Supplementary Information

Supplementary Methods (Complete description)

Animals:

Rats were handled according to the NIH guidelines for the care and use of laboratory animals and in accordance with protocol approval from the University of California-Irvine Institutional Animal Care and Use Committee. Subjects were progeny of timed-pregnant Sprague-Dawley rats (Harlan, Indianapolis, IN). Rats were housed under a 12 hour light-dark cycle in humidity and temperature controlled rooms, with *ad libitum* access to food and drinking water. Parturition was checked daily, and the day of birth was considered postnatal day (P) 0. For the generation of hypothalamic explant cultures, pups were sacrificed on P7. For handling experiments, pups were mixed among litters on P1, and 10 pups with were assigned at random to each dam. Care was taken to ensure that each dam had equal numbers of male and female pups. The pups experienced one of the following early-life rearing conditions: (1) a 15 minute handling, which took place daily from P2 to P9; or (2) undisturbed controls (non-handled) that remained in cages that were not touched between P2 and P9.

Augmentation of maternal care via brief daily handling and observation of maternal care:

Cages were brought into a separate room in the animal facility daily at 8:30 am. The dam and the pups were placed into separate bedded cages. The pups were kept euthermic via a heating pad placed underneath the cage. After 15 min, pups were placed back into their home cage, followed by the dam, and returned to the home room. Undisturbed litters remained in the residence room from P2 to P9. For all experimental groups, cage change did not occur during this time. After pups and the dam were returned to the home room, maternal behavior (including licking and grooming) was observed for both handled and non-handled litters as based on a protocol used by.[5,8,9,17] Each maternal observation session consisted of 30 minutes of continuous observation. Animals were either sacrificed on P10 between 8:00 am and 10:00 am,

or maintained under standard animal facility rearing conditions, weaned on P21, and grown three to four per cage into adulthood (~100 days).

Hypothalamic organotypic / explant procedures:

Cultures were prepared according to a modified stationary hypothalamic slice culture protocol as previously described.[13] Rat pups were decapitated on P7-P8 (day *in vitro* (DIV) 0), brains were removed, and hypothalamic blocks were dissected and cut into 350 μm coronal sections on McIlwain tissue chopper in ice cold prep media (MEM (Thermo Fisher Scientific, Waltham, MA), L-Glutamine (Thermo Fisher Scientific, Waltham, MA), Hepes Buffer (Thermo Fisher Scientific, Waltham, MA), glucose, and cell culture grade water (GE Healthcare, Little Chalfont, UK) in a laminar flow hood. Sections containing the PVN were then maintained on 0.4 μm, 30 mm diameter cell culture inserts (Merck Millipore, Darmstadt, Germany) in six well plates with culture media (Minimum Essential Medium, Hank's balanced salt solution, heat-inactivated horse serum, HEPES, glucose, glutamine, ascorbic acid, insulin, NaHCO3, and cell culture grade water). Explants were maintained for 8 days at 37°C in 5% CO_2 enriched air in an incubator, with the media refreshed every 48 hours (h). Explants were kept in serum-containing medium from DIV 0 to DIV 6, which also contained antibiotics (penicillin and streptomycin (Thermo Fisher Scientific, Waltham, MA) until DIV 4. On DIV 6 cultures were transferred to serum free media (Minimum Essential Media, HEPES, glucose, glutamine, ascorbic acid, insulin, NaHCO3, cell culture grade water), and treated with either sterile nuclease-free tissue-culture grade water (vehicle), or 50 μM MK-801 (Sigma-Aldrich, St. Louis, MO) and 50 μM CNQX (Sigma-Aldrich, St. Louis, MO). Media containing either vehicle or glutamate receptor antagonist(s) were refreshed every 12 h for 52 h. Cultures were then harvested on dry ice, and stored at -80^{o}C until further processing.

