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Altered postnatal chromatin development in the nucleus accumbens primes enduring stress sensitivity

Abbreviated title: *Setd7* in juvenile NAc primes stress response

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ABSTRACT

Early-life stress (ELS) sensitizes individuals to subsequent stressors to increase lifetime risk for psychiatric disorders. Within the nucleus accumbens (NAc) — a key limbic and reward-associated brain region — ELS sensitizes both cellular and transcriptional response to later stress, which are programmed by enduring epigenetic changes. Among the histone modifications persistently enriched by ELS in NAc is H3K4me1, which is associated with open chromatin and epigenetic priming of genomic enhancers. Here, we sought to determine whether H3K4me1 enrichment in NAc was sufficient to prime cellular and behavioral responses to adult stress. Viral-mediated overexpression of the histone H3 monomethyltransferase *Setd7* in juvenile NAc of male and female mice induced persistent chromatin changes and predominately opened chromatin at long-range cis-regulatory elements predicted to enhance immediate early-genes and transcriptional regulators of mesolimbic development and synaptic activity. These epigenetic changes altered physiological properties of D2-type medium spiny neurons in NAc to resemble neurons of stressed mice, without significantly altering D1-type neurons. Finally, juvenile — but not adult — *Setd7* overexpression and H3K4me1 enrichment in NAc enhanced behavioral sensitivity to future stress. Together, these data indicate that altered postnatal chromatin development in NAc by H3K4me1 enrichment is sufficient to prime long-lasting transcriptional, physiological, and behavioral stress sensitivity.

SIGNIFICANCE: Early-life stress enriches for H3K4me1 in the nucleus accumbens, which is sufficient to open chromatin, alter medium spiny neuron physiological properties, and prime behavioral response to future stress. This work links multiple levels of analysis and provides neurobiological evidence that altered maturation of the juvenile epigenome encodes long-lasting stress sensitivity.

INTRODUCTION

The neural epigenome continues to mature after birth and its maturation is fine-tuned by the environment (Stroud et al., 2020; Peña, 2025a). Even after cell-fate specification, maturation of chromatin and DNA methylation patterns shape cell-type identity and function, with significant maturation occurring at enhancers — cis-regulatory regions of the genome that can act on genes many kilobases away (Long et al., 2016; Bonifer and Cockerill, 2017). Maturation of enhancers continues through the juvenile period, after which little further age-related change occurs (Franklin et al., 2025; Heffel et al., 2024; Herring et al., 2022; Lister et al., 2013; Simmons et al., 2013; Stroud et al., 2017, 2020). Early perturbations to the epigenome may then become crystalized, with persistent effects on gene regulation across the lifespan (Weaver et al., 2004; Peña et al., 2013; Stroud et al., 2020; Peña, 2025a; Rashford et al., 2025).

Experience of early life stress (ELS) can alter maturation of the epigenome (Burns et al., 2018; Peña, 2025a; Rahman and McGowan, 2022; Kim et al., 2026). Child maltreatment inflicts genome-wide alterations in DNA methylation and histone modifications in peripheral tissues and brain samples (Labonté et al., 2012; Weder et al., 2014; Lutz et al., 2017, 2021; Parade et al., 2021). These epigenetic alterations likely mediate persistent gene expression changes throughout the brain (Kos et al., 2023; Peña et al., 2017, 2019; Parel and Peña, 2020; Short et al., 2023). In turn, these molecular changes are thought to contribute to altered structural maturation of the brain, functional responses to cognitive tasks, stressors, and rewards, heightened stress response, and mediate increased risk for psychiatric disease observed following ELS (Hanson et al., 2021; Peña, 2025a). However, it is not well understood whether altered maturation of the epigenome contributes to greater stress sensitivity and psychiatric disease risk compared to epigenetic changes that may accumulate in adulthood.

Dynamic changes to post-translational histone modifications occur in the nucleus accumbens (NAc) of mice exposed to ELS (Kronman et al., 2021). The NAc is a limbic brain region extensively implicated in the pathophysiology of psychiatric disease (Hanson et al., 2021). At a cellular level, ensembles of NAc neurons that are initially activated by experience of ELS are hyper-reactive to stress across the lifespan (Balouek et al., 2023). At a transcriptional level, ELS induces long-lasting changes to baseline gene expression in NAc that are

relevant for predicting successful antidepressant treatment response versus failure (Parel et al., 2023). Secondarily, ELS induces latent gene expression changes revealed by a second hit of stress in adulthood (Peña et al., 2019), pointing to epigenetic regulatory mechanisms at gene enhancers that may prime latent transcriptional responses (Kim et al., 2026). Epigenetic priming is a form of molecular memory in which developmental or environmental stimuli open chromatin at enhancers to facilitate response to future stimuli, and which is marked by histone-3 lysine-4 monomethylation (H3K4me1) (Calo and Wysocka, 2013). We recently found that H3K4me1 was enriched in ventral tegmental area following ELS (Kim et al., 2026), suggesting similar biological mechanisms may drive concerted epigenetic adaptations across limbic brain regions. However, previous research in NAc has focused on chromatin modifications associated with active gene expression and adult manipulations (Covington et al., 2011; Heller et al., 2014; Sun et al., 2015; Lepack et al., 2016; Kronman et al., 2021; Torres-Berrío et al., 2024), leaving the role of developmental priming completely uncharacterized.

Here, we sought to determine whether developmental alterations in the NAc epigenome following ELS are sufficient to prime heightened stress response. To test this, we used a combination of NAc histone bottom-up mass spectrometry, overexpression of the H3K4me1-specific methyltransferase *Setd7*, ATAC-sequencing, patch-clamp physiology in D1- and D2-type medium spiny neurons, and characterization of behavior before and after sub-chronic adult social defeat stress. Finally, we tested whether NAc enrichment of H3K4me1 in postnatal development versus adulthood uniquely sensitized stress-related behaviors. Our results provide novel insights into how altered development of the epigenome may encode long-lasting stress sensitivity.

