ACF chromatin-remodeling complex mediates stress-induced depressive-like behavior

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Improved treatment for major depressive disorder (MDD) remains elusive because of the limited understanding of its underlying biological mechanisms. It is likely that stress-induced maladaptive transcriptional regulation in limbic neural circuits contributes to the development of MDD, possibly through epigenetic factors that regulate chromatin structure. We establish that persistent upregulation of the ACF (ATP-utilizing chromatin assembly and remodeling factor) ATP-dependent chromatin-remodeling complex, occurring in the nucleus accumbens of stress-susceptible mice and depressed humans, is necessary for stress-induced depressive-like behaviors. We found that altered ACF binding after chronic stress was correlated with altered nucleosome positioning, particularly around the transcription start sites of affected genes. These alterations in ACF binding and nucleosome positioning were associated with repressed expression of genes implicated in susceptibility to stress. Together, our findings identify the ACF chromatin-remodeling complex as a critical component in the development of susceptibility to depression and in regulating stress-related behaviors.

Although MDD is one of the most prevalent and debilitating disorders worldwide, it has been difficult to understand its pathophysiology and to develop more effective treatments1. Epidemiological studies have shown that environmental factors, such as stressful life events, and highly complex genetic variations act as important determinants of both susceptibility and resilience to MDD2–5. Maladaptive transcriptional regulation in response to chronic stress in limbic neural circuits, including reward-processing regions such as the nucleus accumbens (NAc), is thought to be a major contributor to the development of MDD4–10. An understanding of this transcriptional dysregulation is important for providing mechanistic insights into disease, as well as for identifying new therapeutic targets.

Chromatin—histone and non-histone proteins associating with DNA—serves as an organizer of the genome by condensing double-stranded DNA into multiple levels of higher order structures. Nucleosomes, each consisting of an octamer of core histones around which DNA is superhelically wrapped, are the basic packaging units of chromatin and are positioned at precise locations to modulate the accessibility of regulatory proteins to DNA, thereby controlling eukaryotic gene regulation11. As a result, the mechanisms by which chromatin structure and nucleosome positions are specified and maintained in vivo are critical for the regulation of all DNA-dependent processes, including gene transcription. Epigenetic events—in particular, histone modifications—that alter chromatin structure to regulate programs of gene expression have increasingly been associated with depression-related behavioral abnormalities in animal models and depressed humans5,12–20. ATP-dependent chromatin-remodeling complexes also have a key role in regulating nucleosome positioning to control gene expression, but they have not yet been investigated in depression or other psychiatric disorders21–25.

Here we demonstrate that the specific and persistent upregulation of BAZ1A (also known as ACF1), a subunit of the ISWI-family ACF chromatin-remodeler complex, in NAc in several mouse depression models and in depressed humans contributes to susceptibility to stress-induced depressive-like behaviors by regulating nucleosome architecture at transcriptional start sites (TSSs) and repressing the expression of a subset of genes. This identifies ATP-dependent chromatin-remodeling dysregulation as a key mechanism in depression pathophysiology and provides previously unrecognized candidate targets for improved therapeutics for depression and other stress-related disorders.

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RESULTS

Regulation of ACF complex in mouse models and human depression

As ATP-dependent chromatin remodelers have a key role in regulating nucleosome positioning and transcriptional regulation, we screened subunits in four families of remodeler complexes (SWI/SNF, ISWI, CHD and INO80) in NAc of an ethologically validated mouse model of depression, chronic social defeat stress (CSDS)20,26,27. Over 10 consecutive days, we subjected C57BL/6J male mice to daily 10-min aggressive encounters with CD1 mice; these encounters were followed by sensory but not physical contact for the remainder of the day. After CSDS, ~65% of test mice—termed susceptible—exhibited depression-related behavioral abnormalities including social avoidance (Fig. 1a) and reduced sucrose preference; those that behaved similarly to control, nonstressed animals and did not exhibit these behaviors were termed resilient27. Initial profiling showed persistent NAc mRNA-expression changes in subunits from all four families of chromatin-remodeling complexes 10 d after the last CSDS session (Supplementary Fig. 1a). Expression of the ISWI subunit gene Baz1a showed robust induction in NAc of susceptible mice, and thus was the focus of subsequent investigations. BAZ1A associates with the ATPase SMARCA5 (also known as SNF2H) to form the ACF complex, which serves several cellular functions, including transcriptional regulation28.

We characterized regulation of Baz1a and Smarca5, as well as of the closely related ISWI accessory subunit gene Baz1b (also known as Wstf), over time after the last defeat session. Levels of both Baz1a mRNA and BAZ1A protein were upregulated in NAc of susceptible mice 48 h after CSDS compared with levels in nonstressed control animals (Fig. 1b,c). These levels were not different between resilient and control animals (Fig. 1b,c). BAZ1A upregulation in NAc of susceptible animals persisted through 10 and 28 d after the last defeat session (Supplementary Fig. 1b–e), indicating that it is a highly stable adaptation. In contrast, Baz1b and Smarca5 mRNA and protein levels were not altered at any of these time points (Fig. 1bc and Supplementary Fig. 1b–e). Additionally, coimmunoprecipitation experiments showed increased levels of ACF complexes (SMARCA5 coprecipitated BAZ1A) in NAc of susceptible mice (Supplementary Fig. 1f, P < 0.05), with no change observed for BAZ1B–SMARCA5 complexes. Persistent induction of Baz1a required CSDS, as no change in gene regulation was observed 48 h after a single defeat session (Supplementary Fig. 1g). Baz1a induction in susceptible NAc after CSDS was also region specific, as no changes were observed in medial prefrontal cortex (mPFC) of susceptible mice (Supplementary Fig. 1h,i). Similar induction of Baz1a was observed in NAc of another depression model 48 h after 6 d of chronic unpredictable stress (a combination of tail suspension, shock and restraint stress), and this effect was seen in both male and female stressed mice compared with nonstressed controls (Fig. 1d).