For oligodeoxynucleotide (ODN) treatment, previously described phosphothiolated ODNs consisting of either a control randomly ordered sequence (scrambled; SCR) or a sequence coding for NRSF binding site (NRSE), were used.[14,15] Slices were maintained as described above, transferred to serum free media on DIV 6, and exposed to one of the following treatments: [Veh + SCR], [Veh + NRSE], [CNQX/MK-801 + SCR], or [CNQX/MK-801 + NRSE]. Dose-response analysis determined 10 nM as the optimal ODN dose. The ODNs were used only for the first 12 h of treatment. Media containing either vehicle or antagonists were refreshed every 12 h for 52 h. Cultures were then harvested and stored as described above. To visualize ODNs in neurons, dissociated neuronal cultures were generated as previously described,[16] incubated with BODIPY-tagged ODNs, and visualized using fluorescence microscopy.

RNA extraction, reverse transcription, and qRT-PCR:

Samples were thawed on ice, and RNA was isolated using RNeasy Mini Kit (Qiagen, Valencia, CA) as per manufacturer's protocol. RNA was quantified and purity was analyzed using NanoDrop (Thermo Fisher Scientific, Waltham, MA). It was immediately converted to cDNA with random hexamers using first strand cDNA synthesis kit (Roche, Basel, Switzerland) following manufacturer's protocol. qRT-PCR was performed using SYBR Green chemistry (Roche, Basel, Switzerland) on a Lightcycler 96 (Roche, Basel, Switzerland) with primers for specific transcripts. GAPDH served as the internal control, and relative quantification of mRNA expression was determined using the cycle threshold method (2^-ΔΔCt). Minus-reverse transcription and non-template controls were routinely used to eliminate the possibility of genomic contamination or false positive analyses. Primer sequences used for qRT-PCR are provided in Supplementary Table 1.

Semiquantitiative in situ hybridization (ISH) histochemistry:

ISH histochemistry was conducted on hypothalamic explants (*n* = 5-6 per group) using an established protocol for deoxyoligonucleotide probes.[5,8,9,17] *Crh* and *Avp* probes were labeled with 35S using routine terminal deoxynucleotide transferase methodology. Briefly, sections were brought to room temperature from storage in -80°C followed by dehydration and rehydration through graded ethanols. Sections were exposed to 0.25% acetic anhydride and 0.1 triethanolamine (pH 8) for 8 min and were dehydrated through graded ethanols. Pre-hybridization and hybridization steps were performed in a humidified chamber at 42°C in a solution of 50% formamide, 5x SET, 0.2% SDS, 5x Denhart's, 0.5 mg/mL salmon sperm DNA, 0.25 mg/mL yeast tRNA, 100 mM dithiothreitol, and 10% dextran sulfate. Following a 1 h pre-hybridization, sections were hybridized overnight with 0.2 x 106 cpm of labeled *Crh* probe or 0.15 x 106 cpm of *Avp* probe. After hybridization, sections underwent serial washes at 42°C, most stringently at 0.3x SSC for 30 min at room temperature. Sections were then dehydrated through increasing ethanol concentrations, air-dried and apposed to film (Kodak BioMax MR Film, Eastman Kodak Co., Rochester, NY) for 14 days.[5,8,9,17]

ISH histochemistry signals were analyzed on digitized film using ImageTool software program (UTHSC, San Antonio, TX). All analyses were performed without knowledge of treatment group. The linear range of optical densities (ODs) was determined using C14 standards, after correcting for background by subtracting the density of the hybridization signal over the area immediately adjacent to the PVN. Each animal yielded one PVN organotypic slice containing CRH-expressing cells, and that section with maximal OD values was used to represent each PVN in calculating group means ± standard error (SE).[5,8,9,17]

Immunocytochemistry (ICC):