MATERIALS AND METHODS

Experimental design and statistical analysis

Four cohorts of mice were generated for *Setd7* manipulation experiments. 1) For ATAC-seq, six GFP (3M, 3F) and five SETD7 (2M, 3F) samples from individual non-stressed adult control mice were used. Differential chromatin accessibility was analyzed using DESeq2 as described below. 2) For electrophysiological experiments, four groups were generated to test the effects of *Setd7* and stress in a 2x2

design (n=5-8 mice per group). Data was analyzed using a linear mixed effects model, with mouse as a random factor to account for lack of independence between cells recorded from the same mouse, and for successive current steps recorded in the same cell in IO curve and voltage sag. Virus, Treatment and current steps were fixed effects. Parameters were estimated using restricted maximum likelihood (REML). Models were fit using the mixedlm function in the statsmodels package (v3.9) in Python. 3) For behavioral testing after juvenile Setd7 overexpression, mice were either stress-naïve (and subsequently used for ATAC-seq) or underwent repeated testing before and after stress (n=10-19 per group). 4) For behavioral testing after adult Setd7 overexpression, mice underwent repeated testing before and after stress (n=7-10 per group). For both behavioral cohorts, mixed-effects modeling was used to account for repeated measures testing with missing samples as described in more detail below. Post-hoc analyses were corrected for multiple comparisons. Chi-squared contingency analysis was used to assess differences in proportion of resilient indifferent, and susceptible animals across groups. All significance thresholds were set at $p<0.05$.

Histone modification analysis

Analysis of post-translational histone modifications in NAc across three developmental time points (P21, P35, P70-90, n=3 males/group/age) was done by re-analyzing previously published histone post-translational modification mass spectrometry (data from (Kronman et al., 2021). This data represents percentage of each measured histone modification per fragmented histone peptide (for example, percentage of unmodified histone-3 lysine-4, H3K4me1, H3K4me2, and H3K4me3). Data for H3K27me2 and H3K27me3 are summations of the modification of interest alone or in combination with any H3K36 modification measured on the same peptide fragment. For each histone modification of interest, two-way ANOVA was done to assess main effects and interactions of ELS with age. ELS in this study consisted of a combination of maternal separation and low home cage nesting material in a from post-natal day 10 (P10) to P17, as described (Peña et al., 2017; Kronman et al., 2021).

Mice

All protocols were performed in compliance with the Guide for the Care and Use of Laboratory Animals (NIH, Publication 865–23) and were approved by the Institutional Animal Care and Use Committee of Princeton University (chromatin and behavioral experiments), Washington University in St. Louis (electrophysiology experiments). Wild-type C57BL/6J mice were purchased (Jackson Laboratory) and housed in temperature- and humidity-controlled vivarium facilities and maintained on a 12-h light/dark cycle with *ad libitum* access to food and water. Breeding was performed in-house in trios; males were removed after 5-7 days, and females were separated into individual cages 1-3 days prior to parturition. All offspring were weaned at postnatal day P21, with males and females weaned into separate cages, keeping littermates together and only combining pups from different litters of the same experimental condition in order to maintain 3-5 mice/cage. Males and females were used in all experiments. Retired Swiss-Webster male breeder mice (Taconic) were housed individually and used as aggressor mice in social defeat stress.

Stereotaxic surgery

Standard-reared male and female C57BL/6J pups were randomly assigned to either experimental *Setd7* over-expression group or a control *Gfp* group. Surgeries were performed as previously described (Kim et al., 2026) with the exception of NAc-targeting surgical coordinates. Adeno-associated viral vectors (AAV9) carrying a *Gfp*- and *Myc*-tagged *Setd7* transgene (pAAV-EF1a-EGFP-P2A-Myc-Setd7-WPRE-hGHpA) were previously developed (Kim et al., 2026) from cDNA for *Setd7* (#87131; originally gifted from Francesca Spagnoli; (Kofent et al., 2016)) and *Gfp* (pAAV-EF1a-EGFP-WPRE-hGHpA) purchased from Addgene (#60058; originally gifted from Brandon Harvey; (Savolainen et al., 2014)). AAV-*Setd7* and AAV-*Gfp* control (identical, without *Myc-Setd7*) vectors were cloned and packaged by the Princeton Neuroscience Viral Core. AAV9 was chosen for its ability to expresses transgenes within approximately one week, and continual expression for months (Zincarelli et al., 2008).

For juvenile overexpression, P9-12 pups were anesthetized with isoflurane (2-3% induction; 1-3% maintenance) and fitted into dual-arm stereotax with the nose fitted in the nose cone above the bite bar and the head stabilized using the blunt ends of ear bars between the jaw and ear. The bilateral surgical coordinates for pups were +/-1.4 (x = medial/lateral plane), +1.0(y = anterior/posterior plane), -3.15(z = dorsal/ventral plane), with both Hamilton syringes angled at 7˚. The bilateral surgical coordinates for adults were +/-1.7 (x = medial/lateral plane), +1.6(y = anterior/posterior plane), -4.40(z = dorsal/ventral plane), with both Hamilton syringe angled at 10˚. 250-300nL of virus was administered into each hemisphere at a rate of 0.1µL/minute. Both vet glue (Covetrus, cat#: 001505) and two-four sterile sutures (Fisher, cat#: 501180710) were used to ensure complete healing of incisions. Mice were given a perioperative topical dose of bupivacaine (0.25%, 2 mg/kg), and an additional dose 24h following surgery. Pups recovered in a clean cage on a warming pad before being returned to their home cage. To prevent cannibalization of pups by dams following surgery, pups were only returned to the home cage with the dam once completely ambulatory. All mice were monitored for five days post-surgery. Pups were weaned at P21 and aged to adulthood in standard group housing conditions. Adult mice that underwent surgery recovered for 3 weeks prior to stress, behavioral testing, and/or tissue collection. For molecular analysis and adult over-expression behavior experiments, viral targeting was assessed at the time of sacrifice by GFP flashlight (Electron Microscopy Sciences Nightsea flashlight, NC0792511). For juvenile over-expression behavior experiments, viral targeting was assessed by IHC (see below). In all experiments, mice with poor viral targeting were excluded from analysis.

Sub-chronic social defeat stress

In order to determine whether chromatin manipulation increased sensitivity to later stress, male and female mice were subject to sub-chronic adult social defeat stress for 5 consecutive days. Previous studies have found that among males, susceptibility to social defeat stress develops across 10 days (chronic), but is not apparent after only 5 (Dias et al., 2014; Hodes et al., 2015; Muir et al., 2020). Here, both male and female mice were exposed to social defeat but were run in separate cohorts. Males were defeated as described in standard protocols (Golden et al., 2011). Females were defeated in a “non-discriminatory” version of social

defeat, as aggressors do not naturally attack females alone (Yohn et al., 2019; Bennett et al., 2024; Kim et al., 2026). Briefly, on each day of social defeat, experimental mice were placed into the cage of a novel Swiss-Webster retired breeder ("aggressor"; Taconic) mouse. Male lures were introduced first for ~1-2 minutes, then an experimental female mouse was added into the cage for an additional 5 minutes. After defeat, mice were moved across a perforated plexiglass barrier for the remainder of the day for continued sensory contact. Experimental mice were introduced to a new aggressor each day. Control mice were housed in a standard mouse cage in pairs of the same sex, separated by a perforated plexiglass barrier. Behavioral testing began one day after the last bout of social defeat.

