**Materials and Methods**

**Mice:** 10-12 week old C57BL/6 male mice (Taconic Farm) were housed four per cage, maintained on a 12hr light/dark time schedule, and allowed free access to food and water. All testing took place during the light phase in red-light-illuminated testing rooms following protocols approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

**DNA/RNA Extraction:** Tissue derived from the infralimbic prefrontal cortex of FC-No EXT or EXT trained mice was homogenized by Dounce tissue grinder in 500 ul cold 1X DPBS (Gibco). 400 ul of homogenate was used for DNA extraction, and 100 ul was used for RNA extraction. DNA extraction was carried out using DNeasy Blood & Tissue Kit (Qiagen) with RNAse A (5 prime), RNAse H and RNAse T1 treatment (Invitrogen), and RNA was extracted using Trizol reagent (Invitrogen). Both extraction protocols were followed according to the manufacturer’s instructions. The concentration of DNA and RNA was measured by Qubit assay (Invitrogen).

**LC-MS/MS.** Genomic DNA was enzymatically hydrolyzed to deoxynucleosides by the addition of benzonase (25 U, Santa Cruz Biotech), nuclease P1 (0.1 U, Sigma-Aldrich), and alkaline phosphatase from *E.coli* (0.1 U, Sigma-Aldrich) in buffer 10 mM ammonium aetate pH 6.0, 1 mM MgCl2, and 0.1 mM erythro-9-(2-hydroxy-3-nonyl)adenine. After 40 min incubation at 40 °C the samples were added 3 volumes of acetonitrile and centrifuged (16,000 g, 30 min, 4 °C). The supernatants were dried and dissolved in 50 µl 5% methanol in water (v/v) for LCMS/MS analysis of modified and unmodified nucleosides. Chromatographic separation was performed on a Shimadzu Prominence HPLC system, for m6dA and unmodified deoxynucleosides by means of an Ascentis Express F5 150 x 2.1 mm i.d. (2.7 µm) column equipped with an Ascentis Express F5 12.5 × 2.1 mm i.d. (2.7 µm) guard column (Sigma-Aldrich). The mobile phase consisted of water and methanol (both added 0.1 % formic acid), for m6dA starting with a 4-min gradient of 5-50 % methanol, followed by 6 min re-equilibration with 5% methanol, and for unmodified deoxynucleosides maintaining isocratically with 30% methanol. The mobile phase consisted of 5 mM acetic acid and methanol, starting with a 3.5-min gradient of 5-70% methanol, followed by 4 min re-equilibration with 5% methanol. Mass spectrometry detection was performed using an API5500 triple quadrupole (AB Sciex) operating in positive electrospray ionization mode for m6dA and unmodified deoxynucleosides, or negative mode for (m6dA), 257.1/141.1.

**Dot Blot:** 100 ng of total DNA was diluted with 0.1 N NaOH (Sigma) into 2 ul and spotted 2 ul on a nitrocellulose membrane (BioRad). DNA was spot to the membrane and followed by 10 min incubation at room temperature. DNA was hybridized to membrane using 15 min incubation at 80 °C. The membrane was blocked in odyssey Blocking buffer (Licor) for 60 min. The membrane was then incubated with 1:1,000 dilution of m6A (Active Motif) at 4 °C overnight. After three rounds of washes with 1X TBST, membrane was incubated with 1:15,000 Goat anti-rabbit secondary antibody attached with AlexFlour 680 (LiCOR). The membranes were then washed with 1X TBST and imaged, and the intensity score of each dot was analyzed by the Odyssey Fc system and normalized to background.

**qRT-PCR:** Total 500 ng RNA was used for cDNA synthesis using the PrimeScript Reverse Transcription Kit (Takara). Quantitative PCR was performed on RotorGeneQ (Qiagen) cycler with the SYBR-Green Master mix (Qiagen) by using primers for target genes and for Beta-actin as an internal control (Suppl. Table 1). All transcript levels were normalized for each well to beta-actin mRNA using the ΔΔCT method, and each PCR reaction was run in duplicate for each sample and repeated at least twice.

**DNA shearing:** DNA and chromatin was sheared using m220 Ultra-sonicator (Covaris) with an average size about 300 bp. The program set as Peak Power: 50, Duty Factor: 20, Cycle/Burst: 200, Duration: 75 sec and Temperature: 18 °C to 22 °C.