For CRH ICC, hypothalamic slice cultures (*n* = 4-5 per group) were subjected to standard avidin–biotin complex (ABC) methods.[5] Briefly, after several washes with 0.01 M PBS

containing 0.3% Triton X-100, pH 7.4 (PBS-T), slices were treated for 30 min in 0.3% H_2O_2 in PBS, followed by blockade of nonspecific sites with 1% bovine serum albumin (BSA) in PBS for 30 min and incubation for 48 h at 4°C with anti-CRH (1:40,000; gift from Dr. W. W. Vale, Salk Institute, La Jolla, CA) in PBS. After three 5 min washes in PBS-T, slices were incubated in biotinylated rabbit anti-goat IgG (1:200; Vector Laboratories, San Mateo, CA) in PBS containing 1% BSA for 2 h at room temperature. After washing in PBS-T (three times for 5 min), slices were incubated in ABC solution (1:100; Vector Laboratories, San Mateo, CA) for 2 h at room temperature. Slices were then rinsed again in PBS (three times for 5 min). The reaction product was visualized by incubating slices for 8–10 min in 0.04% 3,3-diaminobenzidine (DAB) containing 0.01% H_2O_2. Explants remained on membrane inserts throughout all washes and incubations until DAB incubation.[5]

ICC analysis in organotypic slice cultures was performed as previously described.[5] All counts were performed without knowledge of experimental group. CRH-immunopositive cells were visualized using a Nikon E400 microscope and counted in anatomically matched sections using systematic sampling methods. Analyses were performed using ImageJ (version 1.41; NIH, Bethesda, MD). Because the diameter of the CRH-positive cell nucleus was considerably smaller than the thickness of each slice (cultures flattened to ~70-100 μm in thickness), each cell was counted once only, using the leading edge of the nucleus. The numbers of cells per animal were used to calculate group means ± SE. The intensity of CRH immunoreactivity was analyzed on digital photomicrographs with a standardized light source and exposure. Densities were expressed in OD units after correcting for background by subtracting the density of the immunoreactive signal immediately adjacent to the PVN. As with *in situ* hybridization, each animal yielded one PVN organotypic slice containing CRH-expressing cells, and that section, with maximal OD values was used to represent each PVN in calculating group means ± standard error (SE).[5]

For double-labeling ICC of CRH and vGlut2, control and augmented care rats were anesthetized at P10 with sodium pentobarbital (100 mg/kg, i.p.) and perfused through the ascending aorta with 0.9% saline solution, followed by freshly prepared, cold 4% PFA in 0.1 M sodium phosphate buffer (PB), pH 7.4, cryoprotected in 15 and 30% sucrose/PB solution, and stored at -80 °C. Brains were sectioned at 30 µm using a cryostat, and sections were collected as four series with 90 µm intervals between sections in tissue-culture wells containing 0.1 M PB. Every fourth matched section was subjected to immunocytochemistry (ICC). For each experiment, sections of augmented care and control rat brains were processed concurrently in parallel wells. Sections of enriched care and control rats (n = 3 per group) were washed three times for 10 min in 0.01 M PBS and treated with 1% Triton X-100 in 0.01 M PBS for 30 min. Sections were placed in 2% normal goat serum and incubated for 48 h at 4 °C in a mixture of rabbit anti-CRH serum (1:10,000) and guinea pig anti-VGlut2 serum (1:10,000; Merck Millipore, Darmstadt, Germany). After three 10 min washes in 0.01 M PBS, sections were incubated in a secondary antiserum mixture (1:400; goat anti-rabbit IgG 568 and goat anti-guinea pig IgG Alexa Fluor 488; Invitrogen, Carlsbad, CA). The number of vGlut2 boutons contacting PVN–CRH neurons was assessed by confocal laser-scanning microscopic analysis (n = 66 cells for control, n = 76 cells for experience-augmented rats). Images of the parvocellular division of the PVN (at coronal levels 3.8–3.5 mm anterior to bregma) were collected using an LSM510 confocal scanning system (Zeiss, Oberkochen, Germany). Five 2 µm thick optical sections were collected along the z-axis throughout the thickness of the whole neuron. Images were acquired with an excitation wavelength of 488 nm (green) and 543 nm (red) with a 40 x oil objective using the minimum pinhole size. Images were imported as TIFF files at the resolution of 1024 x 1024 pixels. vGlut2 boutons contacting PVN– CRH neuron were counted through the complete z-axis in each optical section and averaged (vGlut2 boutons/optical section/CRH cell). A vGlut2 bouton was considered to be apposed to the CRH cell body only when there was no visible space

between the CRH cell membrane and the bouton. In addition, cell size was measured in the central optical section for each CRH neuron using LSM Image browser software (version 4.2.0.121; Zeiss, Oberkochen, Germany). Analyzed cells were selected based on the following criteria: (1) CRH immunopositive; (2) fully visible soma within the Z-stack; and (3) a clearly identifiable nucleus. After all of the quantitative analyses were completed, images used for illustration were optimized for brightness and contrast using Adobe Photoshop 7.0.[5]