Behavioral paradigms and analysis

Mice were tested on a set of two behavioral tests twice for within-subject controls: once prior to ("pre") and once after social defeat stress ("post"). Groups are presented as GFP-Pre (AAV-*Gfp*, no social defeat stress: 19M, 14F), GFP-Post (AAV-*Gfp*, social defeat stress: 15M, 10F), SETD7-Pre (AAV-*Setd7* virus, no social defeat stress: 17M, 18F), SETD7-Post (AAV-*Setd7*, social defeat stress: 13M, 14F). Social interaction and open field exploration testing were performed as previously described (Peña et al., 2017; Balouek et al., 2023; Bennett et al., 2024). All behavior was recorded and initially analyzed with Ethovision software.

Social interaction: To assess social avoidance behavior, experimental mice were tested in a two-stage social interaction test. In the first 2.5-minute stage, experimental mice were placed into a novel empty arena (44 x 44 x 20 cm) with an empty enclosure along one wall. Mice were then removed, a novel aggressor mouse was placed into the enclosure in the "social interaction zone," and experimental mice were reintroduced into the arena for an additional 2.5 minutes. Time spent in the interaction zones and in the corners was measured. Social interaction ratio ("SI ratio") was calculated as time spent in the interaction zone with the aggressor present divided by time spent in the interaction zone without the aggressor present. "Susceptible" was defined as having a social interaction ratio <0.9, "resilient" as >1.1, and "indifferent" as in between.

Open field: Each mouse was allowed to explore an empty arena (44 x 44 x 20 cm) for 10 minutes and time spent in the center of the arena and overall distance traveled were measured.

Statistical analysis: Behavioral data were graphed and analyzed using Graphpad PRISM software (v. 10.0.3). Outliers were considered to be >2 standard-deviations away from group mean and were removed to avoid type-II error. Main effects and interactions were determined by mixed effects model to account for within-subject repeated testing, with stress, *Setd7*, sex, and interactions as fixed effects (type III). Šidák's multiple comparisons testing was used for post hoc analyses including sex. Given a lack of significant main effect of sex, mixed-effects modeling was also run without sex as fixed effect, using Fisher's LSD for post hoc analyses (reported in figures). Chi-squared contingency analysis was used to assess differences in proportion of resilient indifferent, and susceptible animals across groups. All statistical analyses for behavioral results are included in **Supplemental Table 3**.

Nuclear isolation and ATAC-sequencing

To determine whether juvenile H3K4me1 enrichment by *Setd7* overexpression caused persistent changes in chromatin opening, juvenile mice (P9-12) received bilateral AAV-*Gfp* or AAV-*Setd7* as described above and were aged in control conditions until adulthood. Brains from control adult mice (P60-90) mice were harvested after cervical dislocation and rapid decapitation into ice-cold homogenization buffer (Corces et al., 2017). 1 mm slices of brain tissue were made using a slice matrix from which 14-gauge bilateral punches of the NAc were taken and flash frozen until use. Six GFP (3M, 3F) and five SETD7 (2M, 3F) samples from individual mice were used.

All nuclear isolation reactions were performed on ice. To isolate nuclei, bilateral NAc tissue punches from individual mice were dounce homogenized in 1.5uL of 1X Unstable homogenization buffer (Corces et al., 2017). Homogenate was transferred to 2 mL lo-bind tubes then spun down at 5000G for 5 min (at 4°C). The top 800uL of buffer was aspirated off and the sample was gently resuspended in remaining buffer. Nuclei were collected by iodixanol gradient and counts were collected using a hemocytometer. Yield of nuclei for all

samples was between 3.5-5.8 million nuclei. DNA was then transposed using Illumina's transposition kit (Illumina; cat#20034198) for 30 minutes at 37°C at 1,000RPM. Fragmented DNA was recovered with Qiagen MinElute kits (Qiagen; cat# 28604). Samples were amplified and purified according to (Corces et al., 2017) using Illumina UD indexes (Illumina; cat#20027214 A or B) and AMPure XP beads (ThermoFisher; cat#NC9959336). Resulting yield of DNA fragment sizes was determined by Bioanalyzer dsDNA High Sensitivity protocol and normalized by concentration for sequencing. Samples were pooled for sequencing on a single flowcell (NovaSeq S1 100nt Flowcell v1.5) at the Genomics Core Facility of the Lewis-Sigler Institute at Princeton University.

ATAC-seq processing and analysis

ATAC-seq reads adapters were trimmed using fastp (v0.23.4) with base correction enabled and aligned to mm39 using bowtie2 (v2.4.5) ("--no-unal --no-mixed --no-discordant"). Using samtools (v1.13), reads with MAPQ>30 were retained, followed by samtools collate, fixmate, sort, and markdup to identify PCR duplicates. MACS2 (v2.2.9.1) was used to call narrowpeaks for each sample.

Quality control was assessed using Fastqc (v0.11.9), Picard CollectMultipleMetrics (v2.27.5), and multiqc (v1.17). We found read duplication rates between 33-54%, unique alignment rates between 78-86%, and final average deduplicated library size of 96M reads. We verified that mono- and di-nucleosome sized fragments were visible in all aligned insert size distributions. Using a custom R (v4.4.2) script and package rtracklayer (v1.66.0), we calculated a median of 41% for fraction of reads in MACS2 peaks.

A consensus peak set was generated using GenomicRanges (v1.58.0) "reduce" command in R. ATAC reads were imported using BRGenomics (v1.17.1) "import_bam" with "field=NULL" and unique reads in peaks were counted using "getCountsByRegions". Differential accessibility was assessed using DESeq2 (v1.46.0) using a likelihood ratio test comparing a full model "~sex + condition" (with condition being SETD7 or GFP) to a reduced model of sex alone. Related plots were generated using ggplot2 (v3.5.1). We identified differentially accessible regions (DARs) as peaks with |log2FoldChange| >0.5 and p.adj<0.1.