Next, to validate our findings in mice, we examined NAc of postmortem brains of depressed humans and matched control subjects in two separate cohorts (Supplementary Table 1). In both cohorts, we observed increased BAZ1A mRNA levels in NAc of depressed patients compared with controls, with no change seen for SMARCA5 or BAZ1B mRNA (Fig. 1e). These data together suggest that elevated levels of BAZ1A and the resulting ACF complex in NAc are associated with susceptibility to stress and depression.

Figure 1 Chromatin remodeling after CSDS in mice and in depressed humans. (a) Schematic of CSDS and social interaction (SI) test and representative data for control, susceptible and resilient mice. (b,c) NAc mRNA (b) and protein (c) levels 48 h after CSDS. For b, n = 10 control, 11 susceptible and 7 resilient mice. For c, n = 6–7 control, 7–8 susceptible and 6–7 resilient mice. (d) NAC Baz1a mRNA levels 48 h after 6 d of chronic unpredictable stress (CUS) in male and female mice. An experimental schematic is shown at the top. n = 7 for control male, stressed male and stressed female mice; n = 8 for control female mice. (e) mRNA levels in postmortem human NAC in two separate cohorts (demographic information in Supplementary Table 1). For cohort 1, n = 10–11 control and 9–10 depressed subjects. For cohort 2, n = 9 control and 20 depressed subjects. *P < 0.05 compared with respective controls (post hoc Student’s t-test). Error bars represent s.e.m. Detailed statistics are presented in the Supplementary Notes.
Upstream regulators of Baz1a regulation

We next studied potential upstream mechanisms responsible for the persistent upregulation of Baz1a after CSDS. In cultured mouse striatal neurons, KCl-mediated depolarization (4-h treatment) increased Baz1a mRNA levels, suggesting that the gene's expression is activity regulated (Fig. 2a). Previous studies have shown that increased burst firing of ventral tegmental area (VTA) dopamine neurons is a key mechanism driving susceptibility after CSDS27,29. Therefore, we sought to investigate whether optogenetically controlled stimulation of VTA-to-NAc projections, mimicking the increased burst firing observed in susceptible animals, similarly induces Baz1a and susceptibility to social defeat stress. We injected AAV-CaMKIIe-ChR2 expression vectors into VTA, hippocampus or mPFC of mice and used an implanted optic fiber targeting the NAc shell to stimulate ChR2-expressing terminals. We observed that ten sessions (5 min d⁻¹ over 10 consecutive days) of stimulation of VTA neuronal projections to NAc in control animals mimicked the induction of Baz1a in NAc by CSDS (Fig. 2b). This effect was specific to the VTA-NAc pathway, as stimulation of NAc nerve terminals projecting from mPFC or hippocampus had no effect (Supplementary Fig. 2a,b). In subsequent behavioral analysis, these animals did not exhibit changes in basal social interaction (Supplementary Fig. 2c,d). However, they did display increased susceptibility to an accelerated social defeat stress paradigm (two daily defeats for 4 d, equally robust in inducing susceptibility as CSDS and used here to minimize damage to optogenetic cannula) compared with nonstimulated controls (Fig. 2c). It has also been shown that the release of brain-derived neurotrophic factor (BDNF) in NAc mediates the increased susceptibility caused by increased firing of VTA neurons30. We observed that BDNF application increased Baz1a expression both in primary striatal neurons in vitro and in NAc of control animals in vivo, suggesting that BDNF release in NAc may increase susceptibility in part via Baz1a induction (Fig. 2a,d). These results support the hypothesis that the persistent upregulation of the ACF complex in NAc, mediated at least partly by increased VTA-projection firing and BDNF release in this region, is a key driver of susceptibility to stress and depression.

BAZ1A modulates depressive-like behaviors

To directly test the hypothesis that elevated levels of BAZ1A and resulting increased ACF-complex formation are key drivers of stress susceptibility, we manipulated levels of ACF complex selectively in NAc of adult mice and then examined the animals’ susceptibility to stress using a subthreshold social defeat paradigm known as “microdefeat” to reveal pro-susceptibility effects of experimental manipulation. Herpes simplex virus (HSV)-mediated gene transfer resulted in the rapid overexpression of BAZ1A or SMARCA5 in NAc (Fig. 3a and Supplementary Fig. 3a,b, P < 0.05). After 3 d of virus expression, mice were subjected to microdefeat consisting of three 5-min defeat sessions with 15 min of rest in between within a single day; this protocol does not induce social avoidance or reduced sucrose preference in normal mice27. Mice that received intra-NAc injections of HSV-GFP, or of HSV-BAZ1A or HSV-SMARCA5 alone, showed no behavioral effects of the stress (Fig. 3a). However, overexpression of both BAZ1A and SMARCA5 increased susceptibility; mice in this condition exhibited both social avoidance and decreased sucrose consumption (Fig. 3a). The pro-susceptibility effect of ACF complex (BAZ1A + SMARCA5) overexpression was region specific, as overexpression in mPFC did not affect social interaction or sucrose preference (Supplementary Fig. 3c).