Western Blotting:

PVN cultures treated with either vehicle or CNQX/MK-801 were thawed from -80^{o}C. For detection of transcription factors or nuclear proteins, samples were enriched for nuclear and cytoplasmic fractions using the NE-PER kit (Thermo Fisher Scientific, Waltham, MA) according to manufacturer's instructions. Protein amount was then quantified using Bradford assay (Bio-Rad, Hercules, CA) and 20-30 µg of protein was loaded on 8-12% gradient gels (Lonza, Basel, Switzerland). Following SDS-PAGE separation, proteins were transferred to PVDF membrane and blocked using 5% milk in PBS-T. Membranes were then incubated overnight in primary with either NRSF (1:1000, Santa Cruz; sc-25398, Dallas, TX) or β-Actin (1:10000, Sigma-Aldrich; Ab8227, St. Louis, MO) at 4°C while shaking. Membranes were washed in PBS-T to remove loosely bound primary antibody, blocked using 5% milk in PBS-T, and then incubated in species specific appropriate secondary antibody (anti-Rabbit HRP (1:5000, GE Healthcare; NA934-1ML, Little Chalfont, UK) for 1 h at room temperature. Membranes were washed again in PBS-T, incubated in ECL substrate (Thermo Fisher Scientific, Waltham, MA), and subjected to a series of developing incubation times. Densitometry was performed to quantify protein expression using ImageJ software.

Chromatin immunoprecipitation (ChIP):

PVN cultures treated with either vehicle or CNQX/MK-801 were thawed from -80^{o}C, cross linked with formaldehyde for 10 min while shaking at room temperature in PBS. The cross-linking was then neutralized by addition of glycine. Tissue was homogenized in homogenization buffer (50 mM HEPES pH 8.0, 140 mM NaCl, 1 mM EDTA, 0.4% Igepel CA-630, 0.2% Triton X-100, and a cocktail of protease inhibitors), and nuclei harvested by centrifugation. Nuclei were sonicated for 25 min at the 'High' setting on the 30 s on / 30 s off using Diagenode sonicator (Denville, NJ) to an average size of 500 bp, and centrifuged to remove cellular debris. The lysate was precleared overnight with Protein-A/G (Santa Cruz, Dallas, TX) at 4^{o}C and then incubated with either 5 µg or 10 µg of either control non-immune serum (IgG) (Cell Signaling; 2729S, Danvers, MA), 10 µg of anti-NRSF (Santa Cruz; sc-25398x, Dallas, TX), 5 µg of anti-MeCP2 antibody (Abcam, Ab2828, Cambridge, MA), anti-Histone3 Lysine9 dimethyl antibody (Abcam; Ab1220, Cambridge, MA), anti-Histone3 Lysine27 tri-methyl antibody (Abcam; Ab6002, Cambridge, MA), or anti-Histone3 Lysine9 tri-methyl antibody (Abcam; Ab8898, Cambridge, MA) overnight at 4^{o}C in buffer containing 20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA and protease inhibitors. Protein A/G beads precleared with salmon sperm DNA (400 µg/ml) were added to the lysate for 2 h. The beads were washed several times to remove non-specifically bound protein, and then eluted using a buffer containing 2% SDS and 0.2 M sodium bicarbonate. After reversal of cross-linking at 65^{o}C overnight, the bound DNA was purified and eluted using the QiaQuick MinElute PCR purification kit (Qiagen, Valencia, CA). Quantitative PCR (qPCR) amplification was done using SYBR Green chemistry (Roche) on a Lightcycler 96 (Roche) with primers for specific genes as described above. Primer sequences used for ChIP analyses are provided in Supplementary Table 2.