ChIPseeker (v.1.36.0) was used to find genomic features overlapping differentially accessible regions (DARs), and to nominate putative gene contacts by taking the list of closest genes for the 43 most open regions (p.adj<0.1) and running gene ontology (GO) analysis. We used geneontology.org to perform GO term enrichment analysis on the putatively gene contacts of DARs.

To visualize tracks, RPM/CPM-normalized bigWig files were also generated in R using GenomicRanges “coverage” via BRGenomics “getStrandedCoverage” with strand information removed, and exported with rtracklayer.

Patch-clamp electrophysiology

Male and female *Drd1a-tdTomato* mice were stereotactically injected in the NAc with AAV-*Gfp* or AAV-*Setd7* between P9-P12 as described above. Afterwards, animals were returned to the home cage, weaned at P21, and aged to adulthood in standard group-housing conditions. At >P60, female mice underwent 3 days of mild variable stress and were subsequently patched on the fourth day. The mild variable stress consisted of tail hang for one hour on the first day, tube restriction for one hour on the second day, and foot shock for one hour (100 random mild foot shocks at 0.45 mA) on the third day, to be consistent with prior stress work in females (Peña et al., 2019; Kim et al., 2026). At >P60, male mice were subjected to 10 days of subthreshold adult social defeat stress as described above and were patched on the eleventh day. Use of chronic social defeat stress for male mice allowed us to align electrophysiological results with prior published work recording in NAc after social defeat in male mice (Francis et al., 2014).

Mice were deeply anesthetized with isoflurane, rapidly decapitated, and brains removed into ice-cold choline cutting solution (composition in mM: 0.5 CaCl2, 110 C5H14ClNO, 25 C6H12O6, 25 NaHCO3, 7 MgCl2, 11.6 C6H8O6, 3.1 C3H3NaO3, 2.5 KCl and 1.25 NaH2PO4). 210um coronal sections were made through the NAc on a vibratome (Leica VT 2100) and allowed to recover in carboxygenated artificial cerebrospinal fluid (aCSF; composition in mM: 119 NaCl, 2.5 KCl, 1.3 MgCl2, 2.5 CaCl2, 1.0 Na2HPO4, 26.2

NaHCO3, and 11 glucose) at 32°C for 15 minutes. Slices were then maintained at room temperature in aCSF until recording.

For whole cell patch clamp recordings, hemisected slices were superfused with aCSF containing 100 uM picrotoxin and 2 mM kynurenic acid at 30 ± 2°C in the recording chamber. The NAc core, medial to the anterior commissure was targeted for patch clamp recordings. D1-MSNs were identified by fluorescence, while non-fluorescent cells were recorded as putative D2-MSNs. For putative D2-MSNs, we avoided cells with large soma sizes and excluded any neurons that exhibited spontaneous firing or burst firing in response to current injection, consistent with striatal interneurons (Kawaguchi, 1993; Johansson and Silberberg, 2020).

Glass recording pipettes were pulled to a tip resistance of 3 - 5 MΩ and filled with a potassium gluconate-based internal solution (composition in mM: 130 potassium gluconate, 10 phosphocreatine disodium salt, 4 $MgCl_2$, 3.4 Na_2ATP, 0.1 Na_3GTP, 1.1 EGTA, 5 HEPES). All recordings were made in whole cell configuration. To generate an input-output curve of neuronal excitability, 600 ms current steps were injected in increments of 25 pA from −150 to 500 pA and the numbers of spikes were quantified for each step. The current step at which the cell fired the first action potential was determined as the rheobase. Voltage sag was also measured from the same recordings using the negative current steps. Input resistance was calculated based on the average voltage change in response to 20 successive -10 pA current injections. Recordings were excluded if the series resistance varied by more than 20% over the course of recordings. Patch-clamp electrophysiology data acquisition was completed using Clampex 11.4 software (Molecular Devices).

Immunohistochemistry and imaging

To confirm viral targeting of the NAc following behavioral experiments, a subset of mice was anesthetized by i.p. injection of 100 mg/kg ketamine and 10 mg/kg xylazine, then transcardially perfused with ice-cold saline followed by 4% paraformaldehyde (PFA). Brains were dissected and submerged in fresh 4% PFA at 4°C overnight, then stored in 30% sucrose solution for an additional 2 days (until the tissue became isotonic). Brains were then kept at -80°C until slicing. For slicing, brains were embedded in Tissue-Plus™

O.C.T. Compound (Fisher Scientific) and sliced in 20µm sections on a cryostat at -20°C. Slices were mounted directly onto Superfrost™ Plus microscope slides (Fisher Scientific) and stored at -20°C until staining.

Immunohistochemistry entailed the following steps (at room temperatures unless noted): (1) Rinsing the slices in 1 X phosphate-buffered saline (PBS) 3 times for 5 min each. (2) Incubating the slices in 1.5% normal donkey serum (NSD) in 1 X PBS-Triton X-100 solution for 1 h. (3) Incubating the slices in primary antibody [1:500 Anti-Green Fluorescent Protein (Chicken) Antibody (Aves Labs); 1:150 SETD7 Mouse Monoclonal Antibody (OriGene); and 1.5% NSD in 1 X PBS-Triton X-100 solution] overnight at 4°C. (4) Rinsing the slices in 1 X PBS 3 times for 5 min each. (5) Incubating the slices in a secondary antibody solution [1:500 Alexa Fluor® 488 AffiniPure Donkey Anti-Chicken IgY (IgG) (H+L) (Jackson ImmunoReseach Labs); 1:500 Cy™3 AffiniPure Donkey Anti-Mouse IgG (H+L) (Jackson ImmunoReseach Labs); and 1.5% NSD in 1 X PBS-Triton X-100] for 1 h. (6) Rinsing the slices in 1 X PBS 3 times for 5 min each. (7) Incubating the slices in 0.0025% DAPI solution for 5 min. (8) Rinsing the slices in 1 X PBS 3 times for 5 min each. Following the last rinse, Fluoromount-G® mounting media (SouthernBiotech) was used to coverslip the immune-stained slices. Samples were kept at 4°C until imaging. Fluorescent imaging was performed with a Nanozoomer S60 slide scanner (Hamamatsu). All images were processed using the ImageJ (version 1.2.0) software containing the FIJI plugin (NIH, Bethesda, MD, USA).

