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Cell-Type-Specific Epigenetic Editing at the *Fosb* Gene Controls Susceptibility to Social Defeat Stress

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Chronic social defeat stress regulates the expression of *Fosb* in the nucleus accumbens (NAc) to promote the cell-type-specific accumulation of ΔFosB in the two medium spiny neuron (MSN) subtypes in this region. ΔFosB is selectively induced in D1-MSNs in the NAc of resilient mice, and in D2-MSNs of susceptible mice. However, little is known about the consequences of such selective induction, particularly in D2-MSNs. This study examined how cell-type-specific control of the endogenous *Fosb* gene in NAc regulates susceptibility to social defeat stress. Histone post-translational modifications (HPTMs) were targeted specifically to *Fosb* using engineered zinc-finger proteins (ZFPs). *Fosb*-ZFPs were fused to either the transcriptional repressor, G9a, which promotes histone methylation or the transcriptional activator, p65, which promotes histone acetylation. These ZFPs were expressed in D1- *vs* D2-MSNs using Cre-dependent viral expression in the NAc of mice transgenic for Cre recombinase in these MSN subtypes. We found that stress susceptibility is oppositely regulated by the specific cell type and HPTM targeted. We report that *Fosb*-targeted histone acetylation in D2-MSNs or histone methylation in D1-MSNs promotes a stress-susceptible, depressive-like phenotype, while histone methylation in D2-MSNs or histone acetylation in D1-MSNs increases resilience to social stress as quantified by social interaction behavior and sucrose preference. This work presents the first demonstration of cell- and gene-specific targeting of histone modifications, which model naturally occurring transcriptional phenomena that control social defeat stress behavior. This epigenetic-editing approach, which recapitulates physiological changes in gene expression, reveals clear differences in the social defeat phenotype induced by *Fosb* gene manipulation in MSN subtypes.
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INTRODUCTION

While acute exposure to stressful or rewarding stimuli transiently induces many Fos family transcription factors in the nucleus accumbens (NAc), chronic stimulation causes the stable accumulation of ΔFosB, a truncated product of the *FosB* gene (Hope *et al*, 1994), which mediates the bidirectional regulation of hundreds of ΔFosB-target genes in this brain region (McClung and Nestler, 2003; Robison and Nestler, 2011). Studies to elucidate the mechanism of *Fosb* activation by chronic stimuli have uncovered a crucial role of epigenetic remodeling. Drug and stress exposure regulate histone post-translational modifications (HPTMs) and transcription factor binding at the *Fosb* locus to control its expression (Maze *et al*, 2010; Vialou *et al*, 2010a). Recently, we demonstrated that targeting HPTMs specifically to *Fosb* in NAc neurons was sufficient to bidirectionally regulate *Fosb* gene expression as well as drug- and stress-evoked behaviors (Heller *et al*, 2014).

The NAc is composed mainly (~95%) of GABAergic projection medium spiny neurons (MSNs), which are segregated into two subtypes based on their specific enrichment of many genes, including dopamine receptor D1 (*Drd1*) or dopamine receptor D2 (*Drd2*), and by their differential outputs to subcortical structures (Graybiel, 2000; Kupchik *et al*, 2015; Lobo *et al*, 2006). Studies have revealed a role of ΔFosB accumulation in D1-type MSNs in mediating rewarding behaviors. ΔFosB induction in response to chronic administration of drugs of abuse predominates in D1-MSNs (Lobo *et al*, 2013), and its overexpression in this cell type causes plasticity at glutamatergic synapses in NAc (Grueter *et al*, 2013; Robison *et al*, 2013) as well as increased locomotor responses to cocaine (Kelz *et al*, 1999), increased conditioned place preference to both cocaine and morphine (Zachariou *et al*, 2006), and enhanced cocaine self-administration (Colby *et al*, 2003).

Cell-type-specific *Fosb* regulation also appears to be a critical mediator of depressive disorders, given that ΔFosB

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expression is reduced (Vialou *et al*, 2010b), and repressive histone methylation is increased at *Fosb* in the NAc of human depressed patients (Heller *et al*, 2014). The chronic social defeat stress (CSDS) model relies on territorial aggression, a natural mouse behavior, to produce core symptoms of depression as assessed by deficits in social interaction and reward preference (Donahue *et al*, 2014; Krishnan *et al*, 2007). Recently, Lobo *et al* (2013) found that ΔFosB expression is increased in the NAc after CSDS specifically in D2-MSNs of susceptible mice and in D1-MSNs of resilient mice. While ΔFosB overexpression in D1-MSNs of NAc promotes resilience to CSDS (Vialou *et al*, 2010b), the consequences of its induction in D2-MSNs remains unknown, and the reliance on overexpression systems may produce non-physiological results. Moreover, the causal relationship between stress-related behaviors and alterations in HPTMs at a single gene locus within these specific MSN subtypes remains unexplored. We have recently reported that *Fosb*-targeted histone H3 lysine 9 dimethylation (H3K9me2) in all NAc neurons promotes stress susceptibility (Heller *et al*, 2014), yet studies have found opposing functional roles of the G9a-mediated repressive histone modification, H3K9me2, in D1- and D2-MSNs (Maze *et al*, 2014; Schaefer *et al*, 2009). We therefore sought to uncover the cell-type-specific function of HPTM-mediated repression of *Fosb* in responses to CSDS.

To examine the role of epigenetic remodeling at the *Fosb* gene specifically in D1 and D2 NAc MSNs, we injected Cre-dependent viral vectors into the NAc of bacterial artificial chromosome (BAC) transgenic mice that selectively express Cre recombinase in either of these cell types. While previous studies have demonstrated that FosB/ΔFosB protein expression is differentially regulated in the two MSN cell types, the basal level of FosB/ΔFosB protein in the D1 and D2-Cre transgenic lines is the same (Lobo *et al*, 2013). Furthermore, while there is evidence that a subset of NAc MSNs express both D1 and D2 receptor proteins (Hasbi *et al*, 2009; Shetreat *et al*, 1996; Surmeier *et al*, 1996; Yung *et al*, 1995), extensive characterization of the D1 and D2-Cre transgenic mice used in this study find virtually no overlap of the Cre-expressing populations (Bateup *et al*, 2008; Lobo *et al*, 2013). We targeted HPTMs specifically to the *Fosb* promoter using engineered zinc-finger proteins (ZFPs), fused to either the transcriptional repressor, G9a, or transcriptional activator, p65 (Heller *et al*, 2014). We cloned our ZFP constructs into plasmids containing a lox-stop-lox cassette (LS1L) and packaged these plasmids into herpes simplex virus (HSV). We then delivered the HSVs into the NAc of BAC transgenic mice that selectively express Cre recombinase in either D1- or D2-MSNs, thus allowing for cell-type-specific expression of our ZFPs and, in turn, cell-type-specific epigenetic editing. Using this approach we found that epigenetic remodeling of the *Fosb* gene has opposite roles in these two NAc MSN subtypes in mediating the depressive-like phenotype evoked by social defeat stress.

MATERIALS AND METHODS

Animals and Treatments

Adult male 7–8-week-old mice and 6-month-old CD1 retired breeders (CD1 aggressors) were housed at 22–25 °C in a 12-h light–dark cycle and provided food and water *ad libitum*. All tests were conducted during the light cycle. Members of the same cage were randomly assigned to different experimental groups for behavioral studies. Experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at Mount Sinai.

Transcription Factor Engineering

All ZFPs were manufactured by the CompoZr ZFN Operations Group at Sigma-Aldrich Biotechnology and cloned in frame N-terminal to the p65 activation (Liu *et al*, 2001), or G9a repression domain (pre-SET and SET domains only) (Snowden *et al*, 2002). The *Fosb*-ZFP recognizes a 19 bp motif in the *FosB* promoter (5′-GATCCCCTCCCGCGAAGCC-3′), which is unique to the mouse genome (Heller *et al*, 2014).