Restraint Stress and Corticosterone Assay

Young adult male rats from the control and enriched care groups animals were subjected to a 30 min restraint stress,[8,17] and corticosterone assay was performed as previously described using commercial RIA kits (INCSTAR Corp., Stillwater, MN, and ICN, Irvine, CA).[8,17]

Elevated plus maze test:

'Anxiety-like' behaviors, manifest as reduced time and entries into the open arm of the EPM, were tested on young adult male rats without knowledge of early-life treatment as previously described.[18-20] The elevated plus maze consisted of two open arms (50×10 cm) and two enclosed arms (50×10×40 cm), and was elevated 50 cm above the floor. The maze was arranged such that the two arms of each type were opposite each other. Animals were placed in the center of the maze facing an enclosed arm at the start of the experiment. Each rat was allowed one 5 min trial in the maze, and the maze was cleaned with 70% ethanol after each trial. Decreased time spent on open arms relative to closed arms was used as an index of anxiety, and locomotion and the number of times a rat entered the open or closed arms were recorded as a measure of activity level. Behaviors were analyzed without knowledge of treatment groups.

Porsolt forced swim test:

'Depression-like' behaviors were tested on young adult male rats without knowledge of early-life experience.[20] The forced swim test consisted of two sessions separated by 24 h, in a room illuminated with dim lighting. The habituation session (Day 1), lasted 15 min. Rats were placed in a glass cylinder (20 cm in diameter and 60 cm high) containing water (23-25ºC) filled to a depth of 45 cm. The test session occurred 24 h later, and rats were placed in the cylinder for 5 min. Behavior was monitored using a video camera. The duration of immobility was scored. Water was replaced and containers cleaned between trials.[20]

ChIP sequencing (ChIP-seq):

ChIP protocol described above was modified for ChIP-seq experiments. Hypothalamic explants were treated with vehicle or CNQX/MK-801, as previously described. Explants from each animal were processed separately until sonication. Material from 10 animals was pooled for each treatment after sonication. Samples were cross-linked using methanol-free formaldehyde (Thermo Fisher Scientific, Waltham, MA), and processed as before. They were sheared using a Covaris S2 Focused Acoustic Shearer (Woburn, MA) to 200 bp. Samples were pooled after sonication, and precleared overnight at 4^{o}C Protein A/G (Santa Cruz, Dallas, TX). Beads were removed as before, and samples were immunoprecipitated with anti-NRSF (Santa Cruz; sc-25398x, Dallas, TX) antibody overnight at 4^{o}C while rotating. 400 µg/ml BSA (Sigma-Aldrich, St. Louis, MO) was used to preclear protein A/G beads. Samples were incubated with precleared beads for 2 h, and washed, eluted, and reverse cross-linked as described above. The bound DNA was purified and eluted using the QiaQuick MinElute PCR purification kit (Qiagen, Valencia, CA). DNA concentration, quality, and sonication profile was verified by Bioanalyzer (Agilent, Santa Clara, CA) and Qubit. Libraries were prepared using NextFlex Rapid DNA seq kit (Bioo Scientific, Austin, TX) as per manufacturer's protocol, and adapters verified using qPCR. Sequencing was performed through University of California Irvine Genomics High Throughput Facility (UCI GHTF) using Illumina HiSeq 2500. The libraries multiplexed to 4 per lane, and SR50 run was performed. Sequencing data was analyzed as described.[21]

ChIP-sequencing (ChIP-seq) Analysis:

Sequencing data was analyzed for quality control using FastQC. ChIP and input sequencing reads were aligned using Bowtie2 v.2.2.3[53] with default parameters to the genome assembly rat rn5. After alignment, NRSF-bound regions (or peaks) were detected using MACS2 v.2.0.10[54] with default parameters except '-p 0.001'. Peaks were considered reproducible when they were

identified in both replicates. The ChIP-seq data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE87709 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE87709).