To extend our finding beyond acute paradigms (i.e., microdefeat) to chronic paradigms of social defeat, we used the accelerated social defeat protocol, which induces behavioral deficits in control mice overexpressing HSV-GFP within the time frame of maximal HSV-mediated transgene expression (Fig. 3b)27. Animals overexpressing ACF in NAc showed greater reductions in social interaction and sucrose preference. Given the high level of comorbidities between MDD and anxiety disorders31, we examined anxiety behavior on the elevated plus maze and found that ACF overexpression increased anxiety-like behavior (decreased time spent on the open arms) compared with that seen in GFP controls after accelerated defeat (Fig. 3b). Thus, induction of the ACF complex in NAc during a course of social defeat stress increases susceptibility.

To examine whether ACF-complex induction is also required for susceptibility, we virally overexpressed a microRNA (miRNA) in mice that specifically reduced BAZ1A protein levels in NAc by ~50% (Supplementary Fig. 3d, P < 0.05). Mice that received AAV-BAZ1A miRNA in NAc exhibited increased social interaction after CSDS compared with control animals, indicative of a pro-resilience effect (Fig. 3c). Animals expressing AAV-BAZ1A miRNA also showed increased sucrose preference and a trend for decreased anxiety-like behavior (Fig. 3c). Additionally, there was a negative trending correlation between Baz1a expression levels and both social interaction (r = −0.23, P = 0.16) and sucrose preference (r = −0.39, P < 0.05) (Supplementary Fig. 3e,f), providing further support for ACF’s role in mediating stress susceptibility and depressive-like behaviors.
that had already undergone CSDS or in stress-naive mice (Fig. 3d and Supplementary Fig. 4a,b). These findings suggest that ACF’s pro-susceptibility effect occurs very specifically in the context of stress exposure. Additionally, overexpression of ACF in NAc of control mice did not alter several other domains of behavior, including locomotor activity and freezing behavior in contextual fear conditioning (Supplementary Fig. 4c,d).

To determine whether persistent BAZ1A induction is necessary to maintain the susceptible phenotype, we knocked down BAZ1A in NAc of susceptible animals through the use of AAV-BAZ1A miRNA after CSDS. Whereas control animals continued to display social avoidance 4 weeks after the viral infusion, as shown previously, those that received the AAV-BAZ1A miRNA showed reversal of this behavioral abnormality (Fig. 3e), indicating that suppression of ACF in NAc exerts antidepressant-like effects in previously stressed mice. Together, these data suggest that ACF in NAc is a necessary and causal component driving susceptibility to stress and depression.

**Increased ACF binding and nucleosome remodeling after CSDS**

Relatively little is known about the action of chromatin-remodeling complexes in mammalian brains. We used chromatin-immunoprecipitation sequencing (ChIP-seq) to determine the locations of BAZ1A and SMARCA5 binding genome-wide in NAc of susceptible and resilient mice after CSDS compared with controls. First, these samples all had good normalized strand coefficients and relative strand coefficients that exceeded ENCODE standards, suggesting good antibody quality and good signal-to-noise ratios (Supplementary Table 2). Second, Pearson correlation analysis showed that these samples generated consistent peaks and signals across samples, suggesting that the enrichments relative to the input were meaningful signals, not random noise (Supplementary Table 3). Third, close examination of ChIP-seq tracks identified many coincident BAZ1A and SMARCA5 peaks (Pearson correlation between BAZ1A and SMARCA5
ChIP-seq replicates: $r = 0.41$, adjusted $P < 2.2 \times 10^{-16}$ throughout both genic and intergenic regions, with significant enrichment relative to the input (Supplementary Fig. 5a,b). Whereas some regions of the genome showed more defined peaks of BAZ1A and SMARCA5 binding, other regions exhibited more diffuse signals across broad regions (Supplementary Fig. 5c–f). To address both types of binding, we used ChromHMM software to identify sites with high coincident binding of both BAZ1A and SMARCA5 (i.e., ACF complex). In agreement with our biochemical data of upregulated ACF in NAc of susceptible mice (Fig. 1 and Supplementary Fig. 1), this analysis showed more than twice the number of condition-specific ACF complex–binding sites in NAc of susceptible mice (691) compared with both control (329) and resilient (288) mice (Fig. 4a; genomic loci in Supplementary Fig. 4). Heat map analysis (Fig. 4b) and close examination of ChIP-seq tracks (Supplementary Fig. 5c–f) confirmed increased coincident binding of BAZ1A and SMARCA5 at identified loci in both genic (~30%) and intergenic (~70%) regions (Supplementary Fig. 6a).

Because ISWI complexes regulate nucleosome repositioning, we also generated a nucleosomal map from total H3 ChIP-seq data in NAc under control and CSDS conditions. We identified more than 70,000 occupancy changes (altered density of nucleosomes) and 4,000 shift changes (altered position of nucleosomes), with the majority occurring between susceptible and control animals (Fig. 4c and Supplementary Table 5), consistent with dynamic nucleosome remodeling in NAc of susceptible animals after CSDS. Approximately two-thirds of both occupancy and shift changes occurred in intergenic regions, and the rest occurred in genic regions (Supplementary Fig. 6b,c). The majority of occupancy changes represented decreased occupancy in NAc of susceptible or resilient mice compared with controls, and there was an equal distribution of upstream and downstream shift change events (Supplementary Fig. 6b,c).

