Supporting Information

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SI Methods

Animals. Male Sprague–Dawley rats were obtained from Charles River Laboratories and were approximately 2-mo-old (225–250 g) at the start of the studies. Upon arrival, rats were housed in groups of three and were given 2 wk to habituate before the start of the experiment. Animals were kept on a 12-h/12-h light/dark cycle and had ad libitum access to chow and water, except during experimental manipulations. Experiments were performed between 9:00 AM and 11:00 AM. All animals used in this study were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory animals and protocols were approved by the Institutional Animal Care and Use Committee at The Rockefeller University.

RT-PCR. Real-time PCR Sybr green primers for Rat intracisternal-A particle (IAP), B2 short interspersed elements (SINE) and L1 long interspersed elements (LINE) were designed with the aid of Repbase (1) and L1Base (2). Primer sequences: RNIAP Fwd: AATTTCTTGAACGGCTCAGCCAGG, Rev: TGTTAGCAAACGGTCAGTCGTCCT; B2_RN-Fwd: GAAGGCTTCCAGGCCTCAAGTTTA, Rev: AAGTCTGTGAGGTCTTCTCCCTCA; L1_RN- Fwd: ACACTCCTCCATTGTTGGTGGGAT, Rev: AGCTGGATCCTCAGGCAGTTCAAT; β-actin Fwd: CACAGCTGAGAGGGAAATC, Rev: TCAGCA ATGCCTGGGTAC. Because of the lack of apparent silencing response in L1 expression, a total of four primers were constructed, two raised to orf1 and two to orf2 of L1_RN; the results here are for one of the orf2 primers, but the results from the other primers did not differ significantly. RNA was extracted from fresh-frozen hippocampal tissue using a Qiagen RNAeasy Lipid tissue kit and reverse-transcribed using a Transcriptor cDNA kit (Roche). Samples were then quantified using an Applied Biosystems 7900HT sequence detection system (Applied Biosystems). All RT-PCR results were analyzed using Prism 5.0 software using Student *t* test with Bonferonni correction.

Acute Stress and Tissue Processing. Rats were placed in plastic bag restrainers for 30 min then returned to their home cages for 1 h to recover before sacrifice for the experiment. For ChIP-sequencing, after killing by rapid decapitation, whole hippocampi were dissected out by a trained investigator, and processed following the procedure for ChIP outlined in ref. 3. Briefly, tissue was finely chopped, fixed in formaldehyde for 15 min, washed in PBS, and then flash frozen at –80 °C until ChIP. For RT-PCR and Western blotting, hippocampal tissue was extracted and flash frozen, then stored at −80 °C until processing.

Western Blotting. Western blots were performed using 5–20% (wt/vol) bis-Tris gels (BioRad) with hippocampal tissue lysates containing 5 μg protein per lane. After transfer, membranes were incubated with Rabbit anti-H3K9me3 (Cell Signaling) at a dilution of 1:10,000 (vol/vol) overnight at room temperature. After washing in TBST, the membranes were incubated with goat anti-rabbit HRP- (ThermoFisher) coupled secondary antibody at a dilution of 1:50,000 (vol/vol). Blots were then visualized using Pierce Dura reagent (ThermoFisher) and exposed to Kodak MR film for 30 s to 5 min. Protein loading consistency was confirmed by stripping the blots and reprobing with a pan-histone H3 antibody (Millipore) at a concentration of 1:100,000 (vol/vol). All Western results were analyzed using Prism 5.0 software using ANOVA with Tukey's post hoc.

ChIP of H3K9me3. ChIP was performed using previously validated methods (4, 5); hippocampal tissue was thawed, sonicated briefly, and then syringe-passaged three times through a 26-gauge needle. The tissue was then subjected to sonication and DNA shearing in a Bioruptor (Diagenode) for 30 min. Tissue was then processed using an EZ-ChIP kit (Millipore) following the manufacturer's instructions and, after setting aside input samples, chromatin was incubated overnight with 5 μg of H3K9me3 antibody (Cell Signaling) and eluted for sequencing. Four control and four stressed hippocampi were pooled to produce each of the samples for sequencing.

Sequencing was performed on an Illumina GAIIx per the manufacturer's protocols. Reads were quality filtered and trimmed to 50 bp for subsequent analyses (14,421,756 reads for the input sample, 46,727,008 reads for the control sample, and 60,243,937 reads for the stressed sample). All analyses used the rn4 build of the rat genome downloaded from the University of California at Santa Cruz (UCSC) genome browser (6). Reads were aligned using the Burrows-Wheeler Aligner (BWA) (7). Only reads aligning to one potential genomic location were used for subsequent analyses. Differential enriched region finding was performed using methods described in refs. 6 and 8. SICER was run on both of the ChIP libraries as well as an input library as a reference. SICER parameters were set at a window of 5,000, a gap of 5,000 and a false-discovery rate of 0.001 (the window of 5,000 was chosen based on the average length of longer retrotransposons, analyses at shorter and longer window and gap settings returned similar proportions of up and down islands).