Motif calling

Known canonical NRSE motif PSFM (position specific frequency matrix)[55] was divided into PSFMs for two half-sites, i.e. NRSE-left, from position 1 to 10 and NRSE-right, from position 12-21. PSFMs for two half-sites were used to scan within NRSF-bound regions (or peaks) using MEME v.4.9.0_4[56] with parameters 'fimo --thresh 5e-3 --parse-genomic-coord' respectively. Half-sites having >50% motif occurrence score were accepted. To find both canonical and non-canonical NRSF motifs, we defined the distance between two half-sites is between position 6 of NRSE-left and position 16 of NRSE-right (inclusive). This gives an 11 bp distance in canonical NRSE motif and 10, 16-19 bp distance in non-canonical NRSE motifs. Any canonical or non-canonical NRSE motifs were accepted for each peak. Identified motifs were further performed with unambiguous motif analysis in peaks. We used the following procedure to confirm only one motif in each peak: (1) if canonical NRSE lies in peak, the one with highest occurrence score was accepted; (2) if non-canonical NRSE lies in peak but no canonical NRSE, the highest score non-canonical NRSE was accepted; (3) if peak only has half-sites, the highest score half-site was accepted.

Peak annotation and gene ontology (GO) analysis

Peaks were annotated according to their proximity to transcription start sites (TSSs) using Homer[57]. Peaks within 20kb of TSSs were collected and performed gene ontology (GO) analysis. GO analysis were performed by using Metascape[58] with p-value cutoff 0.05. Homer was also used to determine unique and overlapping peaks in each treatment group.

Statistical considerations:

Rats were assigned to groups randomly, and group sizes were determined *a priori* based on expected effect size and variance. All analyses were performed without knowledge of treatment group. Grubbs or confidence-limit outlier tests were used to determine outliers. Beyond ChIP-seq analysis, we employed the Prism Graphpad software package (San Diego, CA). One-way or two-way ANOVAs or Student's *t*-test were used to assess statistical significance as appropriate. For ChIP analysis of NRSF activity, 2-way repeated measure ANOVA was used to account for batch effects in the assay. Following a significant *F* value for an interaction in a 2-way ANOVA, Bonferroni *post hoc* test was used to distinguish among groups. Following a significant *F* value in a one-way ANOVA, a Dunnett's *post hoc* test was conducted to compare experimental groups to control. For behavioral tests, in the Porsolt swim test, one-way ANOVA with *post hoc* Bonferroni tests were used. In elevated plus maze the two groups were compared using a *t*-test. Significance for all analyses was set at $P<0.05$, and data are expressed as mean and standard error of the mean (SEM) unless noted otherwise.

Supplementary References

53. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 2012; **9:** 357-359.

54. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE *et al*. Model-based analysis of ChIP-Seq (MACS). *Genome Biol.* 2008; **9:** R137.

55. Mathelier A, Zhao X, Zhang AW, Parcy F, Worsley-Hunt R, Arenillas DJ *et al.* JASPAR 2014: an extensively expanded and updated open-access database of transcription factor binding profiles. *Nucleic Acids Res.* 2014; **42:** D142-147.

56. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L *et al*. MEME Suite: Tools for motif discovery and searching. *Nucleic Acids Res.* 2009; **37:** 202-208.

57. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P *et al*. Simple Combinations of Lineage-Determining Transcription Factors Prime cis-Regulatory Elements Required for Macrophage and B Cell Identities. *Mol. Cell.* 2010; **38:** 576-589.

58. Tripathi S, Pohl MO, Zhou Y, Rodriguez-Frandsen A, Wang G, Stein DA *et al*. Meta-and Orthogonal Integration of Influenza "OMICs" Data Defines a Role for UBR4 in Virus Budding. *Cell Host Microbe* 2015; **18:** 723-735.