Sites of ACF binding across the different conditions overlapped significantly with differential nucleosome occupancy and shift changes; this overlap was particularly evident for sites with enriched ACF-complex binding and altered nucleosome positioning (shift) in susceptible mice (Fig. 4d). These findings led us to speculate that genes that show increased ACF binding in NAc of susceptible animals might also show altered nucleosome architecture. We therefore plotted the promoter nucleosome distribution for this subset of genes with susceptible enriched ACF binding (Supplementary Table 4) under control, susceptible and resilient conditions (Fig. 4e; overlay in Supplementary Fig. 6d), as promoter nucleosome architecture has the best-characterized role in transcription. In control animals, these genes showed a strongly positioned +1 and −1 nucleosome in addition to a well-defined nucleosome-depleted region (NDR) (Fig. 4e). Susceptible animals, in which ACF binding is enriched, lacked a well-defined NDR and a strongly positioned −1 nucleosome, and the occupancy of the +1 nucleosome was lower than that in control animals. These findings suggest a possible redistribution of the −1 and, to a lesser extent, the +1 nucleosome across TSSs in susceptibility (Fig. 4e and Supplementary Fig. 6d). In contrast, the promoter nucleosome positioning in resilient animals after CSDS largely resembled that in control animals, with a strongly defined +1 nucleosome and a well-defined NDR. The occupancy of the +1 nucleosome in resilient animals, however, was lower compared with that in control animals, with a −1 nucleosome that was shifted farther from the TSS. To quantify this difference in NDR between susceptible and control or resilient animals, we calculated the ‘height’ of the NDR (top of the +1 nucleosome − bottom at
the TSS) and found that the magnitude in susceptible animals was approximately half that in control and resilient animals for the subset of genes that showed increased ACF enrichment in susceptibility (Supplementary Fig. 6d).

To further test whether the change in nucleosome architecture was associated with altered ACF binding, we examined a group of approximately 500 randomly generated genes (Supplementary Table 6) that do not exhibit ACF-complex enrichment in NAc of susceptible animals. Unlike for the subset of genes that showed increased ACF-complex enrichment in susceptible animals, the promoter nucleosome positioning for these randomly generated genes did not differ among control, susceptible and resilient animals, and all of the groups exhibited strongly positioned +1 and −1 nucleosomes as well as a well-defined NDR (Supplementary Fig. 6d). Additionally, the NDR height did not differ among the different groups (Supplementary Fig. 6d). However, similar to the subset of genes that showed increased ACF complex enrichment in susceptible animals, the nucleosome occupancy of these randomly generated genes seemed decreased in susceptible and resilient animals relative to that in controls, in particular at the +1 nucleosome (Supplementary Fig. 6d). These results together suggest that certain aspects of altered nucleosome occupancy and positioning (in particular the lack of a well-defined NDR and −1 nucleosome) are associated with increased ACF enrichment in the NAc of susceptible animals, whereas other aspects (in particular decreased occupancy at the +1 nucleosome) in susceptible and resilient animals seem to be more global phenomena that are not specific to genes that show increased ACF enrichment.

**ACF target genes mediate susceptibility to stress**

Previous studies reported that ACF represses gene transcription.

Here, in NAc of mouse brain, we observed that BAZ1A binding at promoters was inversely correlated with gene expression (Supplementary Fig. 6e). This suggested that increased ACF binding at target genes, with associated altered nucleosome architecture at the respective promoters, likely mediates the repression of target gene transcription in NAc of susceptible animals after CSDS.

To test this hypothesis, we screened 25 randomly selected genes from among the ∼100 testable genes that showed increased ACF binding in susceptibility (Supplementary Table 4; 150 genic loci mapping to 145 genes; 22 were unannotated, and ∼30 had low to no expression in NAc) and observed that 15 of the 25 showed significant or trending decreased mRNA expression in NAc of susceptible mice (Fig. 5a). To directly demonstrate that ACF mediates repression of these genes, we examined NAc of animals that underwent CSDS with and without BAZ1A knockdown by use of AAV-BAZ1A miRNA. Compared with controls, BAZ1A knockdown in NAc reversed the
FIG. 5b. Furthermore, the AAV-BAZ1A miRNA–injected animals that showed reversal of gene repression also showed reversal of social avoidance behavior (Fig. 3c), suggesting that the repression of genes regulated by ACF contributes to stress susceptibility.