Viral-Mediated Gene Transfer

Fosb-ZFPs were cloned into bicistronic herpes simplex viral vector (HSV-LS1L-*Fosb*-ZFP-p65/G9a) (Grueter *et al*, 2013), which expresses GFP under the CMV promoter. *Fosb*-ZFPs were inserted following a stop codon surrounded by loxP sites driven by the IE 4/5 promoter allowing expression of GFP in all transduced cells and expression of *Fosb*-ZFPs only in cells also expressing Cre recombinase. For visualization of Cre+ cells, mCherry was cloned into HSV vector with a Cre-dependent transcription cassette (HSV-LS1L-mCherry). Expression of the HSV transgene is maximal by 3–5 days after infusion and dissipates by days 8–10. Social defeat experiments were thus designed within this time frame. HSV vectors were selected because, unlike other types of viral vectors, HSVs have sufficient capacity to package the large ZFP constructs (>4.5 kb) and specifically infect neurons *in vitro* and *in vivo* (Neve *et al*, 2005). There has been extensive prior characterization of HSV-mediated transduction of the NAc, and it has been observed that the vast majority of infected neurons in the NAc (~95%) are MSNs (Barrot *et al*, 2002).

Stereotaxic Surgery

Mice were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and positioned in a small animal stereotaxic instrument. NAc was targeted bilaterally using the following stereotaxic coordinates: +1.6 (anterior/posterior), +1.5 (medial/lateral), and −4.4 (dorsal/ventral) at an angle of 10° from the midline (relative to Bregma). A total of 1 μl of virus was delivered on each side over a 5-min period, followed by 7 min of rest. In all behavioral experiments, proper NAc targeting of virus infusion was confirmed *post hoc* by preparing brain slices and visual confirmation of both needle track and GFP expression by stereoscopic fluorescence microscopy. Depending on the defeat paradigm (either accelerated or subthreshold), mice were allowed to recover for either 3 or 6 days post surgery, to standardize the amount of time between surgery and social interaction testing.

Immunohistochemistry

Mice were injected intra-NAc with an HSV cocktail containing a mixture of HSV-LS1L-*Fosb*-ZFP-G9a and

HSV-LS1L-mCherry according to the above protocol. Brain tissue was cleared via transcardial perfusion of chilled PBS for 30 s, followed by 5 min of 4% paraformaldehyde (PFA). Brains were postfixed overnight at 4 °C in 4% PFA. Immunofluorescence was performed using a mouse anti-FosB (ab11959, 1:500; Abcam), a rat anti-mCherry (M11217, 1:1000; Invitrogen), and a goat anti-GFP (ab5450, 1:1000; Abcam) primary antibody and corresponding secondaries (705-545-147, 711-175-152, and 712-165-150, 1:200, Jackson Immunoresearch Labs). Fluorescent images were visualized on an Olympus FluoView 1000 Filter-based Laser Scanning Confocal Microscope. Resulting images were analyzed using the ImageJ software (NIH) for pixel intensity of the Cy5 channel (FosB staining) in the nucleus of cells selected by a double-blinded experimenter.

Social Defeat and Behavior Testing

Two social defeat paradigms were used to calibrate the effects of the social defeat and reveal shifts in susceptibility or resilience of mouse cohorts. For the accelerated social defeat, mice underwent 4 days of social defeat stress as described previously (Berton *et al*, 2006; Dias *et al*, 2014; Krishnan *et al*, 2007). Mice were exposed for four consecutive days to a novel aggressive CD1 retired breeder for 10 min in a large hamster cage two times daily (AM and PM). Between bouts, mice were housed separated from the aggressor by a perforated divider to maintain sensory contact. Alternatively, subthreshold social defeat was used to detect mechanisms that promote a susceptibility to social stress (Chaudhury *et al*, 2013; Krishnan *et al*, 2007). Mice were exposed to a novel CD1 male aggressor three times for 5 min each, with 15 min intervals between each exposure. Mice were tested for social interaction 24 h after the last social defeat interaction according to published protocols (Berton *et al*, 2006; Krishnan *et al*, 2007). Based on the interaction ratio, defined as time spent with target/time spent with no target, mice were characterized as susceptible (<1) or resilient (>1) (Krishnan *et al*, 2007). Mice were tested in a standard elevated plus maze (EPM) for 10 min, monitored by Ethovision XT. Note that as with previously published protocols (Chaudhury *et al*, 2013; Heller *et al*, 2014; Heller *et al*, 2016; Walsh *et al*, 2014), this paradigm does not include a home-cage (non-defeated) control, as comparisons being made are within stressed groups.

Sucrose Preference Test

Immediately following the social interaction and EPM tests, mice were individually housed and given both a bottle containing water and a bottle containing a 1% sucrose solution with the left/right location balanced across animals. No previous food or water deprivation was applied before the test. Water and sucrose solution intake was measured daily by weighing the bottles. The positions of two bottles were exchanged every 24 h, to avoid position preferences. After 3 days of habituation, the sucrose preference on the fourth day was expressed as $(\Delta\text{weight}_{\text{sucrose}})/(\Delta\text{weight}_{\text{sucrose}} + \Delta\text{weight}_{\text{water}}) \times 100$.

Statistics

The appropriate statistical test was determined based on the number of comparisons being carried out. Student's *t*-tests were used for comparison of two groups, in the analysis of social interaction data, sucrose preference, and EPM. Effects were considered significant at $p<0.05$. Data are expressed as mean ± SEM. All experiments were carried out one to three times, and data replication was observed in instances of repeated experiments.

RESULTS

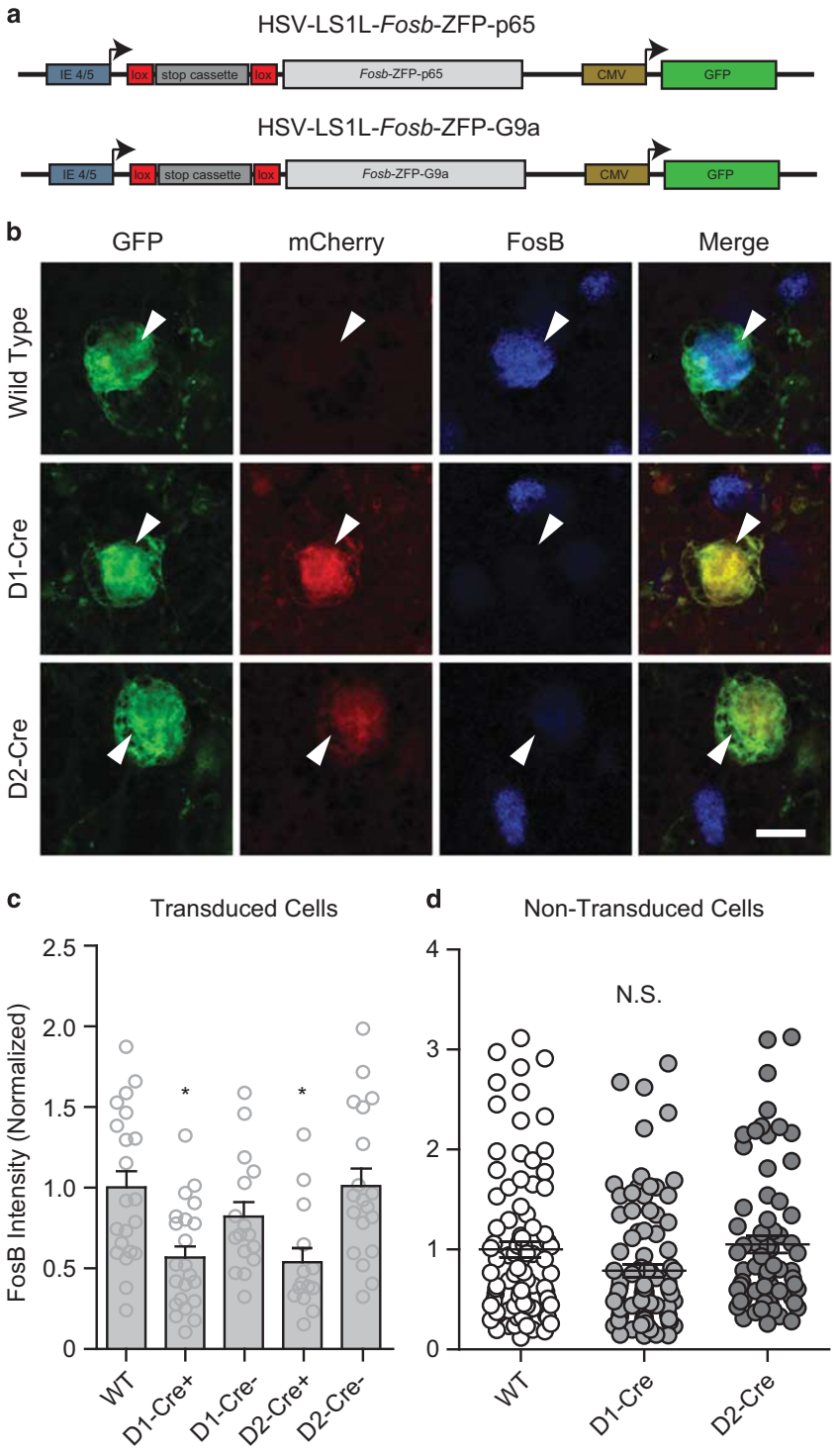
In vivo Validation of Cre-Dependent Expression of Engineered Transcription Factors

