1cc* genes (right) 2 h after fear conditioning; *n* mice per group: N = 2; C = 4; S = 3; CS = 5. N, naive; C, context; S, shock; CS, context plus shock. Data are expressed as mean $\pm$ s.e.m. *Follow-up comparisons with $P < 0.05$.

Extended Data Figure 8. Acetylated H2A.Z binding at the –1 and +1 nucleosomes 7 days after fear conditioning in the mPFC

H2A.Zac binding was investigated at the –1 nucleosome (displayed in the first column for each set of genes) and the +1 nucleosome (displayed in the second column for each set of genes) of *Egr1*, *Egr2*, *Arc* and *Ppp3ca* (left) and *Npas4*, *Fos*, *Bdnf IV* and *Ppp1cc* genes (right) 7 days after fear conditioning; *n* mice per group: N = 4; C = 3; S = 4; CS = 4. N, naive; C, context; S, shock; CS, context plus shock. Data are expressed as mean $\pm$ s.e.m.

*Follow-up comparisons with $P < 0.05$.

Extended Data Figure 9. Open field test in mice receiving intra-cortical scramble or H2A.Z AAV

a, Summary of experimental design. **b**, There were no differences in locomotor activity between H2A.Z mice and scramble controls. **c**, No group differences were found in movement velocity. **d**, No differences were found in vertical activity. **e**, There were no differences in the time spent in the centre, a widely used index of anxiety ($n = 8$ mice per group). Data are expressed as mean $\pm$ s.e.m.

Extended Data Table 1

The list of qPCR primers for genomic and complementary DNA

Nucleosome	Gene	gDNA	
		Forward	Reverse
+1	<i>Egr1</i>	CCCCTGCCCCAGCCT	CACTGCGGGGAGTGTAGGT
+1	<i>Egr2</i>	CGAGGGGACACACTGACTG	ACTGACTCTCTCCTGCCTG
+1	<i>Bdnf IV</i>	GCGCGGAATTCTGATTCTGGTAAT	GAGAGGGTCCACGCTGCCTTGACG
+1	<i>Arc</i>	TGCCACACTCGCTAAGCTCC	AACTCCTCTGAGGCAGAAGCC
+1	<i>Fos</i>	AGTGTCTACCCCTGGACCC	GCGTTGAAACCCGAGAACATC
+1	<i>Npas4</i>	AGCAAGAGCCTGAGCGAAAA	AGCACCTGCGATCCTTTCC
+1	<i>Ppp3ca</i>	CTGGAGATGTCCGAGCCCAA	GCTTACCTTTACCACCCTGT
+1	<i>Ppp1cc</i>	GGCGGCCATCTTGTCTCTCT	GCCACGAGCCCCACG
-1	<i>Egr1</i>	ACTGCTGCTGTCCAATACTAGG	CATCCAAGAGTGGTGGGCA
-1	<i>Egr2</i>	CTGCAAATCGTTCCTGGCG	CAGCTTTTGCCGTACATGG
-1	<i>Bdnf IV</i>	CCAGAACCTAGTCATGTAACATGAT	GTGGGTAGCTCACTAAGCCC
-1	<i>Arc</i>	TCCCGGTGGGAGGCG	GTGCCCTCAAGGACCCG
-1	<i>Fos</i>	AGGAGACCCCTAAGATCCC	CTGTCGTCAACTCTACGCCC
-1	<i>Npas4</i>	GAAGTCTGGGAGGGAGGAGG	GGAGGTGGGCTAAAGCAA
-1	<i>Ppp3ca</i>	GCCCCGTCCCCAAGAATAAA	CGCGTGTGTGCTGGTTATTT
-1	<i>Ppp1cc</i>	TCTATTTCCCCCGCCGTTT	GCGGAGACGGTTGAGCG
-1	<i>H2afz</i>	ACTCCGCTGTGCGTTCTC	ACCAATGGTTGCCTCCCG

	Gene	cDNA	
		Forward	Reverse
	<i>Egr1</i>	AGCGCCTTCAATCCTCAAG	TTTGCTGGGATAACTCGTC
	<i>Bdnf IV</i>	CCAGAGCAGCTGCCTTGATGTTA	TGCCTTGTCCTGGACGTTACTT
	<i>Arc</i>	ACGATCTGGCTTCCTCATCTGCT	AGGTTCCTCAGCATCTCTGCTTT
	<i>Fos</i>	AATGGTGAAGACCGTGTGAGGA	TTGATCTGTCTCCGCTGGAGTGT
	<i>Hprt1</i>	GGAGTCCTGTTGATGTTGCCAGTA	GGGACGCAGCAACTGACATTTCTA
	<i>Rpl13a</i>	ATGTCCCCTCTACCCACAG	TGAACCAATAAAGACTGTTTGC

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 2. H2A.Z depletion in CA1

a, Design of behavioural experiments. **b**, AAV spread. **c**, *H2afz*-AAV knockdown (Scrambled (scr) $n = 9$; H2A.Z $n = 10$). **d**, **e**, *H2afz*-AAV enhanced memory 24 h (**d**; Scr $n = 18$; H2A.Z $n = 14$) and 30 days (**e**; Scr $n = 5$; H2A.Z $n = 6$) after training. **f**, Design of gene expression experiments. **g–n**, Effect of *H2afz*-AAV on mRNA levels of memory-associated genes in untrained and trained mice (n mice per group for *Fos*, *Egr2*, *Ppp3ca*, *Ppp1cc*, *Npas4*: scr, untrained $n = 3$; scr, trained $n = 3$; H2A.Z, untrained $n = 7$; H2A.Z, trained $n = 7$; n per group for *Bdnf IV*, *Egr1* and *Arc*: scr, untrained $n = 6$; scr, trained $n = 3$; H2A.Z, untrained $n = 8$; H2A.Z, trained $n = 4$). Data expressed as mean $\pm$ s.e.m. *Follow-up comparisons with $P < 0.05$.

Figure 3. RNA sequencing data depicting genome-wide transcriptional impact of AAV-mediated H2A.Z depletion

a, A comparison of untrained animals two weeks after stereotaxic delivery of scrambled or H2A.Z AAV into CA1. **b**, Comparison of gene expression in mice receiving H2A.Z AAV with and without fear conditioning. Samples were taken 30 min after training. **c**, Venn diagram depicting the overlap between genes mediated by H2A.Z AAV at baseline and in response to fear conditioning. **d**, Gene ontology analysis of differentially expressed genes in untrained scrambled versus H2A.Z AAV and of H2A.Z AAV mice with and without training. **e**, Example of individual genes modified by training. FPKM, fragments per kilobase of exon per million mapped reads; kb, kilobase. *n* = 3 mice per group.

Figure 4. H2A.Z depletion in mPFC

a, Experimental design for behaviour. **b**, AAV spread. **c**, H2A.Z-AAV reduced H2A.Z protein and mRNA expression ($t_3 = 6.91$, $P = 0.006$; scr $n = 2$; H2A.Z $n = 3$). **d-f**, Freezing behaviour at 30 days (**d**; $n = 8$ mice per group), 7 days (**e**; $n = 5$ mice per group) or 24 h (**f**; $n = 5$ mice per group) after training. **g**, Experimental design for mRNA measurement. **h-o**, Effect of AAV treatment on mRNA levels of memory-associated genes in untrained (naive) and fear-conditioned mice. (n mice per group: scr, untrained $n = 5$; scr, trained $n = 3$;

H2A.Z, untrained $n = 4$; H2A.Z, trained $n = 4$). Data are expressed as mean $\pm$ s.e.m.

*Follow up comparisons with $P < 0.05$.

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