We thus investigated directly whether decreased expression of such ACF targets contributes to the susceptible phenotype. Overexpression of two validated targets, RAB3B and AGTR1B, in NAc partially reversed the social avoidance and sucrose-preference deficits in mice susceptible to CSDS (Fig. 5c,d and Supplementary Fig. 7a–c, P < 0.05), suggesting that ACF repression of these genes contributes to depressive-like abnormalities. Additionally, RAB3B expression levels were positively correlated (r = 0.6461, P < 0.01) with the extent of susceptibility reversal (Supplementary Fig. 7d). We further confirmed our results in human postmortem NAc and observed a significant decrease in RAB3B mRNA levels and a trend for decreased AGTR1 (the mouse genome encodes Agtr1a and Agtr1b at distinct chromosomal loci, whereas the human genome encodes only AGTR1 at a single locus) mRNA levels in depressed patients compared with controls (Fig. 5e). To further demonstrate the specificity of ACF gene repression in susceptible animals, we examined ten genes from the randomly generated gene list that had no ACF enrichment (Supplementary Table 6) or promoter nucleosome architecture change in susceptible animals and found that none of the genes showed gene expression regulation in NAc by CSDS or by BAZ1A knockdown (Supplementary Fig. 7e,f). Moreover, overexpression of one of those genes, PRMT5, in NAc of susceptible animals did not rescue social avoidance behavior (Supplementary Fig. 7g).

DISCUSSION

Here we show that the ISWI–complex subunit BAZ1A and the resulting ACF complex are persistently and selectively upregulated, possibly as a result of increased VTA neuronal activity and BDNF release, in NAc of mice that are susceptible to chronic social stress, as well as in NAc of depressed humans. We further establish that ACF induction is necessary and causal for susceptibility to stress-induced depressive-like behaviors. Using ChIP-seq, we found that increased ACF binding in NAc of susceptible animals after chronic stress was correlated with altered nucleosome positioning, particularly around the TSSs of affected genes. These alterations in ACF binding and nucleosome positioning were associated with repressed expression of a subset of genes in NAc of animals that were susceptible to chronic stress. Together, these findings establish that the ACF complex, presumably through its effects on chromatin remodeling, is a key mediator of gene repression that contributes to stress susceptibility (Fig. 5f).

Our study is in agreement with previous work demonstrating that yeast homologues of ISWI and other families of chromatin-remodeling complexes repress transcription by altering nucleosome positioning, in particular around TSSs23,24,37. Several studies in other systems have further confirmed ACF’s transcriptional repressive role.36,38–41

BAZ1A and ACF upregulation alone, without co-occurring stress, seems to be insufficient for inducing depressive-like behavior, but it is probably one of several key mechanisms important in mediating depression pathophysiology (more detail is provided in the Supplementary Discussion). We show that elevated BAZ1A levels in NAc were required for the induction and maintenance of depressive-like phenotypes, which indicates that reversal of this elevation may have therapeutic potential (Fig. 3e). Specific inhibitors of reader domains such as bromodomains have become increasingly popular for the treatment of several cancers; specific inhibitors of BAZ1A’s bromodomain may exert antidepressant efficacy. However, as the ACF complex is also involved in other important cellular processes such as DNA replication and repair, ACF may not be an ideal therapeutic target. Thus, identification of ACF target genes—by studying the location of the ACF complex and its regulation of TSS nucleosome repositioning—could reveal new therapeutic targets that might have fewer off-site effects. Several bona fide targets (Rab3b, Prdm16, Zbb7c, Agtr1b, Plcz1 and Espn1) were identified here on the basis of the following criteria: increased binding of ACF under susceptible conditions, specific suppression of gene expression under susceptible conditions and reversal of gene suppression upon BAZ1A knockdown. The rescue of social avoidance and sucrose-preference phenotypes by overexpression of RAB3B or AGTR1B in NAc of susceptible mice and the significant decrease in RAB3B mRNA levels observed in NAc of depressed humans offer further support for the potential of our approach in identifying new therapeutic targets through ChIP-seq analysis of the ACF complex.

Our analysis of chromatin-remodeling factors revealed several other regulators that may contribute to stress susceptibility or resilience (Supplementary Fig. 1a), and our nucleosome maps before and after CSDS showed many changes not accounted for by ACF. Therefore, this study is only the beginning of examining ATP-dependent nucleosome remodelers in the pathophysiology of depression. Together, these studies elucidate a previously unrecognized mechanism of ATP-dependent chromatin remodeling in the gene regulation implicated in the persisting pathophysiology of depression. The studies thereby provide a new approach for the identification of novel therapeutic targets for the treatment of stress-related disorders.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. ChIP-seq data for BAZ1A, SMARCA5 and nucleosome positioning (total H3 MNase-seq) have been deposited in GEO with the accession code GSE54263.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

H.S. and E.J.N. conceived the study and wrote the manuscript. H.S. performed some experiments with the help of D.M.D.-W., K.N.S., C.D., J.R., J.W.K., H.S. and E.J.N. conceived the study and wrote the manuscript. H.S. performed some experiments with the help of D.M.D.-W., K.N.S., C.D., J.R., J.W.K., E.K., R.C.B., F.H.A., M.E.C., B.L., E.M., E.A.H., H.C., S.A.G., S.J.R., P.J.K., I.M. and D.M.D. N.-Y.S., S.A., P.V.-W. and L.S. conducted bioinformatics analysis. K.G., G.T. and C.T. provided post-mortem human brain samples. R.N. provided the HSV overexpression vectors. C.D.A. and P.V.-W. provided plasmid and antibody reagents.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Animals and treatments. For all experiments, 7–8-week-old C57BL/6j male and female mice (The Jackson Laboratory, Bar Harbor, ME, USA) were housed in a colony room set at a constant temperature (23 °C) on a 12-h light/dark cycle (lights on from 07:00 to 19:00 h) with ad libitum access to food and water. All protocols involving mice were approved by the Institutional Animal Care and Use Committee at the Icahn School of Medicine at Mount Sinai (New York, NY, USA).