Our group has previously reported the development and validation of engineered transcription factors in their ability to modify chromatin states proximal to targeted, high-affinity DNA binding sites (Heller *et al*, 2014, 2016). Specifically, we use engineered ZFPs targeted to the *Fosb* promoter and fused to either the transcriptional repressor, G9a, which catalyzes the deposition of H3K9me2, or the transcriptional activator, p65, which mediates the enrichment of H3K9/14Ac at the *Fosb* locus. We have demonstrated that these epigenetic modifications, when delivered globally to neurons in the NAc, are sufficient to alter addiction- and depression-related behaviors of mice (Heller

Figure 1 Cre-dependent *Fosb*-ZFP viral vectors target Cre+ neurons *in vivo*. Wild-type (WT) ($n=5$), D1-Cre ($n=3$), and D2-Cre ($n=3$) mice were injected intra nucleus accumbens (NAc) with a herpes simplex virus (HSV) mixture of HSV-LS1L-*Fosb*-ZFP-G9a and HSV-LS1L-mCherry (to visualize Cre+ cells) and were allowed to recover for 3 days. Brain tissue was cleared, formaldehyde crosslinked, and immunohistochemistry was performed. (a) Vector map cartoons of the HSV-LS1L-*Fosb*-ZFP-p65 and HSV-LS1L-*Fosb*-ZFP-G9a used in these studies. (b) Green fluorescence indicates successful transduction of the HSV-LS1L-*Fosb*-ZFP-G9a vector in WT, D1-Cre, and D2-Cre animals. Green fluorescent protein (GFP) is expressed under the control of a separate, non-Cre-dependent CMV promoter on the *Fosb*-ZFP expression vector (first column). mCherry indicates the presence of Cre+ neurons in D1- and D2-Cre mice, but not WT mice (second column). FosB expression levels in both Cre+ and Cre− cells are represented in the third column, with the merged channels shown in the fourth column. Scale bar = 10μm. (c) Among all virally infected neurons, FosB expression is altered only in Cre+ cells. FosB expression is not altered in Cre− cells in D1- and D2-Cre mice, or in WT mice, indicating that *Fosb*-ZFP-G9a functions only in Cre+ cells (WT, $n=21$; D1-Cre+, $n=22$; D1-Cre−, $n=16$; D2-Cre+, $n=14$; D2-Cre−, $n=19$. *$p<0.05$; one-way analysis if variance (ANOVA) followed by Tukey's multiple comparison test: WT *vs* D1 Cre+: mean diff., 0.4352, 95% confidence interval (CI) of diff., 0.09659–0.7738, *$p<0.05$; WT *vs* D1 Cre−: mean diff., 0.1800, 95% CI of diff., −0.1883 to 0.5483, NS; WT *vs* D2 Cre+: mean diff., 0.4644, 95% CI of diff., 0.08150–0.8474, *$p<0.05$; WT *vs* D2 Cre−: mean diff., −0.01034, 95% CI of diff., −0.3618 to 0.3411, NS). (d) There is no significant difference in FosB expression in non-infected cells in WT, D1-Cre, or D2-Cre mice (WT, $n=89$; D1-Cre, $n=91$; D2-Cre, $n=68$. Not significant by one-way ANOVA followed by Tukey's multiple comparison test: WT *vs* D1 Cre: mean diff., 0.2129, 95% CI of diff., −0.03077 to 0.4565, NS; WT *vs* D2 Cre: mean diff., −0.05225, 95% CI of diff., −0.3155 to 0.2110, NS). NS, not significant.

et al, 2014). In this study, we aimed to deliver these ZFPs to specific neuronal subpopulations, namely D1- and D2-MSNs in the NAc, by stereotaxic injection of Cre-dependent HSV vectors expressing *Fosb*-targeted ZFPs.

To confirm the Cre-dependent expression of our constructs, we validated the HSV-LS1L-*Fosb*-ZFP-G9a vector *in vivo* in WT, D1-Cre, and D2-Cre mice (Figure 1). First, we confirmed viral transduction in both Cre+ and Cre− neurons by GFP expression, which is driven by a separate, non-Cre-dependent promoter in our viral plasmid (Figures 1a and b). Second, we confirmed Cre-regulated gene expression via coinjection of HSV-LSIL-mCherry, which expresses mCherry selectively in Cre+ neurons, allowing us to identify Cre+ and Cre− neurons. Third, to confirm that only Cre+ (mCherry+) neurons express functional LS1L-*Fosb*-ZFP-G9a vectors, we quantified the FosB expression



levels in these Cre+ cells. Indeed, we find that only D1- and D2-Cre+ cells, but not Cre− cells, show reduced FosB expression relative to WT levels (Figures 1b and c). We observe no difference in FosB expression in non-transduced cells in WT, D1-Cre, or D2-Cre mice (Figure 1d and Supplementary Figure 1). These data validate our approach of targeted, cell-type-specific control of the *Fosb* gene via epigenetic editing through Cre-dependent expression of our engineered ZFP constructs.

Fosb-Targeted Histone Acetylation in D2-MSNs Promotes Susceptibility to Social Stress

We first investigated the effects of *Fosb*-targeted histone acetylation exclusively in D2-expressing NAc MSNs. Given that ΔFosB is induced in D2-MSNs in mice susceptible to CSDS (Lobo *et al*, 2013), we hypothesized that ZFP-mediated activation in D2-MSNs would be prodepressive. We injected HSV-LS1L-*Fosb*-ZFP-p65 into the NAc of D2-Cre mice or their WT littermates. Six days after the HSV injection, mice were subjected to one day of subthreshold social defeat stress (Figure 2b), a paradigm that does not produce a depressive, social-avoidant phenotype in control animals (Chaudhury *et al*, 2013), and would thus be useful to identify a prodepressive effect of D2-MSN-specific *Fosb* histone acetylation and gene activation. Indeed, we found that WT mice injected with HSV-LS1L-*Fosb*-ZFP-p65 display a characteristic increase in the amount of time spent in the interaction zone when the novel target mouse is present, indicating the subthreshold social defeat stress was not of sufficient intensity to induce a susceptible, socially avoidant phenotype in control mice. D2-Cre mice injected with HSV-LS1L-*Fosb*-ZFP-p65 do not demonstrate this increased social interaction when a novel target mouse is present (Figure 2c), indicating that D2-MSN-specific histone acetylation at *Fosb* induces susceptibility to social defeat stress. To more fully explore this possibility, we determined each mouse's social interaction ratio, defined as time spent in the interaction zone when the target mouse is present/absent, and defined the mice as susceptible (ratio <1) or resilient (ratio >1) in accordance with previously published methodology (Krishnan *et al*, 2007). We observed that, while 83% of WT mice were resilient to subthreshold social defeat stress, only 55% of D2-Cre littermates were resilient, supporting our hypothesis that histone acetylation exclusively at the *Fosb* locus within D2-MSNs promotes susceptibility to social defeat stress (Figure 2d).