RESULTS

Enriched H3K4me1 following ELS

To determine whether ELS altered development of chromatin modifications associated with enhancer priming and activity in NAc, we took advantage of published bottom-up mass spectrometry data that profiled more than 200 individual and combinatorial post-translational histone modifications in the NAc at P21, P35, and P70-80 of male mice following ELS (Kronman et al., 2021). We found both a main effect of age (two-way ANOVA, $F(2,12)=42.86$, $p<0.0001$), and a main effect of ELS on H3K4me1 ($F(2,12)=4.759$, $p=0.049$), such that after weaning both aging and ELS increased H3K4me1 (**Figure 1A**). This early enrichment suggests that ELS augments age-related enrichment of H3K4me1.

In contrast, di- and tri-methylation at H3K4 — histone modifications associated with active gene expression — decreased with age [main effect of age for H3K4me2: $F(2,12)=11.87$, $p=0.0014$; main effect of age for H3K4me3 $F(2,12)=22.69$, $p<0.0001$], but were unaffected by ELS (**Figures 1B-C**). Active enhancers are further distinguished from primed enhancers by the presence of H3K27Ac. Although this modification was below detection levels at P21 and P35, at P60, H3K27Ac was enriched in standard-reared NAc but not ELS (Welch's two-tailed $t=14.50$, $p=0.0002$; **Supplemental figure S1A**). We also found an interaction between ELS and age for H3K27me2, a histone modification associated with protecting enhancers from spurious activation (Ferrari et al., 2014), such that this modification was decreased following ELS but elevated in adulthood (**Supplemental Figure S1B**; $F(2, 12)=5.874$, $p=0.017$). There were no main effects or interactions with ELS for H3K27me3 or H3K36me3, both associated with inhibition of gene expression (**Supplemental Figures S1C-D**). Taken together, our findings that ELS augments and accelerates H3K4me1 accumulation in the NAc without simultaneous changes in markers of active gene expression are consistent with both premature development and priming of chromatin architecture.

Juvenile Setd7 overexpression alters long-lasting chromatin accessibility in NAc

Given the specific enrichment of H3K4me1 following ELS and the role of H3K4me1 in priming enhancers (Heintzman et al., 2007; Mercer et al., 2011; Griffith et al., 2024), we sought to test the impact of early accumulation of H3K4me1 in NAc on chromatin accessibility state. To do this, we took advantage of an epigenome editing tool recently validated by our group to over-express the histone mono-methyltransferase *Setd7* to enrich for H3K4me1 (Kim et al., 2026). AAV-*Setd7* broadly enriches for H3K4me1 without inducing further H3K4 methylation or other histone modifications associated with active gene expression, such as H3K27Ac. We hypothesized that juvenile *Setd7* overexpression and consequent H3K4me1 enrichment would induce a long-lasting open and accessible chromatin state associated with epigenetic priming. To test whether *Setd7* overexpression opened chromatin, we bilaterally overexpressed AAV-*Setd7* or AAV-*Gfp* (**Figure 2A**) in juvenile NAc at postnatal day P9-12 (n=5-6/group) and collected tissue punches for ATAC-sequencing in adulthood (P70-80).

Analysis of differentially accessible regions (DARs) showed that juvenile *Setd7* overexpression and H3K4me1 enrichment induced long-lasting changes in chromatin accessibility relative to *Gfp* controls, with nearly twice as many regions of opened vs closed chromatin (DESeq2 p.adj <0.1, $|\log_2 FC| > 0.5$; **Figure 2B-C; Supplemental Table 1**). Most differentially open chromatin regions and all closed chromatin regions were long-range cis-regulatory regions and putative enhancers (>1kb away from a transcription start-site and not within an exon; **Figure 2C**). Examples of both opened and closed intergenic regions are shown in **Figure 2D**. To determine the genes these DARs may be regulating (as enhancers do not always regulate their nearest gene neighbors; (Schoenfelder and Fraser, 2019)), we used a long-range target gene predictor tool based on publicly available histone modification and gene expression data (Grant and Bailey, 2021). Putatively primed genes (genes predicted to be regulated by open DARs) include immediate early genes and transcriptional regulators important for mesolimbic development and synaptic activity (*Fos, Homer1, Gadd45g, Cebpb, Cdkn1c, Prrx1*, and *Eif4ebp2*), genes encoding transmembrane receptors and channels (*Kcnq3, Grin1, Gab2*), and epigenetic regulators (*Tet1*) (**Supplemental Table 2**). Gene ontology (GO) analysis identified an enrichment of genes related to synaptic plasticity, neuron development and differentiation, and gene expression (**Figure 2E**). Putatively repressed genes (regulated by closed DARs) are enriched for those regulating various homeostatic processes, signal transduction, and axon guidance, among other processes (**Figure 2F, Supplemental Table 2**). Together, these results suggest developmental enrichment of H3K4me1 through *Setd7* overexpression in the NAc may facilitate response to future stimuli by driving a more open chromatin landscape and priming genes that promote transcriptional and synaptic activity.

Juvenile Setd7 overexpression in NAc mimics stress phenotype in D2-MSNs

A majority of neurons of the NAc are medium spiny neurons (MSNs) that can be distinguished by their expression of either type 1 (*Drd1*; D1) or type 2 (*Drd2*; D2) dopamine receptors. D1 and D2-MSNs play opposing roles in mediating stress-relevant avoidance and appetitive behavior, with stimulation of D2-MSNs exerting lateral inhibition over D1-MSNs and generally promoting avoidance (Lobo et al., 2010; Kravitz et al., 2012; Francis et al., 2014; Dobbs et al., 2016; Lemos et al., 2016; Peña, 2017; Fang and Creed, 2024). To test

the hypothesis that juvenile H3K4me1 enrichment via *Setd7* overexpression may alter neuronal physiology, we overexpressed *Setd7* in juvenile (P9-12) Drd1a-tdTomato mice and performed patch-clamp electrophysiology in D1- and D2-MSNs, distinguished based on presence (D1-MSNs) or absence of red fluorescence (D2-MSNs) (Pascoli et al., 2014; Creed et al., 2015). Putative D2-MSNs were also confirmed by absence of spontaneous or burst firing which may indicate interneurons (Kawaguchi, 1993; Johansson and Silberberg, 2020), see methods).