Sample and subject selection and general experimental practices. Sample sizes for all experiments were predetermined on the basis of extensive laboratory experience with these end points. All samples and subjects that made it through the endpoint of the experiments successfully were included in the analysis. Values were excluded (one maximum per group) only if they were considered outliers on the basis of the Grubbs’ test. This criterion was pre-established. For all molecular and behavioral experiments, animals were randomly assigned to groups. For all molecular experiments, tissue collected from treated animals was randomly pooled to provide ample tissue for biochemical procedures and to minimize variance across cohorts. Additionally, the investigator was blinded to the treatment group until all data had been collected.

NAc RNA isolation and quantitative RT-PCR. Bilateral punches of NAc and mPFC were obtained at various times after the last defeat session and frozen on dry ice. Samples were then homogenized in TRIzol and processed according to the manufacturer’s instructions (Life Technologies, NY, USA). RNA was purified with RNAeasy Micro columns (Qiagen, CA, USA) and reverse transcribed using an iScript kit (Bio-Rad, Hercules, CA, USA). cDNA was quantified by quantitative PCR using SYBR green. Each reaction was performed in duplicate and analyzed according to the standard ΔΔC_{t} method using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a normalization control. Supplementary Table 7 contains a complete list of primers.

Western blotting. Frozen NAc tissue was homogenized in 30 µl of RIPA buffer containing 10 mM Tris, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, and protease inhibitors (Roche, Basel, Switzerland) using an ultrasonic processor (Cole Parmer, Vernon Hills, IL, USA). Protein concentrations were determined using a DC protein assay (Bio-Rad), and 50 µg of protein were loaded onto 4–15% gradient Tris–HCl polyacrylamide gels for electrophoresis fractionation (Bio-Rad). Proteins were transferred to nitrocellulose membranes, blocked with Odyssey blocking buffer (Li-Cor, Lincoln, NE, USA), and incubated overnight at 4 °C with primary antibodies (BAZ1A: A301-318A, Bethyl, 1/500; BAZ1B: ab51256, Abcam, 1/500; SMARCA5: ab37479, Abcam, 1/1,000) in Odyssey blocking buffer. After thorough washing with 1× Tris-buffered saline plus 0.1% Tween-20, membranes were incubated with IRDye secondary antibodies (1/5,000 to 1/10,000; Li-Cor) dissolved in Odyssey blocking buffer for 1 h at room temperature. For analysis, the blots were imaged with the Odyssey Infrared Imaging system (Li-Cor) and quantified by densitometry using ImageJ (NIH, Bethesda, MD, USA). The amount of protein blotted onto each lane was normalized to levels of GAPDH (Cell Signaling 2118, 1/30,000).

Immunoprecipitation. Anti-SMARCA5 (Bethyl, A301-018A) was incubated overnight with anti-rabbit magnetic beads (Invitrogen) at 4 °C. Frozen NAc tissue was homogenized with plastic pestles in 100 µl of RIPA buffer as described above and combined with 220 µl of immunoprecipitation buffer containing 16.7 mM Tris, 167 mM NaCl, 1.2 mM EDTA, 0.01% SDS, 1.1% Triton X-100, and protease inhibitors. The antibody–bead mixture was then added to the tissue lysate and incubated overnight at 4 °C. After three washes with buffer containing 20 mM Tris, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, and 0.1% SDS, the pulldown was dissociated from the beads with elution buffer (50 mM Tris, 10 mM EDTA, 1% SDS) at 65 °C and analyzed by western blotting as described above.

Cell culture. E16.5 mouse striata were dissected and dissociated in PBS and grown for 8 d in culture in neurobasal media supplemented with B27 (Gibco), glutamax (Gibco), and Pen-Strep (Gibco). Neurons were stimulated for 4 h with either BDNF (50 ng/mL, PeproTech) or KCl. Neurons were lysed and RNA was collected as described above.

Optogenetics. For circuit-specific stimulations, mice were injected with AAV-CAMKIIr-ChR2 or control vectors with the following coordinates: VTA, −3.3 anterior-posterior (AP), +1.05 mediolateral (ML), −4.6 dorsal-ventral (DV); hippocampus, −3.6 AP, +3.05 ML, −4.85 DV; and mPFC, +1.9 AP, +0.5 ML, −3.0 DV. After 9 weeks of recovery to allow for expression in terminals, we performed a second stereotaxic surgery to implant an optic fiber targeting the NAc shell (+1.4 AP, +1.5 ML, −4 DV). After 1 week of recovery, mice underwent 10 d of daily 5-min stimulation sessions. Stimulation parameters were 20 Hz, 40 ms, five spikes over 10 s (VTA), 20 Hz, 30 pulses per burst, with 10 s between bursts (hippocampus); or 30 Hz, 90 pulses per burst, 10 s between bursts (mPFC).

BDNF infusion. Recombinant human BDNF was infused into NAc (AP, 1.5; ML, ±1.5; and DV, −4; 10° angle). An infusion volume of 0.5 µL was delivered using a 5-µL Hamilton syringe over the course of 5 min (at a rate of 0.1 µL/min). The infusion needle remained in place for at least 5 min after the infusion before being removed to prevent backflow of the injection.