**DpnI-Seq:** DpnI-seq library prep was modified from previous published protocol (*1*). Frozen mPFC tissues were homogenized and fixed with 1 % methanol free PFA (Thermo) at room temperature for 5 mins. Final concentration of 0.125 mM of glycine was used to stop fixation. Then, cells were washed with 1X cold PBS for three times. The cell suspension was treated with DNaseI (Thermo) for 15 mins at 4 °C followed by 1ml of 1X cold PBS wash. Then, cell suspension was blocked by using FACS blocking buffer (1X BSA, 1X normal Goat Serum and 1% TritonX) for 15 min at 4 °C with end-to-end rotation. After 15 min blocking, cell suspension was incubated with 1:150 dilution of preconjugated Arc antibody (Bioss) and 1:300 dilution of preconjugated NeuN antibody (Bioss) at 4 °C for 1hr with an end to end rotation. End of incubation, two rounds of 1ml 1X cold PBS washes were applied. Then, the cell pellets were resuspended with 500ul of 1X cold PBS, and 1:2000 diluted of DAPI was added. The FACS sorting was done by using BD FACSAriaII (BD Science). Pheno/choloroform approach was used to extract DNA from FACS sorted cells. The sequencing run was performed on Hiseq4000 by UCI genome sequencing facility.

**Sequencing Data analysis:** The Illumina pair-end sequencing data was aligned to the mouse reference genome (mm10) using BWA (v0.6.2) (*2*). Samtools (v0.1.17) (*3*)was then used to convert “SAM” files to “BAM” files, sort and index the “BAM” files, and remove duplicate reads. Reads with low mapping quality (<20) or reads that were not properly paired-end aligned to the reference genome were excluded from the downstream analysis. These steps ensure that only high-quality alignments were used for the analysis of DpnI cleavage sites (Suppl. Table 2). After alignment, we applied a similar approach that infers potential DpnI cleavage sites based on the position of 5’ends as described in a previous study (*1*). Briefly, a binominal distribution model was assumed that each read could be randomly sheared and aligned to the genome with a probability *p* = 1/gs (gs = genome size) or cleaved by DpnI. For each individual sample, let *n* be the total number of reads. The *P* value of each genomic locus supported by *x* number of reads was calculated as . Bonferroni correction was then applied for multiple testing correction. A genomic locus was determined as a real DpnI cleavage site if it satisfies the following criteria: i) the corrected *P* value < 0.01 in at least 2 of the 3 biological replicates in one condition or both conditions, and ii) the locus is not in the mm10 empirical blacklists identified by the ENCODE consortium (*4*).

The detected DpnI cleavage sites in each condition as well as merged data were used for motif analysis, separately. A DpnI cleavaged “GATC” site was determined as a differentially methylated site between RC and EXT conditions if it satisfies i) the DpnI cleavage site are supported by at least two biological replicates in one condition (e.g. condition A) but at most one replicate in the other condition (e.g. condition B), ii) all three biological replicates in condition A should have 5’ end(s) supporting the DpnI site, and iii) the number of 5’end supported reads in condition A is at least two-folds more than that in condition B. Genes with differentially methylated GATC sites near the TSS region (+/- 500 bp) were parsed for GO enrichment analysis using DAVID (version 6.8) (*5*, *6*).

**m6dA-DIP:** 1 ug of genomic DNA was diluted to 130 ul ultrapure water (Invitrogen) and sheared with an average size about 300bp prior to the capture. m6dA captured was performed using a m6dA antibody (Active Motif) to capture m6dA enriched genomic regions. The procedure was adapted from manufactory’s protocol for Methyl DNA immunoprecipitation (Active motif). 500 ng of sheared DNA and 4 ug of m6dA antibody was used for each immunoprecipitation reaction and all selected targets (GATC site proximal BDNF P4: Chr2: 109692436-109692774; distal GATC site: Chr2: 109691953-109692103) were normalized to input DNA and then to their own controls by using the ΔΔCT method, and each qPCR reaction was run in duplicate for each sample and repeated at least twice.

**DpnI-qPCR:** 300 ng of sheared DNA was treated with 200 units of DpnI (NEB) for 16 hours at 37°C and followed by heat inactivation using 80 °C for 20 min. Treated DNA was then used in qPCR reactions. All selected targets were normalized to its own untreated control by using the ΔΔCT method, and each PCR reaction was run in duplicate for each sample and repeated at least twice. A schema is included in Supplementary figure 1B.

**N6amt1 knockdown constructs.** Lentiviral plasmids were generated by inserting either N6amt1, N6amt2 shRNA or scrambled control fragments (supplementary table 1) immediately downstream of the human H1 promoter in a modified FG12 vector (FG12H1, derived from the FG12 vector originally provided by David Baltimore, CalTech) as previously described (*7*). Lentivirus was prepared and maintained according to protocols approved by the Institutional Biosafety Committee at the University of California Irvine.