Mice that exhibit susceptibility to CSDS also display behaviors reminiscent of anxiety and natural reward anhedonia (Berton *et al*, 2012; Krishnan *et al*, 2007). To determine whether *Fosb* gene-specific histone acetylation promoted the full complement of these behavioral abnormalities, we tested animals in the EPM, to study the anxiety-related effects of this manipulation. We found that *Fosb*-targeted histone acetylation specifically in D2-MSNs significantly decreased the percent time mice spent exploring the open arms in the EPM (Figure 2e), with no effect on percent time in closed arms, percent time in center, or total locomotor distance traveled during the EPM test (Supplementary Figure 2A). Further, utilizing a sucrose preference paradigm as a measure of anhedonia, we found that *Fosb*-targeted histone acetylation specifically in D2-MSNs decreased preference for sucrose consumption relative to WT littermates (Figure 2f). Our manipulations had no effect on the gross body weights of the animals (weights of mice post defeat; WT: 19.0 ± 0.7 g, $n=6$; D2-Cre: 17.8 ± 0.8 g, $n=11$; expressed as mean ± SEM; $p>0.05$ by Student's *t*-test). Taken together, these findings indicate that site-specific epigenetic editing of the *Fosb* gene in a single cell type—increasing permissive histone acetylation in D2-MSNs—is sufficient to promote the full complement of depressive- and anxiety-like behaviors following subthreshold social defeat stress.

Fosb-Targeted Histone Methylation in D2-MSNs Promotes Resilience to Social Stress

We next investigated the role of *Fosb*-targeted repressive histone methylation specifically in D2-MSNs in NAc to determine if the results would reflect the converse of that seen following *Fosb* gene activation in this cell type (Figure 3a). We selected an accelerated defeat paradigm sufficient to produce a depressive-like, social-avoidant phenotype in control animals while matching the time course of HSV expression (Dias *et al*, 2014). Three days before subjecting mice to accelerated social defeat stress (Figure 3b), we injected the NAc of D2-Cre mice with HSV-LS1L-*FosB*-ZFP-G9a, which methylates *Fosb*-associated histones (H3K9me2), leading to *Fosb* gene repression (Heller *et al*, 2014). We found that WT mice injected with HSV-LS1L-*FosB*-ZFP-G9a and subjected to an accelerated social defeat demonstrated a significant reduction in the amount of time spent in the interaction zone when a novel target mouse is present (Figure 3c). Conversely, D2-Cre mice do not demonstrate a significant reduction in the time spent in the interaction zone, indicating an increased resilience to the social stress (Figure 3c). Analysis of the percentage of susceptible and resilient animals shows an increase in resilience in the population of D2-Cre mice relative to their WT littermates (Figure 3d).

We next sought to determine whether *Fosb*-specific histone methylation selectively in D2-MSNs was sufficient to repress additional measures of stress susceptibility. We found that there was no difference in open-arm exploratory behavior (Figure 3e), time in closed arms, time in center, or total locomotor distance traveled in the EPM (Supplementary Figure 2B). Note that while both groups of mice display only 5% exploratory behavior in open arms, which may preclude our ability to measure a further reduction in this measure, previous studies have documented percent time in open-arm below that reported here (Krishnan *et al*, 2007). We also observed no difference in body weight (weights of mice post defeat; WT: 20.4 ± 0.7 g, $n=9$; D2-Cre: 18.8 ± 0.6 g, $n=9$; expressed as mean ± SEM; $p>0.05$ by Student's *t*-test). However, consistent with our social interaction observations, mice with targeted histone methylation at the *Fosb* locus selectively in D2-MSNs displayed a greater preference for sucrose consumption compared with their WT littermates, demonstrating resistance to the anhedonic effects of social defeat (Figure 3f). Taken together, these data indicate that *Fosb*-targeted repressive histone methylation in D2-MSNs confers resilience specifically to the socially avoidant and anhedonic-like elements of social defeat stress.

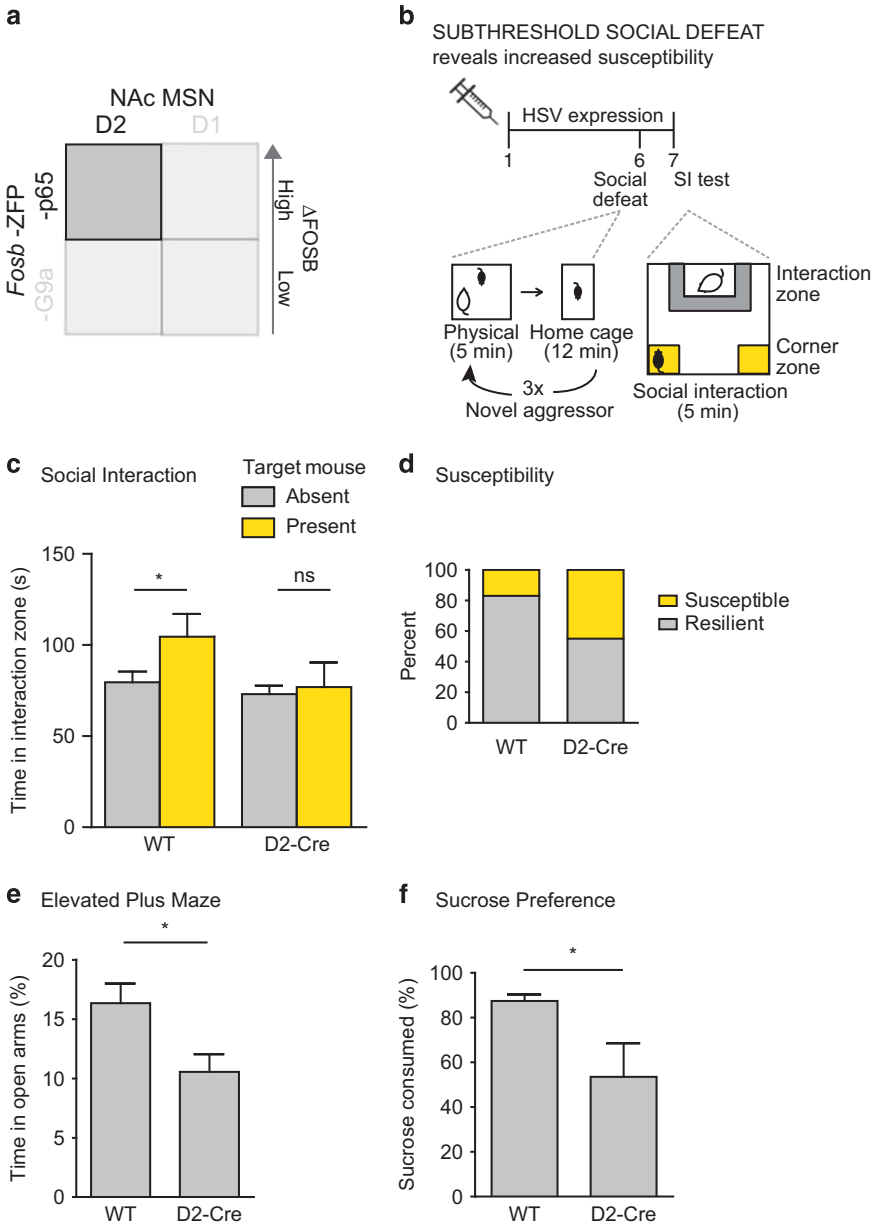


Figure 2 *Fosb*-ZFP-p65 mediated histone acetylation selectively in D2-medium spiny neurons (MSNs) promotes susceptibility to social stress. (a) The experiments conducted in this study are depicted as a matrix in which the dark grey quadrant specifies the particular experimental mouse and ZFP studied in that figure. The matrix is organized such that the targeted cell type is displayed horizontally and the ZFP construct is displayed vertically. The ZFP effect on ΔFosB expression is indicated along the right vertical axis. (b) Wild-type (WT) ($n=6$) or D2-Cre ($n=11$) mice were injected intranucleus accumbens (NAc) with HSV expressing Cre-dependent *Fosb*-ZFP-p65 and allowed to recover for 6 days before being subjected to a subthreshold social defeat paradigm. After 1 day of social defeat stress, which consisted of three 5-min bouts with a novel aggressor mouse, mice were tested for stress phenotypes in social interaction, sucrose preference, and elevated plus maze (EPM) tests. (c) Social interaction tests revealed that WT mice subjected to subthreshold social defeat displayed a significantly increased preference for the interaction zone when a novel mouse was present. This increase in social preference was not observed in mice that received *Fosb* targeted histone acetylation in D2-MSNs, indicating a prosusceptible effect ($^*p<0.05$; Student's *t*-test). (d) Quantification of the social interaction (SI) ratio and determination of the resilience (SI ratio > 1) or susceptibility (SI ratio < 1) of each mouse in the WT and D2-Cre populations revealed an increase in susceptibility in the D2-Cre population. (e) D2-Cre mice injected with HSV-LS1L-*Fosb*-ZFP-p65 spent less time exploring the open arms of the EPM relative to their WT littermates ($^*p<0.05$; Student's *t*-test). (f) D2-Cre mice injected with HSV-LS1L-*Fosb*-ZFP-p65 displayed decreased consumption of a 1% sucrose solution relative to WT mice ($^*p<0.05$; Student's *t*-test). Data are presented as mean ± SEM. NS, not significant.