Supplementary Figure Legends

Supplementary Figure 1. Augmented maternal care *in vivo*, and blockade of ionotropic glutamate receptors *in vitro,* does not regulate the expression of another stress mediator *Avp*. (**a**) qRT-PCR analysis of AVP expression in the PVN of control and augmented care rats at P10 (*n* = 5 per group). (**b**) qRT-PCR analysis (*n* = 8-10 per group) and (**c**) ISH (*n* = 5 per group) for AVP mRNA expression in hypothalamic explants incubated with vehicle or CNQX/MK-801. ChIP followed by qPCR was performed to assess NRSF binding to *Avp* gene in (**d**) hypothalamic explants incubated with vehicle or CNQX/MK-801 (*n* = 6 per group, presented as $\%input^{-1}$), and (**e**) PVN of control and augmented care animals at P10 (*n* = 5 per group, presented as $\%input^{-2}$)

Supplementary Figure 2. Interfering with NRSF activity rescues CRH repression, but does not affect AVP expression. (**a**) qRT-PCR for CRH expression in vehicle or CNQX/MK-801 incubated hypothalamic explants exposed to 2 nM, 10 nM, or 100 nM ODNs (*n* = 3-5 per group). (**b**) qRT-PCR analysis for AVP expression in ODN incubated hypothalamic explants (*n* = 10 per group).

Supplementary Figure 3. Augmented maternal care *in vivo*, and blockade of ionotropic glutamate receptors *in vitro,* does not promote epigenetic modulation of *Avp*. ChIP followed by qPCR to assess MeCP2 binding at *Avp* gene in (**a**) hypothalamic explants incubated with vehicle or CNQX/MK-801 (*n* = 6 per group, presented as %input^{-1}), and (**b**) PVN of control and augmented animals at P10 (*n* = 4 per group, presented as %input^{-2}). ChIP followed by qPCR was used to assess H3K27 trimethylation at the *Avp* gene in (**c**) hypothalamic explants incubated with vehicle or CNQX/MK-801 (*n* = 5 per group), (**d**) PVN of P10 (*n* = 5-6 per group) and (**e**) adult (*n* = 6 per group) animals that experienced control or enriched care early in life. ChIP followed by qPCR was used to measure H3K9 dimethylation (*n* = 6 per group) at the *Avp* gene in (**f**) hypothalamic explants incubated with vehicle or CNQX/MK-801 (*n* = 4 per group), and (**g**) in PVN of adult control and augmented care animals (*n* = 5 per group).

Supplementary figure 4. QC: Correlation coefficient of reads coverage in overlapped peaks in each ChIP-seq replicate.

Correlation coefficient of reads coverage in consolidated peaks between ChIP-seq replicates of vehicle exposed group (left) and glutamate receptor blockers incubated group (right). Reads coverage for peaks was normalized based on sequencing depth for each replicate into reads per 1 million reads mapped (RPM).

Supplementary figure 5. QC: Representative ChIP-seq signals, demonstrating the quality of the ChIP-seq derived from hypothalamic explants.

Track views generated using UCSC Genome Browser to depict representative NRSF peaks in each group. NRSF peak on **(a)** *Nefm* promoter that is unique to vehicle, and (**b**) *Crh* intron that is unique to glutamate receptor blockers group. **(c)** *Npas4* contains several NRSF bound peaks, one (right) is unique to the glutamate receptor blockers group, while the other (left) is an

overlapping peaks between the two groups. **(d)** NRSF bound peak in *Snap25* intron which is overlapping in both groups.

Supplementary Tables

Supplementary Table1 List of primer sequences used for qRT-PCR analyses to assess mRNA levels of various genes.