We found no change in the excitability metrics of D1-MSNs (**Figure 4A-G**) or their passive membrane properties (**Figure 4A-G**) induced by *Setd7* virus expression or adult stress. In contrast, *Setd7* overexpression in D2-MSNs resulted in a leftward shift in the input-output curve of spikes per current injection [**Figure 3A;** virus x current injection Coef= -0.011, Std.Err=0.003, z=-4.207, p<0.001], indicating subtle hyperexcitability of D2-MSNs. While D2-MSNs from GFP-injected mice that had undergone adult stress exhibited a similar leftward shift in the IO curve relative to unstressed GFP-controls [Effect of Stress x current step: Coef=0.032, StErr=0.013, z=2.436, p=0.014], there was no additional effect of *Setd7* expression [**Figure 3B**; Coef=-4.009, StErr=1.977, z=-2.028, p=0.043]. Together, this suggests that *Setd7* mimics the effect of adult stress on current firing of D2-MSNs, with further occlusion of the *Setd7* effect in stressed mice. Bolstering this interpretation, the input resistance of D2-MSNs was significantly increased by *Setd7* overexpression (p=0.006, U=53.00; Mann-Whitney test), an effect which was again occluded following adult stress *(***Figure 3D**; Virus x Treatment: Coef=-117,099, StErr=56.072, z=2.088, p=0.037, post-hoc t=-2.441, p=0.0216). There was no change in rheobase (Control: p=0.2219, U=69.00; Stress: p=0.4023, U=91.50, Mann-Whitney test) or resting membrane potential (**Figure 3C, E;** Control: p=0.2577, U=93.50; Stress: p=0.6498, U=75.00, Mann-Whitney test). Finally, *Setd7* overexpression increased the voltage sag elicited by hyperpolarizing current injections (**Figure 3F**), an effect which was again mimicked by adult stress, with no additive effect of *Setd7* overexpression [**Figure 3G**; Effect of virus; Coef=0.047, StErr=0.012, z=4.032, p<0.001]. Given the opposing roles of D1- and D2-MSNs in mediating avoidance behavior, these findings lead to a prediction that *Setd7* overexpression in the NAc may subtly shift the balance between D1- and D2-MSNs towards promoting avoidance behavior.

Epigenetic priming via postnatal H3K4me1 enrichment in NAc increases sensitivity to future stress

We next tested whether developmental enrichment of H3K4me1 primes behavioral response to future stress. We again overexpressed AAV-*Setd7* or AAV-*Gfp* in NAc at P10 to match the start of ELS (**Figure 5A-B**; (Peña et al., 2017)). We used a within-subject design and mixed-effect modeling to test main effects and interactions of sex, virus, and stress (**Figure 5C**) on behavioral tests before and after five days of sub-chronic adult social defeat stress (Balouek et al., 2023; Francis et al., 2014; Yohn et al., 2019). Although we powered this study to detect sex differences (n=10-19/sex/group), there were no significant main effects of sex and results are presented together for simplicity. All statistical results are in **Supplemental Table 3**.

Across males and females, both stress and postnatal epigenetic priming by *Setd7* decreased social interaction time, a measure of depression-like behavior and stress sensitivity [**Figure 5D-E;** main effect of stress: $F(1,44)=72.15$, $p<0.0001$; main effect of *Setd7*: $F(1,64) = 4.88$, $p=0.0306$; interaction between stress and sex: $F(1,44) = 6.54$, $p=0.014$); no main effect of sex]. Similarly, both stress and *Setd7* decreased social interaction ratio [**Figure 5F;** main effect of stress: $F(1,44)=20.61$, $p<0.0001$; main effect of *Setd7*: $F(1,64) = 5.148$, $p=0.027$; interaction between stress and sex: $F(1,44) = 2.959$, $p=0.0925$)]. Time spent huddling in corners, an additional measure of heightened vigilance and stress sensitivity (Duque-Wilckens et al., 2020), was increased by stress in combination with *Setd7* and sex [**Figure 5G**; main effect of stress: $F(1,44)=56.34$, $p<0.0001$; interaction between stress and *Setd7*: $F(1,44) = 7.705$, $p=0.008$); interaction between stress and sex: $F(1,44) = 4.229$, $p=0.046$); main effect of sex: $F(1,63) = 3.111$, $p=0.0826$); main effect of *Setd7*: $F(1,63) = 2.669$, $p=0.10$)]. Collectively, *Setd7* and stress impacted the proportion mice categorized as susceptible, indifferent, and resilient based on social avoidance behavior (**Figure 5H**; $X^2=66.13$, df=6, $p<0.0001$).

Time spent exploring the center of an open field (**Figure 5I**), a more generalized measure of threat avoidance, was sensitive to stress but not primed by juvenile *Setd7* overexpression [**Figure 5J**; main effect of stress: $F(1,46)=22.93$, $p<0.0001$; no main effects of *Setd7* or of sex]. Ambulatory behavior was not impaired by *Setd7* [**Figure 5K**; main effect of stress: $F(1,42)=21.33$, $p<0.0001$; no main effects of *Setd7* or of sex].

Setd7 overexpression in adult NAc minimally influences stress response

We next sought to determine whether the impact of epigenetic priming was specific to developmental enrichment of H3K4me1, or whether *Setd7* overexpression in adulthood might similarly heighten sensitivity to later stress. We therefore expressed AAV-*Setd7* or AAV-*Gfp* in adult NAc (P60-70), waited three weeks, and tested behavior before and after sub-chronic social defeat stress as before (**Supplemental Figure S2A-C**). All statistical results are in **Supplemental Table 3**.

In contrast to juvenile *Setd7* overexpression, there was no main effect of stress but there was a main effect of adult *Setd7* expression to decrease social interaction time, but this effect did not further interact with stress [**Supplemental Figure S2D;** main effect of *Setd7*: $F(1,30)=5.709$, $p=0.023$; interaction between stress and sex: $F(1,28) = 11.17$, $p=0.002$; interaction between *Setd7* and sex: $F(1,30) = 9.941$, $p=0.004$)]. Adult *Setd7* overexpression also increased time spent in arena corners, a classic metric of avoidance behavior, but did not further interact with stress [**Supplemental Figure S2F;** main effect of *Setd7*: $F(1,30)=4.022$, $p=0.050$].There were no main effects of stress, *Setd7*, or sex on social interaction ratio (**Supplemental Figure S2E**), and the percent of mice categorized as susceptible was unchanged (**Supplemental Figure S2G**). Interestingly, adult *Setd7* overexpression modestly increased rather than decreased time spent exploring the center of an open field [**Supplemental Figure S2H;** main effect of *Setd7*: $F(1,30)=4.178$, $p=0.050$] but did not alter distance traveled (**Supplemental Figure S2I**). These results indicate that *Setd7* overexpression in adulthood is insufficient to increase sensitivity to subchronic social defeat stress. Taken together, juvenile — but not adult — *Setd7* overexpression and H3K4me1 enrichment in NAc heightens sensitivity to stress later in adulthood, suggesting postnatal development is particularly sensitive to epigenomic perturbations.