Fosb-Targeted Histone Acetylation in D1-MSNs Promotes Resilience to Social Stress

Given that ΔFosB induction after CSDS occurs specifically in D1-MSNs in resilient mice (Lobo *et al*, 2013), we hypothesized that ZFP-mediated *Fosb* activation in D1-MSNs would be antidepressive. We injected HSV-LS1L-*FosB*-ZFP-p65 into the NAc of D1-Cre mice or their WT littermates 3 days before subjecting mice to 4 days of accelerated social defeat stress (Figures 4a and b). As before, this paradigm produced a social avoidance phenotype in WT

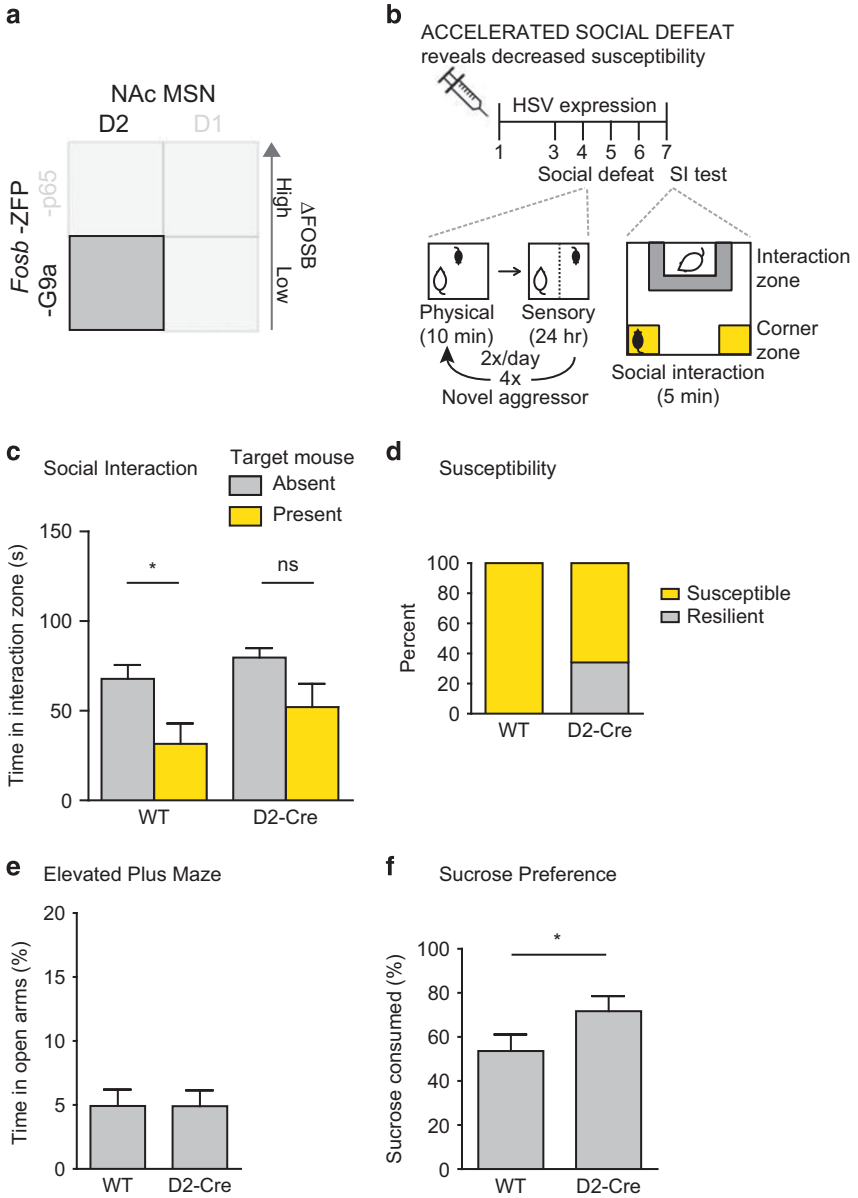


Figure 3 *Fosb*-ZFP-G9a mediated histone methylation selectively in D2-medium spiny neurons (MSNs) promotes resilience to social stress. (a) See description in Figure 2a. (b) Wild-type (WT) ($n=9$) or D2-Cre ($n=9$) mice were injected intranucleus accumbens (NAc) with herpes simplex virus (HSV) expressing Cre-dependent *Fosb*-ZFP-G9a and allowed to recover for 48 h before being subjected to an accelerated social defeat paradigm. Following 4 days of social defeat stress, mice were tested for stress phenotypes in social interaction, sucrose preference and elevated plus maze (EPM) tests. (c) Social interaction tests revealed that stressed WT mice spent significantly less time in the interaction zone when a novel mouse was present, whereas mice that received *Fosb* targeted histone methylation in D2-MSNs did not, indicating a proresilience effect (*$p<0.05$; Student's *t*-test). (d) Quantification of the social interaction (SI) ratio revealed a greater percentage of resilient animals in the D2-Cre population. (e) WT and D2-Cre mice displayed no difference in anxiety-like behavior as determined by percent time exploring the open arm of the EPM. (f) D2-Cre mice injected with HSV-LS1L-*Fosb*-ZFP-G9a demonstrated a greater preference for consumption of a 1% sucrose solution (*$p<0.05$; Student's *t*-test). Data are presented as mean ± SEM. NS, not significant.

animals (Figure 4c). Importantly, targeted histone acetylation at the *Fosb* gene in D1-MSNs promoted resilience to social defeat stress, as seen by a decrease in social avoidance behavior in the D1-Cre mice (Figure 4c) and a shift towards greater resilience in the D1-Cre mice as quantified by the percentage of animals displaying a resilient or susceptible phenotype in WT and D1-Cre populations (Figure 4d).

To determine whether *Fosb*-associated histone acetylation in D1-MSNs also protected animals from the anxiety-like effects of social defeat stress, we tested mice in the EPM. Surprisingly, we found a significant decrease in the amount of time D1-Cre mice spent exploring the open arms (Figure 4e) with no effect on percent time in closed arms, percent time in center, or total locomotor distance traveled during the EPM test (Supplementary Figure 2C). Using the sucrose preference paradigm as a measure of anhedonia, we observed that *Fosb*-targeted histone acetylation in D1-MSNs led to a significant increase in preference for sucrose relative to plain water (Figure 4f)