Primer	**Forward**	**Reverse**
CRH	5'-GAAACTCAGAGCCCAAGTACGTTGAG-3'	5'-GTTGTTCTGCGAGGTACCTCTCTCAG-3'
NPAS4	5'-GTGTCCTAATCTACCTGGGCTTTGAGCG -3'	5'-GAATATCTCCATTTTCAGCCAACAGGCGG -3'
KCC2	5'-GCACGATGCTCACAGCCATTTCCATG-3'	5'-GCAAAGGTAGTGCCCAGGTAGAAGCAG -3'
NPPB	5'-GAACAATCCACGATGCAGAAGCTGCTG -3'	5'-GAAGTTCTTTTGTAGGGCCTTGGTCCTTTGAG -3'
VGF	5'-GAGGATTGCGAGCGTTCTCTGACCATTTG -3'	5'-GGATGAGTAGAAGGAAGCAGAAGAGGACGG -3'
TRPC7	5'-GTCAAGAAGTTCGTTGCCCACCCTAA	5'-GAGCAATCCAATAGGCTATGGCGAGA

	CTG -3'	AAAGG -3'
LRP11	5'- GACGACCACGCCATTGTCCTTTATGA GTG -3'	5'- GGAAGCACAGTCACAGATACATTG TCGGAG -3'
SNAP2 5	5'- GAAACCTCCGTCATATGGCCCTAGAC ATG -3'	5'- GCACGTTGGTTGGCTTCATCAATTCT GG -3'
CHGA	5'- CAGAGAGAAGGCTGGGCCTAAAGAA GTC -3'	5'- CCTGAGACTCCGACTGACCATCATCA TC -3'
GLRA1	5'- CCCAAGGTGTCCTACGTGAAAGCTAT TGAC -3'	5'- CTTGTGTTGCCGGGACACAAAGTTGA C -3'
GRIN1	5'- GAGGCAGTAAACCAGGCCAATAAGC G -3'	5'- GTGGCTAACTAGGATAGCGTAGACCT GG -3'
DRD2	5'- GCAGGATTCACTGTGACATCTTTGTC ACTCTGG -3'	5'- GGAGCTGTAGCGTGTGTTATACAGCA TGG -3'

NEFM	5'-CTATCAAGGAGGAAATCAAGGTCGAGAAGCC -3'	5'-CCTTGGCTACCTCCTTCTTCTCCTCAAC -3'
NEUROD2	5'-GAACTACATCTGGGCTCTCTCGGAGATCTTG -3'	5'-GAAGTTACGAGAGTTCAGCTGCAGGCAG -3'
NTSR2	5'-CAGAGCCATCGTGGCTGTGTATGTCATC -3'	5'-CTGAGCTGACATAGAAGAGCGTGTTGGTC -3'
GAPDH	5'- ATGCCATCACTGCCACTCAGA -3'	5'- ACCAGTGGATGCAGGGATGAT -3'

Supplementary Table 2 List of primer sequences used for qPCR analyses following ChIP to assess occupancy at specific gene regions.

Primer	**Forward**	**Reverse**
CRH NRSE	5'-GGAAGACTTAGGAAGAGGAGTCAGGG -3'	5'-GGTTAGAGTTTAGCTCAGCTGGAAGGTG -3'
CRH Promoter	5'-CTGTCAAGAGAGCGTCAGCTTATTA	5'-CTCTTCAGTTTCTCAACGTACTTGGGC

	GGC -3'	TC -3'
AVP +1.1	5'- GACTCTGTCAAAGCAGGAGAGGGT TTT G -3'	5'- GATGCTCTGGCTCTATCCAGGAGAAG -3'

Supplementary Table 3 Reads alignment for each sample (QC)

Sample	Aligned reads (%)
Vehicle IP R1	59920882 (85.43%)
Vehicle IP R2	29362996 (74.98%)
Vehicle Input R1	69240802 (87.48%)
Vehicle Input R2	29725759 (76.39%)
Glutamate receptor blockers IP R1	68515453 (85.10%)
Glutamate receptor blockers IP R2	34236699 (75.92%)
Glutamate receptor blockers Input R1	77168762 (87.10%)
Glutamate receptor blockers Input R2	41534003 (75.43%)

Supplementary Table 4 Peak calling for replicates and motif calling for overlapped peaks (QC)

Sample	Peaks	Overlapped peaks between Rep1 and Rep2	Overlapped peaks having NRSF motifs
Vehicle IP R1	2196	277	259
Vehicle IP R2	3843		
Glutamate receptor blockers R1	2083	407	352
Glutamate receptor blockers R2	2174		