Human postmortem brain tissue. For cohort 1, NAc tissue, obtained from the Dallas Brain Collection at UT Southwestern, was analyzed for control and depressed subjects matched for age, postmortem interval, RNA integrity, and pH. A standard dissection technique was used, and the tissue was snap-frozen and stored at −80 °C. The UT Southwestern institutional review board (IRB) reviewed and approved the collection of this tissue for research use, and informed consent was obtained from all subjects or next of kin.

For cohort 2, brain tissue was obtained from the Quebec Suicide Brain Bank (QSBB; Douglas Mental Health Institute, Verdun, Québec, Canada). All individuals were group-matched for age, pH, and post-mortem interval. Inclusion criteria for both suicide completers and controls were as follows: the subject had to be of European ancestry and of French Canadian origin and had to have died suddenly without a prolonged agonal state. NAc was stored at −80 °C. This study was approved by the Douglas IRB, and signed informed consent was obtained from next of kin. Detailed demographic information for both cohorts is provided in Supplementary Table 1.

Generation of viral constructs. Human BAZ1A (ACF1)–pEGFP and SMARCA5 (SNF2H)–Flag cDNAs were cloned into HSV vectors. Briefly, we first cloned BAZ1A–pEGFP into pENTR1A cut with the same enzymes. Gateway cloning technology (Invitrogen) was then used to recombine it into HSV p1006 GW. SMARCA5–Flag was cut out of the original vector with NheI (5′) and SalI (3′). Its 5′ end was blunted with polymerase and cloned into HSV p1005+ with EcoRV (5′) and XhoI (3′). Mouse Rab3β (MC203236) and Agtr1b (MC208118) cDNAs were obtained from Origene (Rockville, MD). Rab3β was cut out of the original vector with EcoRI (5′) and XhoI (3′) and cloned into HSV p1005+, Agtr1b was cut out of the original vector with KpnI (5′) and XhoI (3′) and cloned into HSV p1005+. PRMT5 was excised from a human pcDNA3 plasmid using BamHI (5′) and EcoRI (3′) and cloned into HSV p1005+.

Viral-mediated gene transfer. HSV expression plasmids were packaged into high-titer viral particles as described previously26. Viral titers for these experiments were between 3 × 10^{10} and 4 × 10^{10} particles/ml. Mice were positioned in small animal stereotaxic instruments under ketamine (100 mg/kg)–xylazine (10 mg/kg) anesthesia, and their cranial surfaces were exposed. Thirty-three gauge syringe needles were bilaterally lowered into NAc (from bregma: AP, +1.6; ML, +1.5; DV −4.4 mm; 10° angle) or PFC (from bregma: AP, +1.8; ML, +0.75; DV −2.7 mm; 15° angle) to infuse 0.5 µL of virus. Infections occurred at a rate of 0.1 µL/min. Animals receiving HSV injections were allowed to recover for at least 24 h after surgery.

CSDS and behavior testing. CSDS was performed as described previously26,27. Briefly, an experimental C57BL/6j mouse was placed into the home cage of a CD1 mouse for 10 min, during which time it was physically defeated by the CD1 mouse. After the physical interaction, the CD1 and experimental mouse were
kept in sensory contact for 24 h by means of a perforated Plexiglas partition dividing the resident home cage in two. The experimental mice were exposed to a new CD1 mouse on each of 10 consecutive days. Subthreshold defeat involved exposing experimental mice to a novel CD1 male aggressor for 5 min three times, with 15-min intervals between exposures. A social-interaction test was performed 24 h after the last defeat. Mice were placed in an open field that included an interaction zone and two opposing corner zones. A social target (novel CD1 mouse) was placed in a metal mesh-plastic box in the interaction zone that allowed sensory but not physical interaction. Ethovision XT (Noldus) tracking software was used to measure the time that the test mouse spent in the interaction zone with and without the target CD1 mouse present (2.5 min). Subsequently, test animals were studied in a standard EPM for 5 min while being monitored by Ethovision XT. They were also monitored for their sucrose consumption in a sucrose-preference test over 24 h. Briefly, a solution of 1% sucrose or drinking water was provided in 50-ml tubes with stoppers fitted with ballpoint sippers. The weights of solutions each mouse consumed were recorded, and sucrose preference was calculated as a percentage (100 × volume of sucrose consumed/total volume consumed).

**Locomotor activity.** Locomotor behavior was tested in an open field box 3 d after viral infusion. Animals were placed in the box for 2.5 min, and Ethovision XT was used to track and analyze the distance traveled by each mouse.

**Contextual fear conditioning.** Animals were put into a sound-proof, contextually distinct chamber 3 d after viral infusion for 5 min. Three shocks (0.7 mA/s for 2 s) were delivered 2, 3 and 4.5 min after the animal was placed into the chamber. 24 h after training, the animal was put back into the same chamber and recorded for 5 min. Freezing behavior was scored.