DISCUSSION

Our findings presented here provide evidence that changes in the postnatal epigenome persist into adulthood and can impact neurophysiological and behavioral response to experiences such as stress. We show that ELS accelerates and augments postnatal accumulation of H3K4me1, and that postnatal enrichment of H3K4me1 through overexpression of the histone monomethyltransferase *Setd7* alters physiological

properties of D2-MSNs in the NAc and sensitizes behavioral response to adult social stress. These results help elucidate the mechanisms through which ELS programs long-lasting stress sensitivity and stress-induced risk for psychiatric disease through changes in molecular brain development.

Both acute and chronic stress dynamically alter post-translational histone modifications (Covington et al., 2011; Levine et al., 2012; Howerton et al., 2013; Heller et al., 2014; Pusalkar et al., 2015; Sun et al., 2015; Lepack et al., 2016; Bastle and Maze, 2019; Farrelly et al., 2019; Lepack et al., 2020; Morrison et al., 2020; Kronman et al., 2021; Torres-Berrío et al., 2024; Rashford et al., 2025; Kim et al., 2026). Through analysis of mass spectrometry data of histone modifications in NAc, we find that ELS augments levels of H3K4me1, a chromatin modification associated with epigenetic priming, without concomitant increases in histone modifications associated with active gene expression. ELS also increases H3K4me1 levels in VTA (Kim et al., 2026) and drives open chromatin in ELS-activated cells of VTA (Rashford et al., 2025) suggesting epigenetic priming as a concerted response to ELS across limbic brain regions.

Examination of histone modifications across multiple ages also indicates that H3K4me1 accumulation may represent accelerated epigenetic development, given that levels of this histone modification increase across age. Similarly, ELS accelerated NAc accumulation of histone variant H3.3, associated with activity-dependent neuronal transcription, after a period of initial suppression (Lepack et al., 2016). ELS has also been found to accelerate other aspects of brain development, such as connectivity between amygdala and prefrontal cortical regions (Gee et al., 2013) and markers of hippocampal development including parvalbumin interneuron maturation, myelination, and synaptic plasticity (Bath et al., 2016). Accelerated development within some cell types or brain regions decouples otherwise tightly-controlled neurodevelopmental processes, which has been hypothesized to prematurely reduce plasticity and the ability to appropriately learn from expected environmental experiences (Reh et al., 2020; Rakesh et al., 2023). Our results support an interpretation that these effects may arise from accelerated epigenetic development.

Our findings intriguingly show that developmental accumulation of H3K4me1 in NAc, but not adult-specific accumulation of H3K4me1, is sufficient to prime heightened behavioral sensitivity to stress (Peña, 2025b). This may be due to prolonged developmental maturation and plasticity of the epigenome through the

postnatal juvenile period that finally crystalizes in adulthood (Franklin et al., 2025; Heffel et al., 2024; Herring et al., 2022; Lister et al., 2013; Simmons et al., 2013; Stroud et al., 2017, 2020). However, it has yet to be determined whether adult-specific *Setd7* overexpression also fails to broadly alter chromatin opening or whether perhaps a different set of genomic regions may be altered based on distinct genomic regions accessible to the enzyme at different ages. Future studies may also test whether transient *Setd7* enzymatic activity in the juvenile period (as opposed to the long-term expression of AAVs) would become crystalized in the epigenome and carried forward to have similar persistent effects on physiology and behavior.

While *Setd7* overexpression here was not cell-type specific, prior single-nucleus RNA-seq within NAc shows that *Setd7* is expressed in nearly every cell type, with highest levels in D1 and D2 neuronal clusters and lowest levels in microglia and astrocytes (Phillips et al., 2023; Ianov and Day, 2024). Nevertheless, our results indicate a D2-specific neurophysiological mechanism. This is in line with prior work showing that repeated activation of D2-MSNs in stress-naïve mice induces sensitivity to later subthreshold social defeat stress (Francis et al., 2014). However, other studies have reported greater changes on D1-MSNs relative to D2-MSNs following chronic social defeat stress (Francis et al., 2014; Pignatelli et al., 2021), which we did not detect here. This may be due to the use of exclusively male mice in prior studies, owing to challenges in applying the chronic social defeat stress models in females. Moreover, prior changes in excitability were observed only in response to suprathreshold stress, with resilient individuals showing much more subtle physiological changes, consistent with the subtle adaptations we report following subchronic social defeat stress here. Another consideration is that our recordings were targeted to the NAc core, while prior work has not distinguished between subregions or may differentially define these territories (Francis et al., 2014; Heshmati et al., 2018; LeGates et al., 2018). Likewise, while our viral injections were targeted to the NAc core, the possibility that spread of the virus to adjacent areas may contribute to the observed behavioral effects of Sedt7 overexpression. Importantly, while different adult stressors were used for males and females across physiological and behavioral testing, our results indicate that juvenile *Setd7* sensitizes response to multiple forms of adult stressors across outcome measures, which relates to the wide variety of stressors humans experience. Finally, it is still unclear whether physiological adaptations on MSNs following chronic stress manipulations may reflect compensatory or homeostatic adaptations, since stimulating D1-MSNs promotes

resilience, despite the finding of increased excitability associated with stress susceptibility (Lobo et al., 2010; Francis et al., 2014; Francis and Lobo, 2016). In this context, our results are consistent with a large body of prior work suggesting that the balance between D1- and D2-MSNs mediates approach and avoidance behavior, with increased D2-MSN activity relative to D1-MSN activity being predicted to promote avoidance behavior as we see here (Kravitz et al., 2012; Creed et al., 2016; Francis and Lobo, 2016; Peña, 2017; Creed, 2018; Bariselli et al., 2019; Matikainen-Ankney et al., 2023).

In sum, these data provide novel evidence that altered postnatal chromatin maturation is sufficient to prime sensitivity to future stress. Our research provides an important foundation for additional research to develop therapeutic strategies targeting chromatin to ameliorate the impact of ELS on brain development and psychiatric disease risk.