**Cannulation surgery and lentiviral infusion.**  A double cannula (PlasticsOne) was implanted in the anterior posterior plane, +/- 30 degrees along the midline, into the infralimbic prefrontal cortex (ILPFC), a minimum of 3 days prior to viral infusion. The coordinates of the injection locations were centered at +1.80 mm in the anterior-posterior plane (AP), and -2.7 mm in the dorsal-ventral plane (DV). 1.0 ul of lentivirus was introduced bilaterally via 2 injections delivered within 48 hours. For knockdown experiments, mice were first fear conditioned, followed by 2 lentiviral infusions 24 hours post fear condition training, and after a one-week incubation; then, extinction trained.

**Behavioral Tests.** Two contexts (A and B) were used for all behavioral fear testing. Both conditioning chambers (Coulbourn Instruments) had two transparent walls and two stainless steel walls with a steel grid floors (3.2 mm in diameter, 8 mm centers); however, the grid floors in context B were covered by flat white plastic non-transparent surface with two white LED lights to minimize context generalization. Individual digital cameras were mounted in the ceiling of each chamber and connected via a quad processor for automated scoring of freezing measurement program (FreezeFrame). Fear conditioning was performed in context A with spray of vinegar (10% distilled vinegar). Then, actual fear condition protocol was starting with 120 sec pre-fear conditioning incubation; then, followed by three pairing of a 120 sec, 80dB, 16kHZ pure tone conditioned stimulus (CS) co-terminating with a 1 sec (2 min intervals), 0.7 mA foot shock (US). Mice were counterbalanced into equivalent treatment groups based on freezing during the third training CS. For extinction, mice were exposed in context B with a stimulus light on and spray of Almond (10% Almond extracts and 10% ethanol). Mice allowed to be acclimated for 2 min, and then, extinction training comprised 60 non-reinforced 120 sec CS presentations (5-sec intervals). For the behavior control experiments, context exposure was performed for both fear condition and fear extinction training. Animal, inside 3CS-US or 60CS treatment, only exposed into either context A or B with equal times of mice spend there by fear condition or extinguished mice but were not exposed to any 3CS-US or 60CS. For the retention test, all mice were returned to context B and following a 2 min acclimation (used to minimize context generalization), freezing was assessed during three 120 sec CS presentations (120 sec intertribal interval). Memory was calculated as the percentage of time spent freezing during the tests.

**Behavioral Training (for tissue collection):** Naïve animals remained in their home cage until sacrifice. For the other groups, fear conditioning consisted of three pairing (120 sec inner-trial interval ITI) of a 120 sec, 80dB, 16kHZ pure tone conditioned stimulus (CS) Co-terminating with a 1 sec, 0.7 mA foot shock in context A. Mice were matched into equivalent treatment groups based on freezing during the third training CS. Context A exposure group spent an equivalent amount of time in context A without any CS and US. One day later, the fear-conditioned mice were brought to context B, where the extinction group (EXT) was presented with 60 CS presentations (5s ITI). The fear-conditioned without extinction (FC No-EXT) group spent an equivalent amount of time in context B without any CS presentations. Tissue was collected from both of these groups immediately after the end of either context B exposure (FC-No EXT) or extinction training (EXT).

**Immunohistochemistry:** Mice were euthanized with 100 mg/Kg ketamine mixed with 10 mg/Kg xylazine, after which 60 ml of 1X PBS, was pumped through the circulatory system, serving as a vasodilator. To fix the tissue, 4% paraformaldehyde in 1X PBS was used. Following extraction, the brains were stored 4% paraformaldehyde overnight. The brains were then placed in 30% sucrose for a minimum 24hr prior to cryostat slicing. Sectioning at 40um was performed using CM1950 cryostat (Leica), and sections were mounted on Superfrost Plus microscope slides (Fisher Scientific). Briefly, the sections were incubated 1-2hr in blocking buffer, after which primary antibodies (N6amt1 or GFP) were added and the slides incubated at 4 °C overnight. The slides were then washed 3 times with 1X PBS containing 0.02 % Tween 20 (PBS-T), after which secondary antibodies were added (Dylight 488-conjugated AffiniPure sheep anti-goat IgG or Dylight 549-conjugated AffiniPure goat anti-rabbit IgG, Jackson ImmunoReasearch Laboratories). The slides were then incubated at room temperature for 45 min, washed 3 times with 1X PBST away from light, and sealed with Prolong Diamond Antifade Mountants (Life technology).