**ChIP, library preparation, and sequencing.** Three fully independent biological replicates were obtained per mark per condition. For each ChIP-seq replicate, bilateral 14-gauge NAC punctures were pooled from 5–10 mice. Tissue was lightly fixed to cross-link DNA (12 min with 1% formaldehyde followed by 5 min with glycine and eight washes with cold PBS) with associated proteins, and the material was further sheared (for BAZ1A and SMARCA5, a Bioruptor was used to obtain mostly 100–300-bp fragments) or MNase digested (for histone H3–nucleosome, nuclei were digested with 10 U/mL MNase at 37 °C for 10 min, and the reaction was stopped by the addition of EDTA to 20 mM; >80% mononucleosome was obtained) and immunoprecipitated using sheep anti-rabbit magnetic beads (Invitrogen) conjugated to an antibody that specifically recognized BAZ1A (Bethyl, A301-318A), SMARCA5 (Abcam, ab3749) or H3 (Abcam, ab1791). Immunoprecipitated DNA and total (input) genomic DNA were prepared for ChIP-seq using an Illumina kit according to the manufacturer’s instructions. Each experimental condition was analyzed with independent biological triplicates. Briefly, each sample underwent end repair followed by the addition of an A base to the 3’ end. Proprietary adapters were then ligated to the ends, and samples underwent size selection on a 2% agarose gel. The range of excision was 200–300 bp. After DNA cleanup, samples were amplified with 13 (H3) or 19 (BAZ1A and SMARCA5) cycles of PCR. Amplification and size selection were confirmed with a BioAnalyzer. The resulting libraries were sequenced on an Illumina HiSeq 2500 with 100-bp read length.

**ChIP-seq data analysis.** ChIP-seq data were aligned to the mouse genome (mm9) by CASAVA 1.8, and only unique reads were retained for analysis. FastQC ([http://www.bioinformatics.babraham.ac.uk/projects/fastqc/](http://www.bioinformatics.babraham.ac.uk/projects/fastqc/)) was applied for quality control, and then SAMTools ([http://samtools.sourceforge.net](http://samtools.sourceforge.net)) was used to remove potential PCR duplicates. PhantomPeak ([https://code.google.com/p/phantompeakqualtools/](https://code.google.com/p/phantompeakqualtools/)) was applied to estimate the quality and enrichment of the ChIP-seq data set. Additional ENCODE quality metrics, such as the normalized strand coefficient (NSC) and the relative strand correlation (RSC), were calculated. For all samples in our research, NSC ≥ 1.05 and RSC ≥ 0.8. Basic filtering and quality control confirmed that these samples were of strong quality and exceeded ENCODE standards ([Supplementary Tables 2 and 3](#)) (raw and genome browser–compatible files can be accessed at GEO with accession GSE42835). For the H3 MmNase ChIP-seq libraries, 150+ million raw reads were obtained for each replicate. With ~70% reads uniquely mapped and less than 20% duplicate reads, there were ~100 million uniquely mapped, non-redundant reads per replicate. This is an absolute coverage of the mouse genome and allows for sufficient analysis of genome-wide nucleosome mapping.

For visualization of the ChIP-seq data genome-wide, we used ngs.plot ([https://code.google.com/p/ngsplot/](https://code.google.com/p/ngsplot/)) to visualize the data set. All three replicates of the conditions were pooled and normalized to 1 million reads. The density of BAZ1A binding 1 kb up- and downstream of TSSs of coding genes in Ensembl annotations was plotted. The Corrgram package in the R software ([https://www.r-project.org](https://www.r-project.org)) was used to calculate and visualize the correlation between basal BAZ1A and SMARCA5. TDF files (all duplicate/redundant reads >2 removed) were applied in IGV for genome browser views of ChIP-seq tracks.

Coincident binding sites between BAZ1A and SMARCA5 were identified using ChromHMM ([http://compbio.mit.edu/ChromHMM/](http://compbio.mit.edu/ChromHMM/)). First, all BAZ1A and SMARCA5 ChIP-seq data were binarized at 200-bp intervals. The intervals were designated as enriched, or “1,” if the fold-enrichment threshold relative to the input was 2.0 or greater and the Poisson tail P value was ≤ 5 × 10-6. Otherwise, the intervals were designated as not enriched, or “0.” Then the binarized marks were fed into ChromHMM to estimate the states of the 200-bp intervals, and the training was iterated 200 times. The initiating number of states was four, and on the basis of the state-pruning strategy, we defined two states: one representing low to no binding of the two factors, and one representing high binding of both factors. To analyze the effect of CSDS, we extracted sites of high binding of both factors for control, susceptible and resilient conditions. A site was considered enriched or specific in one condition only if no other condition contained that enrichment site within 2 kb.

For analysis of nucleosome position and occupancy, we used DANPOS ([https://sites.google.com/site/danposdoc/](https://sites.google.com/site/danposdoc/)) in the dynamic analysis of nucleosomes ([Supplementary Figure 4](#)). This analysis focused on the position shift and occupancy change events. The false discovery rate (FDR) cutoff was 0.01 for both events. For shift events, the cutoff for the shifting distance was between 50 and 90 bp. For occupancy events, the FDR of the difference between treatment and control was <0.01. The overlap of condition enrichment of the ACF complex and nucleosome events was calculated using Fisher’s tests, with multiple tests corrected by Benjamini-Hochberg analysis.

**Statistical analysis.** Appropriate assumptions of data (for example, normal distribution or similar variation between experimental groups) were examined before statistical tests were conducted. Student’s t-tests were used whenever two groups were compared, and one-way and two-way analysis of variance was done wherever necessary to determine significance for all other data. Significant main effects (P ≤ 0.05) were further analyzed using post hoc tests. Detailed statistics are provided in figure legends and the [Supplementary Notes](#).

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