Author Contributions

The chromatin and behavioral studies were designed by CJP and RLR. ATAC-seq data were collected by RLR and analyzed by RLR and MD. Viral manipulations, behavioral work, and immunohistochemistry were performed by RLR, HJJK, LH, EC and MRB. The physiology experiments were designed by MCC with input from CJP, performed by LZF, JMT, and analyzed by LZF, MRB, JMT, and MCC. The manuscript was written by RLR, MCC, and CJP with input from all authors.

Data Availability

All sequencing data is deposited in NCBI's Gene Expression Omnibus (GEO), accession number GSE268468. Sequencing data was analyzed via standardized pipelines as described.

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778 **FIGURE LEGENDS**

779

780 **Figure 1 | ELS selectively enriches for H3K4me1.** Proportion of H3K1-4 peptide fragments modified with (**A**)
781 H3K4me1, (**B**) H3K4me2, and (**C**) H3K4me3 in male NAc across postnatal development (reanalyzed mass
782 spectrometry data published in (Kronman et al., 2021). Proportion is relative to the sum of unmodified and
783 modified fragments. Mean±SEM; *p<0.05 for main effect of ELS.

784

785 **Figure 2 | Juvenile *Setd7* overexpression alters chromatin accessibility in adult NAc.** (**A**) Viral schematic
786 of AAV9-EF1a-EGFP-P2A-MYC-Setd7; control vector was AAV9-EF1a-EGFP. (**B**) Volcano plot of bulk ATAC-
787 seq showing chromatin regions with gained or lost accessibility with juvenile *Setd7* (n=5) vs. *Gfp* (n=6)
788 overexpression. Significant genomic regions (p.adj.<0.1 and $|\log_2 FC| > 0.5$) are shown in purple (more open
789 with *Setd7*) and gray (more closed). (**C**) Percentage of DARs opened vs closed (top). Among open (middle)
790 and closed (bottom) DARs, proportion mapping to long-range cis-regulatory elements (putative enhancers) or
791 to promoter regions. (**D**) Example RPM-normalized track overlays of an open region (left [chr10:59072341-
792 59072625]) and closed regions (center [chr18-86218908-86219130], and right [chr4:27247388-27247650]). (**E**)
793 Gene ontology enrichment for genes within or nearest to opened DARs. (**F**) Gene ontology enrichment for
794 genes within or nearest to closed DARs.

795

796 **Figure 3**: **Juvenile *Setd7* overexpression increases the excitability of D2-MSNs, mimicking**
797 **adulthood stress. A-B**. (Left) Input-output plot of action potentials elicited in response to successive
798 600ms current injections from (**A**) control and (**B**) mice that had undergone stress. (Right)
799 Representative traces of action potential firing in response to a 50 pA (top), 150 pA (middle), and 250
800 pA (bottom) current injection. There was a significant interaction between virus and treatment group
801 (Coef=-4.01, z=-2.028, p=0.043), and direct comparison of the AUC of the ascending limb of the IO
802 curve revealed greater firing in GFP mice following stress (Coef=0.032, StErr=0.013, z=2.436,

803 *p*=0.014). **C-E**. The effect of *Setd7* overexpression on intrinsic membrane properties, (**C**) rheobase,
804 (**D**) input resistance, and (**E**) resting membrane potential, of D2-MSNs. *Setd7* overexpression does
805 not alter the rheobase or resting membrane in either control or stressed mice but did alter input
806 resistance (Coef=117.099, StErr=56.072, z=2.088, *p*=0.037), post-hoc test revealed this was driven
807 by an increase in input resistance following Setd7 in control mice (*t*=-2.441, *p*=0.0216). (**F-G)**. The
808 peak change in voltage in response to successive 600ms hyperpolarizing currents from (**F**) control
809 and (**G**) mice that had undergone stress. *Setd7* overexpression increases the peak voltage sag in
810 control, but not stressed mice.

811 **p*<0.05; all data are depicted as mean ± SEM.

812

813 **Figure 4**: **Juvenile *Setd7* overexpression in NAc does not significantly alter D1-MSN currents.**

814 **A-B.** (Left) Input-output plot of action potentials elicited in response to successive 600ms driving
815 currents from control (**A**) and stressed (**B**) mice. (Right) Representative traces depict action potential
816 firing in response to a 50 pA (top), 150 pA (middle), and 250 pA (bottom) driving current. *Setd7*
817 overexpression did not affect action potential firing in D1 MSNs (Effect of virus: Coef=-0.120,
818 StErr=0.825, z=-0.145, *p*=0.885, Effect of virus x current step: Coef=0.001, StErr=0.009, z=0.103,
819 *p*=0.918) under control or stress conditions (Effect of virus x treatment x current step: Coef=0.008,
820 StErr=0.013, z=0.572, *p*=0.567).

821 **C-E**. The effect of *Setd7* overexpression on intrinsic membrane properties of D1 MSNs. *Setd7*
822 overexpression does not alter the rheobase (**C**) input resistance (**D**), nor the resting membrane
823 potential (**E**) of D1 MSNs in control or stressed mice.

F-G. The peak voltage change in response to successive 600ms hyperpolarizing currents from control (**F**) and stressed (**G**) mice. *Setd7* overexpression does not affect the peak voltage sag under control or stress conditions. All data are depicted as mean ±SEM.

Figure 5 | Juvenile *Setd7* overexpression in NAc increases behavioral sensitivity to future stress.

(**A**) Experimental Timeline. (**B**) Representative image and brain atlas showing viral targeting of NAc. Scale bar, 1mm. (**C**) Legend for statistical main effects and interactions and individual male (circle) and female (triangle) data points. (**D**) Social interaction testing schematic: time spent in the interaction zone or corners was measured (left). Heatmaps showing representative trials from GFP and SETD7 groups, respectively (right). Within the social interaction test, (**E**) social interaction time, (**F**) social interaction ratio (lower ratio is associated with greater social avoidance), and (**G**) time spent in corners, among male and female mice before (pre) and after (post) social defeat stress. (**H**) Percent of each group considered resilient, indifferent, or susceptible based on SI ratio. (**I**) Open field testing schematic (left). Heatmaps showing representative trials from GFP and SETD7 groups, respectively (right). Within the open field test, (**J**) time spent in the center of the arena, and (**K**) total distance traveled. Main effects and interactions from mixed-effect modeling are indicated above each plot. Post hoc pairwise comparisons using Fisher's LSD are depicted within each plot. ****p<0.0001; ***p<0.001; **p<0.01; *p<0.05. Data are represented as mean ± SEM.