**Chromatin immunoprecipitation:** Chromatin immunoprecipitation (ChIP) was performed following modification of the Invitrogen ChIP kit protocol. Tissue was fixed in 1% formaldehyde and cross-linked cell lysates were sheared by Covaris in 1% SDS lysis buffer to generate chromatin fragments with an average length of 300bp by using Peak Power: 75, Duty Factor: 2, Cycle/Burst: 200, Duration: 900 Secs and temperature: between 5 °C to 9 °C. The chromatin was then immunoprecipitated using the specific antibody to each target. Previous validated antibody has been selected for H3K4me3 (*8*), YY1 (*9*) and RNA Pol II (*10*). Also, equivalent amount of control normal rabbit IgG (Santa Cruz) was used for non-specificity control. Then, antibody and samples mixture were incubated overnight at 4 °C. Protein-DNA-antibody complexes were precipitated with protein G-magnetic beads (Invitrogen) for 1hr at 4 °C, followed by three washes in low salt buffer, and three washes in high salt buffer. The precipitated protein-DNA complexes were eluted from the antibody with 1% SDS and 0.1 M NaHCO3, then incubated 4hr at 60 °C in 200 mM NaCl to reverse formaldehyde cross-link. Following proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation, samples were subjected to qPCR using primers specific for 200 bp segments corresponding to the target regions.

**Primary cortical neuron, N2A and HEK cell culture:** Cortical tissue was isolated from E15 mouse embryos in a sterile atmosphere. To dissociate the tissue it was finely chopped followed by gentle pipette to create single cell suspension. To prevent clumping of cells due to DNA from dead cells, tissue was treated with 2 unit/ul of DNase I. Cells finally went through the 40um cell strainer (BD Falcon) and were plated onto 6 well plate coated with Poly-L- Ornithine (Sigma P2533) at a density of 1x106 cells per well. The medium used was Neurobasal media (Gibco) containing B27 supplement (Gibco). 1X Glutamax (Gibco), and 1% Pen/Strep (Sigma). N2a cell was maintained in medium contains half DMEM, high glucose (GIBCO), half OptiMEM 1 (Gibco) with 5% serum and 1% Pen/Strep. HEK293t cell was maintained in medium contains DMEM, high glucose (Gibco) with 5% serum and 1% Pen/Strep.

**Western blot**: Protein concentration was determined by using Qubit protein detection Kit (Invitrogen) followed by manufactory’s protocol. Individual samples were run on a single 10 well gel. Briefly, samples were prepared on ice (to final volume of 20 ul) and then vortexed and denatured for 10 min at 90 °C. Gels were run with 1X Tris buffered saline-Tween (TBS-T) and proteins transferred onto nitrocellulose membrane (Bio-rad). The membrane was blocked by blocking buffer (Licor) for 1hr at room temperature, washed with TBS-T for 5 min (3X), and incubated with 5ml of N6amt1 (1:250; Santa Cruz) and Beta-actin (1:500; Santa Cruz) antibodies in blocking buffer (Licor) for overnight at 4 °C. The membranes was washed with TBS-T (3X), incubated for 1hr with anti-mouse secondary antibody (1: 15000; Li-Cor) and anti-rabbit secondary antibody (1:15000; Li-Cor) in blocking buffer (Li-Cor), and washed three times with TBST for 10 min (5X) and 20 min (1X). Optical density readings of the membrane were taken using a Li-Cor FX system followed by manufactory’s protocol.

**Lentiviral knockdown N6amt1 *in vitro*:** 1 ul of N6amt1 shRNA or scramble control lentivirus was dropped on to primary cortical neurons in a 6-well plate. After 7 days incubation, cells were harvested for RNA extraction.

**Statistical Analyses.** In all cases where a t-test was employed, we opted for a one-tailed test with an priori hypothesis that the accumulation of m6dA is permissive for gene expression and memory formation. Therefore, all experiments related to epigenetic and transcriptional machinery were hypothesized to show a positive correlation with m6dA, hence, a one-tailed test was employed.For behavioral analysis,freezing (the absence of all non-respiratory movement) was rated during all phases by automated digital analysis system, using a 5-sec instantaneous time sampling technique. The percentage of observations with freezing was calculated for each mouse, and data represent mean ± SEM freezing percentages for groups of mice during specified time bins. Total session means were analyzed with one-way ANOVA for the data in Supplementary Figure 2 and, in experiments using viral manipulation, by two-way ANOVA for the data in Figures 5, Supplementary Figure 5 and 6. Fisher’s posthoc tests were used where appropriate.

Reference

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