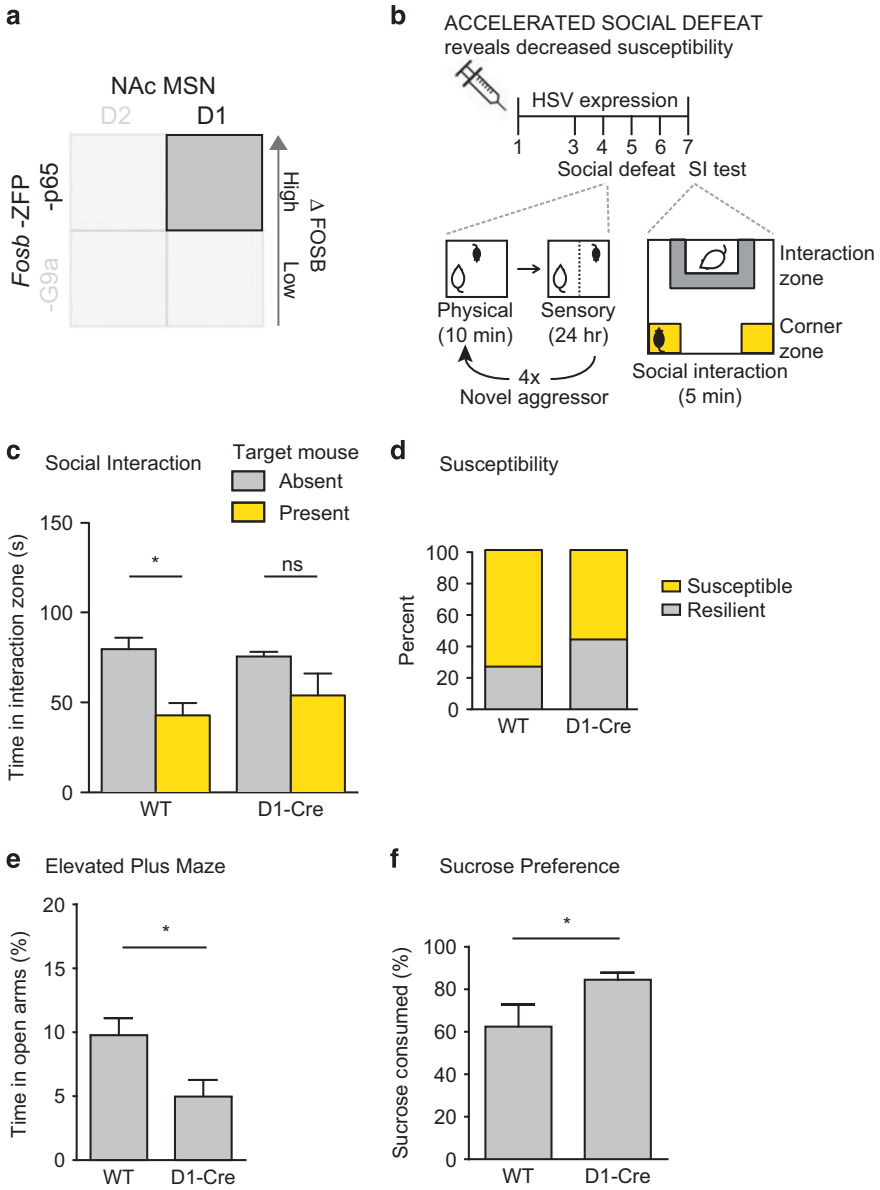


Figure 4 *Fosb*-ZFP-p65 mediated histone acetylation selectively in D1-medium spiny neurons (MSNs) promotes resilience to social stress. (a) See description in Figure 2a. (b) Wild-type (WT) ($n=11$) or D1-Cre ($n=9$) mice were injected intranucleus accumbens (NAc) with herpes simplex virus (HSV) expressing Cre-dependent *Fosb*-ZFP-p65 and allowed to recover for 48 h before being subjected to an accelerated social defeat paradigm. Following 4 days of social defeat stress, mice were tested for stress phenotypes in social interaction, sucrose preference, and elevated plus maze (EPM) tests. (c) Social interaction tests revealed that stressed WT mice spent significantly less time in the interaction zone when a novel mouse was present, whereas mice that received *Fosb* targeted histone acetylation in D1-MSNs did not, indicating a proresilience effect (*$p<0.05$; Student's *t*-test). (d) Quantification of the social interaction (SI) ratio revealed a greater percentage of resilient animals in the D1-Cre population. (e) Relative to WT mice, D1-Cre mice injected with HSV-LS1L-*Fosb*-ZFP-p65 spent less time exploring the open arm of the EPM (*$p<0.05$; Student's *t*-test). (f) D1-Cre mice injected with HSV-LS1L-*Fosb*-ZFP-p65 demonstrated a greater preference for consumption of a 1% sucrose solution (*$p<0.05$; Student's *t*-test). Data are presented as mean ± SEM. NS, not significant.

without affecting body weight (weights of mice post defeat; WT: 22.5 ± 0.4 g, $n=11$; D1-Cre: 21.6 ± 0.3 g, $n=9$; expressed as mean ± SEM; $p>0.05$ by Student's *t*-test). Taken together, these findings indicate that site-specific permissive histone acetylation of the *Fosb* gene in D1-MSNs protects against the social avoidance and anhedonic phenotypes induced by social defeat, but not anxiety traits as measured by exploratory behavior in the EPM (see Discussion).

Fosb-Targeted Histone Methylation in D1-MSNs Promotes Susceptibility to Social Stress

To complete our systematic interrogation of targeted histone acetylation or methylation at the *Fosb* gene in each NAc MSN cell type, we tested whether repressive histone methylation at *Fosb* within D1-MSNs exacerbates the consequences of social defeat stress. We injected the NAc of D1-Cre or WT mice with HSV-LS1L-*FosB*-ZFP-G9a 6 days before subjecting them to subthreshold social defeat