Assignment of aligned reads to gene features was performed by counting the reads whose alignment coordinates either intersected or were contained within the indicated feature's genomic coordinates. Gene deserts and binding enrichment in intergenic regions were identified using the methods outlined in refs. 8 and 9.

H3K9me3 Sequencing. ChIPed Genomic DNA from stressed and control animals, as well as input DNA, was sequenced on an Illumina GaIIx according to the manufacturer's instructions. Following the procedure in ref. 8, we created libraries from each animal and then pooled the result. Two libraries were created per sample and these were processed in separate sequencing runs to ensure adequate depth of sequencing and control for the biases inherent in a single run. Alignments of reads were made with BWA (v0.5.9-r16), which was run allowing for no more than three errors in 50 bp and the resultant alignments converted to bam format for postprocessing. This process yielded the following read and alignment counts:

Input quality-filtered reads: 14,421,756; aligned reads: 13,134,795; reads aligned uniquely: 11,308,746;
Control quality-filtered reads: 46,727,008; aligned reads: 41,492,057; uniquely aligned reads: 29,169,108;
Stress quality-filtered reads: 60,243,937; aligned reads: 50,876,323; reads aligned uniquely: 35,074,912.

Browser tracks for the figures were displayed using the integrative genomics viewer (10), and were created using igvtools (Broad Institute) counting function using 25-bp windows, extending the reads 100 bp in the 3′ direction.

Heat Map Analysis. The 15 M reads from input, control K9me3, and stressed K9me3 ChIPs were aligned using the "instance" method from ref. 11, using their modified version of the BWA aligner against RepeatMasker data from the rn4 genome build. Repeats

were clustered using the Cluster 3.0 package, using Euclidean distance and pair-wise complete-linkage clustering. Data were displayed using Java Treeview.

GR ChIP-Seq. For ChIP analysis 8-wk-old male Sprague–Dawley rats (Harlan) were adrenalectomised as described previously (12) to completely deplete endogenous corticosterone (CORT) levels and ensure there was no glucocorticoid receptor (GR) bound to the DNA. Three days after adrenalectomy, animals received an intraperitoneal injection with either 300 or 3,000 μg/kg CORT-hydroxypropyl-cyclodextrin complex. Animals were decapitated 1 h after injection for ChIP. Experiments were approved by the Local Committee for Animal Health, Ethics, and Research of the University of Leiden. Animal care was conducted in accordance with the European Commission Council Directive of November 1986 (86/609/EEC).

Immediately after decapitation, the hippocampi were isolated, fixed and sonicated as described previously (13). For sequencing, IgG and GR ChIP-samples were prepared according to the protocol supplied with the Illumina Genome Analyzer GA1. In brief, the DNA fragments were blunted and ligated to sequencing adapters after which the DNA was amplified for 18 rounds of PCR. The DNA was size-selected for fragments of 100–500 bp length by gel electrophoresis on a 2% agarose gel. Single-end sequencing of the first 35 bp of the resulting DNA library was performed on the Illumina Genome Analyzer (Leiden Genome Technology Center, Leiden University, The Netherlands).

For ChIP-Seq data analysis, the obtained 35-bp reads were aligned to the rat genome (rn4) using the BWA, controlling for unique tags, mismatch, and DNA-gaps. BED-files were generated using BEDtools and used as input for MACS and to generate wiggle files to visualize the reads on the UCSC genome browser. GR binding sites in the DNA were identified with the MACS peak caller (14), using a *P*-value cutoff of 1.00e-05, a model fold of 30 and a λ-set of 1,000/5,000/10,000 to determine the significantly GR-bound DNA-regions relative to IgG background. Refseq genes in the vicinity of the genomic binding sites were determined using Galaxy (http://main.g2.bx.psu.edu/) (15). Data were visualized by uploading wiggle-files containing the raw ChIP-Seq data on the UCSC genome browser (http://genome.ucsc.edu).

In Situ Hybridization. In situ hybridization was performed as described previously (16), using S^{35}-labeled oligos against *Suv39h2*: 5′- CCT TGA GTT GCA TTC ATA GAT GGG AGT GCC TGG T -3′; 5′- TCT ACC AAG TGC CTG GCA TCA CAT CAC CTT CT -3′.

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