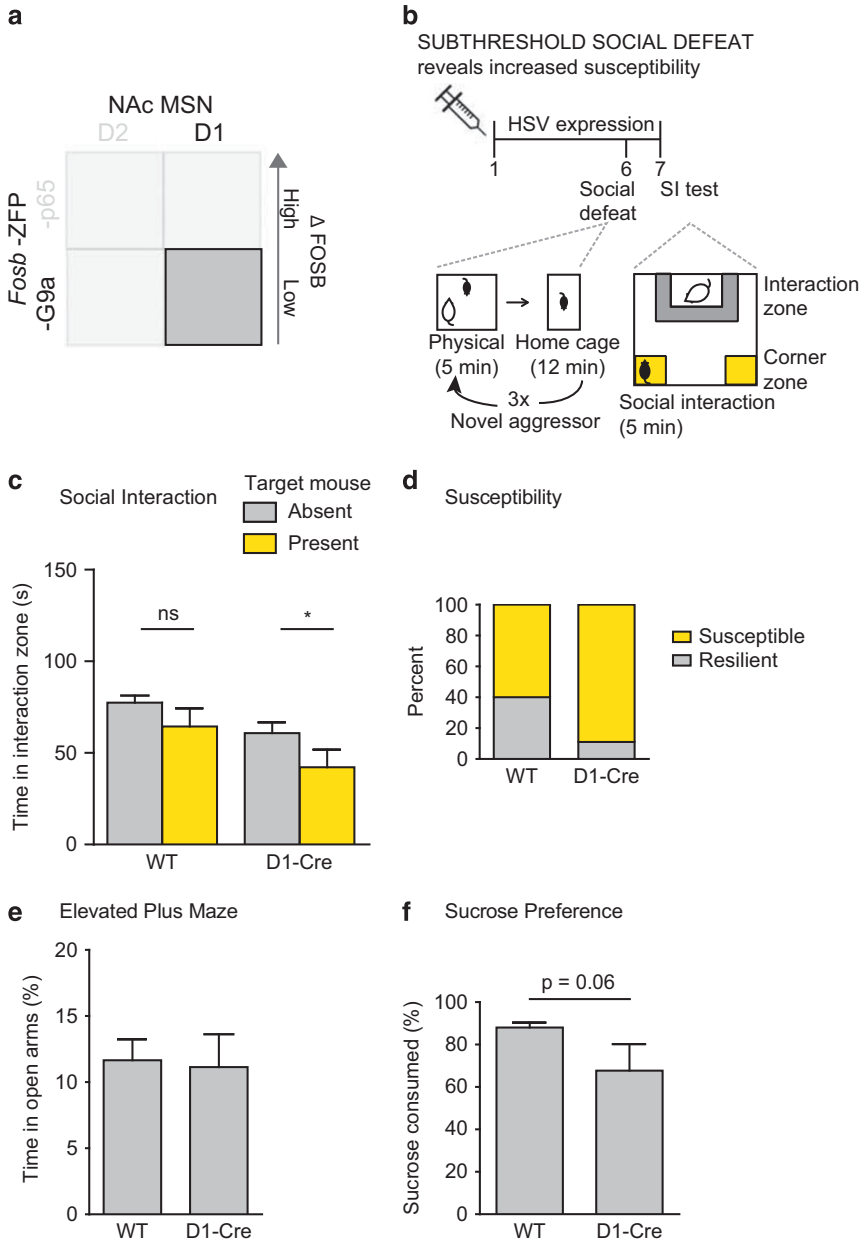


Figure 5 *Fosb*-ZFP-G9a mediated histone methylation selectively in D1-medium spiny neurons (MSNs) promotes susceptibility to social stress. (a) See description in Figure 2a. (b) Wild-type (WT) ($n=10$) or D1-Cre ($n=10$) mice were injected intranucleus accumbens (NAc) with herpes simplex virus (HSV) expressing Cre-dependent *Fosb*-ZFP-G9a and allowed to recover for 6 days before being subjected to a subthreshold social defeat paradigm. After one day of social defeat stress, mice were tested for stress phenotypes in social interaction, sucrose preference, and elevated plus maze (EPM) tests. (c) Social interaction tests revealed that mice that received *Fosb*-targeted histone methylation in D1-MSNs subjected to subthreshold social defeat spent significantly less time in the interaction zone when a novel mouse was present (a prosusceptible effect), whereas their WT littermates did not (*$p<0.05$; Student's *t*-test). (d) Quantification of the social interaction (SI) ratio revealed a greater percentage of susceptible animals in the D1-Cre population than in the WT littermate population. (e) WT and D1-Cre mice injected with HSV-LS1L-*Fosb*-ZFP-G9a exhibited no significant difference in anxiety-like symptoms as assessed by percent time spent on the open arms of the EPM. (f) D1-Cre mice injected with HSV-LS1L-*Fosb*-ZFP-p65 demonstrated a trend towards a lower preference for consumption of a 1% sucrose solution ($p=0.06$; Student's *t*-test). Data are presented as mean ± SEM. NS, not significant.

stress (Figures 5a and b). In these mice, we observed that D1-MSN-specific histone methylation at *Fosb* led to a significant decrease in the amount of time spent in the interaction zone during the interaction test, whereas WT littermates showed no reduction in their social interaction scores (Figure 5c). Relative to WT mice, we did observe a reduction in the amount of time that D1-Cre mice spent in the interaction zone when the target mouse was absent ($p<0.05$ by Student's *t*-test). This effect is not due to an alteration in basal locomotor behavior in D1-Cre mice (total locomotor distance moved when target mouse was absent: WT: 1382 ± 54 cm, $n=10$; D1-Cre: 1507 ± 70 cm, $n=10$; expressed as mean ± SEM; $p>0.05$ by Student's *t*-test). Importantly, upon stress exposure, WT and D1-Cre mice differentially manifest stress responses as seen in time spent in the interaction zone when the target mouse is present.

This difference in stress response was revealed further when we observed that the percentage of mice displaying resilience to social defeat stress decreases in the D1-Cre population (Figure 5d).

We observed no difference between WT and D1-Cre mice in anxiety-like behaviors, as quantified as percentage of time in open arm of EPM (Figure 5e), or time in closed arm, center, or total locomotion scores (Supplementary Figure 2D). Further, we saw no changes in gross body weight (weights of mice post defeat; WT: 19.9 ± 0.4 g, $n = 10$; D1-Cre: 20.2 ± 0.6 g, $n = 10$; expressed as mean ± SEM; $p > 0.05$ by Student's *t*-test). However, when we investigated the effect of D1-MSN-specific histone methylation at the *Fosb* gene in the development of anhedonic-like phenotypes, we observed that mice expressing ZFP-*Fosb*-G9a in D1-MSNs demonstrated a trend towards a decreased preference for consuming a sucrose solution (Figure 5f). Taken together, we found that increased repressive histone methylation at the *Fosb* gene solely in D1-MSNs is sufficient to exacerbate the deficits in social interaction induced by social defeat stress.

DISCUSSION

This is the first study to usee cell-type-specific targeted epigenetic editing *in vivo* in a neuropsychiatric syndrome model. While it is well established that epigenetic modifications contribute to depression (Berton *et al*, 2012; Pena *et al*, 2014; Sun *et al*, 2013), it has been difficult to define the exact cell types in which these modifications function. Using our innovative approach, we analyzed the cellular specificity of *Fosb* transcription in controlling susceptibility *vs* resilience to social defeat stress. These findings are particularly relevant to our understanding of the causal role of epigenetic remodeling in stress responses, which is critical for the development of novel therapeutics for stress-related neuropsychiatric disorders.

We targeted *Fosb* epigenetic remodeling to distinct subpopulations of NAc neurons, given emerging data on the distinct roles of the D1 and D2 subtypes of MSNs. In particular, a prior study from our group found differences in the extent of ΔFosB induction in the two cell types in response to several forms of chronic pharmacological (cocaine, haloperidol, Δ9-THC, heroin, morphine), emotional (environmental enrichment, calorie restriction, sucrose two-bottle choice, social defeat, fluoxetine), and optogenetic stimuli (Lobo *et al*, 2013). Examination of the effect of optogenetic activation of various NAc afferent regions in these two mouse lines found different levels of ΔFosB induction in the two cell types as a function of input stimulation (Lobo *et al*, 2013). Interestingly, an additional study found that optogenetic stimulation of distinct populations of dynorphinergic cells also show specific regulation of emotional behavior. That is, stimulation of dynorphinergic cells in the ventral NAc elicited robust conditioned and real-time aversive behavior, while photostimulation of dorsal NAc dynorphin cells was positively reinforcing (Al-Hasani *et al*, 2015). Taken together, these studies indicate that cell-type-specific regulation of emotional behavior extends beyond MSNs to dynorphinergic, and likely other, cell types. To complement these optogenetic studies, we selected to epigenetically regulate *Fosb* expression in each of the two MSN cell types and examine depressive-like behavior.

Prior studies have described an increase in ΔFosB levels in the NAc of mice both susceptible and resilient to CSDS, with a larger increase seen in the resilient population (Vialou *et al*, 2010b). In addition, while inducible overexpression of ΔFosB in all NAc neurons was shown to mediate directly a state of resilience in the social interaction test (Vialou *et al*, 2010b), subsequent studies indicated that the observed increase in ΔFosB levels in the NAc of resilient mice occurs solely in D1-MSNs, whereas D2-MSNs display increased ΔFosB levels specifically in susceptible mice (Lobo *et al*, 2013). Our study used targeted manipulation of the chromatin state at the *Fosb* gene to regulate ΔFosB expression in each cell type for a direct examination of the causal role of epigenetic modulation in each MSN type in mediating a susceptible *vs* resilient phenotype

Social defeat stress produces several depressive-like behaviors in mice, including social avoidance and anhedonia, as well as anxiety-like symptoms (Berton *et al*, 2006; Donahue *et al*, 2014; Krishnan *et al*, 2007). We found that activation of *Fosb* expression via histone acetylation in D2-MSNs is causal in potentiating a full complement of depressive- and anxiety-like behaviors induced by social stress, including social avoidance in the social interaction test, and increased anhedonia- (sucrose preference) and anxiety- (EPM) like responses. Conversely, *Fosb*-targeted repressive histone methylation in D2-MSNs promotes resilience to social defeat stress, as reflected by protection against the social avoidance and anhedonic phenotypes associated with this paradigm. Interestingly, there was no effect on the exploratory behavior of these animals, suggesting a functional separation between depressive- and anxiogenic-like traits associated with social defeat stress. Such separation of phenotype has been observed previously (Krishnan *et al*, 2007), in that resilient mice do not display depressive-like behavior, as measured by social interaction and sucrose preference, but do display increased anxiety-like behaviors, measured as decreased exploration of the open arms in the EPM (Krishnan *et al*, 2007). This observed separation of behavioral phenotype may account for the inconsistent expression of anxiety-like behavior in the EPM following cell-type-specific epigenetic editing of *Fosb*. Specifically, while two distinct *Fosb*-targeted histone modifications were associated with increased social avoidance behavior, only acetylation of *Fosb* in the D2-MSNs, but not methylation of *Fosb* in D1-MSNs, was associated with decreased open-arm exploration in EPM. Along similar lines, activation of *Fosb* expression in the D1-MSNs promoted resilience in the social interaction test and protected against anhedonia, as predicted by its expression pattern following social defeat stress (Lobo *et al*, 2013), but paradoxically caused an increase in anxiety-like responses. Repression of *Fosb* expression via histone methylation in D1-MSNs promoted susceptibility to social avoidance, but had less of an effect on EPM and sucrose preference behavior. While these results illuminate the differential contributions of cell-type-specific HPTMs at the *Fosb* locus in driving depressive-like behaviors, the inconsistent results in anxiety-like behaviors may be due to similar, yet undefined, mechanisms regulating the emergence of resilient and susceptible phenotypes. Finally, it is important to note

that the behavioral measurements were taken at different times following viral injection, that is, EPM was tested 7–8 days following HSV delivery. It is therefore possible that the inconsistent behavior expressed at this time point is due to diminishing HSV expression. This is not likely to be the case, however, given that HSV-ZFP expression consistently regulates behaviors measured 7–10 days after viral injection (Heller *et al*, 2014, 2016), indicating that the epigenetic manipulations are stable beyond the maximal expression of HSV. Indeed, we do observe decreased open-arm exploratory behavior in mice expressing *FosB*-ZFP-p65 in D2-MSNs, revealing a novel functional role for epigenetic modulation of *Fosb* within D2-MSNs in driving both depressive- and anxiety-like behaviors induced by social stress.

It is important to note that the behavioral effect sizes observed in this study are small relative to more traditional overexpression and knockout strategies. This difference can be attributed to the subtlety of our manipulation, that is, the targeting of a single epigenetic modification to a single locus in a single-cell type. This mode of gene regulation drives physiologically relevant changes in expression level (~1.2–2.0-fold) (Gilbert *et al*, 2013; Heller *et al*, 2014, 2016) that are lower than that seen with exogenous gene expression or gene knockout (Chen *et al*, 1998). Transcriptional activation via HSV expression of *Fosb*-ZFP-p65 results in a ~2-fold induction of *ΔFosb* mRNA in the NAc (Heller *et al*, 2014), which is comparable to the twofold induction in FosB +-immunoreactive cells following social defeat stress (Lobo *et al*, 2013; Vialou *et al*, 2010b). This is markedly different from the expression levels seen in transgenic mice or virally treated mice with inducible ΔFosB overexpression, in which the degree of ΔFosB induction far exceeds levels of the endogenous protein induced in brain by numerous treatments (Chen *et al*, 1998). Thus, our tools make it possible to mirror the endogenous physiology of stress responses by emulating natural epigenetic mechanisms of inducing physiologically relevant transcriptional events within specific cell populations and avoiding potential artifacts seen with non-physiological degrees of gene manipulation.

In addition to more closely modeling physiological levels of gene expression, our approach is the first to elucidate the causal relevance of naturally occurring, stress-induced modes of epigenetic regulation at a single gene. The importance of parsing cell- and gene-specific functions of HPTMs is underscored by the fact that epigenetic alterations associated with chronic stimuli also appear to be cell-type-specific. An immunohistochemical examination of global histone modifications in the striatum following chronic cocaine treatment found that acetylation of histone H4 lysine 5 is increased specifically in D1-MSNs, while H3K14Ac and H4K8Ac are enriched in D2-MSNs (Jordi *et al*, 2013). Histone methylation appeared to be upregulated exclusively in D1-MSNs (Jordi *et al*, 2013). Interestingly, D1- or D2-MSN-targeted deletion of G9a, which, as noted earlier, catalyzes H3K9me2, alters behavioral responsiveness to several pharmacological stimuli in a cell-type-specific manner (Schaefer *et al*, 2009). Additionally, while repeated cocaine exposure reduces G9a expression in both D1- and D2-MSNs, conditional G9a-knockout or adult G9a overexpression in D1- *vs* D2-MSNs produced opposing behavioral responses to cocaine treatment (Maze *et al*, 2014). One prior study investigating the connection between cocaine-repressed histone methylation in MSNs and subsequent stress exposure found that global reduction in G9a and H3K9me2 levels following chronic cocaine exposure increased susceptibility to social defeat stress (Covington *et al*, 2011). Paradoxically, we have measured enrichment of H3K9me2 at the *Fosb* locus in depressed human NAc and found that *Fosb*-targeted H3K9me2 in NAc increases susceptibility to social defeat stress in mice (Heller *et al*, 2014). To determine whether these differences in stress regulation by H3K9me2 are cell-type-specific, in this study, we found that increased H3K9me2 at the *Fosb* gene specifically in D1-MSNs promotes susceptibility to social defeat stress, whereas *Fosb*-targeted H3K9me2 in D2-MSNs increases resilience to social defeat stress. Taken together, these results demonstrate differences in the behavioral effects of global epigenetic modifications and targeted epigenetic editing at single loci within specific cell types. A cell-type-specific examination of *Fosb*-specific histone modifications would greatly enhance our understanding of the relevance of global *vs* gene-specific histone modifications within specific cell populations. Technical innovations that enable ChIP following fluorescence-activated cell sorting (FACS) (Finegersh and Homanics, 2016; Mitchell *et al*, 2017; von Schimmelmann *et al*, 2016) as well as single-cell ChIP sequencing (Rotem *et al*, 2015) are necessary to elucidate the cell-type-specific mechanisms of *Fosb* gene expression in regulating stress- and depressive-like behavior.

Since this study was designed to test the hypothesis that cell-type-specific epigenetic regulation of *Fosb* differentially mediates responses to stress exposure, we did not examine the role, if any, of FosB/ΔFosB on basal emotional behavior. Further study using this paradigm may uncover a role for cell-type-specific epigenetic regulation of *Fosb* in the emotional behavior of stress-naïve mice. Regarding the utility of this approach for studying baseline behaviors, it is important to note that investigators have observed some differences in baseline behavior between the two Cre-transgenic lines used in this study, despite the fact that they share the same genetic background (Kramer *et al*, 2011). For example, we found that the baseline open-arm exploratory behavior (Figures 3e and 4e), baseline social interaction time (Figures 2c and 5c), and percent resilient (Figures 2d and 5d) differ between WT littermates of D1- and D2-Cre transgenic animals. To avoid any confounds due to strain differences, all comparisons in this study are thus made within strain between hemizogous transgenic and wild-type littermates.

Given the role of ΔFosB as a transcription factor, it is likely that the distinct phenotypes revealed by our manipulations are due to unique subsets of downstream gene targets for ΔFosB in D1- *vs* D2-MSNs. Indeed, it is the stable accumulation of ΔFosB that distinguishes its transcriptional function from more labile members of the Fos family of immediate early genes (Nestler, 2008). For example, ΔFosB accumulation is known to regulate downstream target genes associated with synaptic plasticity, such as the AMPA glutamate receptor subunit GluA2 (Vialou *et al*, 2010b) and calcium/calmodulin-dependent protein kinase II (Robison *et al*, 2013, 2014). Moreover, ΔFosB overexpression reduces AMPA current in D1-MSNs, but not D2-MSNs, as would be expected from GluA2 induction (Grueter *et al*, 2013). Further investigation of the expression of glutamate and GABA receptors, as well as transcription factors

regulating their expression, will be needed to elucidate the difference in ΔFosB-target gene regulation following specific modes of epigenetic regulation of the *Fosb* locus in distinct cell types. Furthermore, western blot analysis may reveal cell-type-specific differences in the post-translational modifications of ΔFosB known to modulate its function as a transcription factor (Cates *et al*, 2014). In addition to downstream molecular targets, the regulation of *Fosb* in each of the MSN projection neurons may have distinct physiological effects on basal dopaminergic tone of VTA neurons. This difference in dopaminergic tone would then be amplified through the opposite response in the two MSNs to dopamine signaling, that is, enhanced glutamatergic activation of D1-MSNs and reduced glutamatergic activation of D2-MSNs (Gerfen and Surmeier, 2011; Surmeier *et al*, 2007). Thus, in addition to the Fosb-mediated MSN inhibitory tone, regulation of dopaminergic signaling may contribute to the behavioral phenotypes observed.

Locus-specific epigenetic remodeling allows us to model closely the subtle changes in gene expression that underlie mood regulation. Cell-type-specific epigenetic remodeling of a single gene locus allows direct examination of the causal role of such changes in depression-related behavioral abnormalities. We found that, in the context of *Fosb*-targeted HPTMs in MSN subtypes, these subtle manipulations reveal opposing influences on stress susceptibility depending on the cell type in which they occur. This observation serves to increase the resolution of our understanding of the causal epigenetic mechanisms that contribute to depression-related behaviors.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)