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Fluoxetine Epigenetically Alters the CaMKIIα Promoter in Nucleus Accumbens to Regulate ΔFosB Binding and Antidepressant Effects

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Chronic social defeat stress in mice produces a susceptible phenotype characterized by several behavioral abnormalities consistent with human depression that are reversed by chronic but not acute exposure to antidepressant medications. Recent work in addiction models demonstrates that the transcription factor ΔFosB and protein kinase calmodulin-dependent protein kinase II (CaMKII) are co-regulated in nucleus accumbens (NAc), a brain reward region implicated in both addiction and depression models including social defeat. Previous work has also demonstrated that ΔFosB is induced in NAc after chronic social defeat stress or after chronic antidepressant treatment, wherein it mediates a pro-resilience or antidepressant-like phenotype. Here, using chromatin immunoprecipitation assays, we found that ΔFosB binds the CaMKIIα gene promoter in NAc and that this binding increases after mice are exposed to chronic social defeat stress. Paradoxically, chronic exposure to the antidepressant fluoxetine reduces binding of ΔFosB to the CaMKIIα promoter and reduces CaMKII expression in NAc, despite the fact that ΔFosB is induced under these conditions. These data suggest a novel epigenetic mechanism of antidepressant action, whereby fluoxetine induces some chromatin change at the CaMKIIα promoter, which blocks the ΔFosB binding. Indeed, chronic fluoxetine reduces acetylation and increases lysine-9 dimethylation of histone H3 at the CaMKIIα promoter in NAc, effects also seen in depressed humans exposed to antidepressants. Overexpression of CaMKII in NAc blocks fluoxetine's antidepressant effects in the chronic social defeat paradigm, whereas inhibition of CaMKII activity in NAc mimics fluoxetine exposure. These findings suggest that epigenetic suppression of CaMKIIα expression in NAc is behaviorally relevant and offer a novel pathway for possible therapeutic intervention in depression and related syndromes.
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INTRODUCTION

Current antidepressant treatments provide full relief for fewer than half of the patients diagnosed with major depressive disorder; however, as their mechanisms of long-term action are incompletely understood, development of novel, more effective treatments remains a challenge. ΔFosB is a stable transcription factor that has an essential role in long-term adaptive changes in the brain associated with diverse conditions, including stress resilience and antidepressant treatment. For example, its induction in nucleus accumbens (NAc), a brain reward region, after chronic social defeat stress occurs preferentially in resilient mice and contributes to a state of resilience (Ohnishi *et al*, 2011; Vialou *et al*, 2010). Likewise, its induction in NAc by antidepressants is required for the therapeutic-like effects of these drugs (Vialou *et al*, 2010). Although ΔFosB is known to affect both the morphology (Lee *et al*, 2006; Maze *et al*, 2010; Robison *et al*, 2013) and synaptic physiology (Grueter *et al*, 2013) of NAc medium spiny neurons, identification of its specific mechanisms of action and gene targets remains a goal for the field.

We have identified numerous target genes for ΔFosB through gene microarray or chromatin immunoprecipitation (ChIP) technology (McClung and Nestler, 2003; Nestler, 2008; Renthal *et al*, 2009), although study of the role of specific target genes in depression models is just beginning. The neuronally enriched calcium/calmodulin-dependent protein kinase II (CaMKII) was identified originally as a ΔFosB target in NAc after cocaine exposure using genome-wide approaches (McClung and Nestler, 2003), and we have recently verified this finding by showing that ΔFosB binding to the CaMKIIα gene promoter is both necessary and

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sufficient for the kinase's induction in NAc by cocaine (Robison *et al*, 2013). CaMKII in NAc has been linked to altered synaptic function (Huang and Hsu, 2012; Kourrich *et al*, 2012) and behavioral responses to drugs of abuse (Loweth *et al*, 2008; Pierce *et al*, 1998; Robison *et al*, 2013), but the role of NAc CaMKII in antidepressant action is unknown. Here, we investigated the regulation of NAc CaMKII expression by the antidepressant fluoxetine in the context of chronic social defeat stress. We uncovered a surprising inhibitory epigenetic mechanism at the CaMKIIα promoter that is conserved in human patients on antidepressants, and demonstrate that such paradoxical repression of CaMKII expression in NAc is required for the behavioral effects of fluoxetine.

MATERIALS AND METHODS

Human ChIP

Site-directed qChIP was performed as previously described (Golden *et al*, 2013; Jiang *et al*, 2008) using a micrococcal nuclease (MNase)-based assay allowing for high-resolution mapping of human gene promoters. Briefly, 50 mg of human NAc tissue was lightly homogenized with 550 μl buffer (10 mM Tris-HCl (pH 8.0), 4 mM MgCl2, 1 mM CaCl2) in a 1 ml loose-fit glass homogenizer. The homogenate was then digested with an MNase (2 units/ml) for 10 min in a 37 °C water bath. The digestion reaction was terminated by addition of 10 mM EDTA (pH 8.0). The digested chromatin was then further incubated in SDS lysis buffer (1% SDS, 50 mM Tris-HCl (pH 8.1), 10 mM EDTA) for 60 min on wet ice and lightly vortexed every 10 min. The lysed chromatin was centrifuged at $3000 \times g$ for 20 min at 4 °C, and the supernatant was collected. A volume of 400 μl of the digested chromatin supernatant was used for each ChIP and brought to 500 μl final volume with an incubation buffer (500 mM NaCl, 200 mM Tris-HCl (pH 8.0), 50 mM EDTA (pH 8.0)).

qChIP was performed with 7 μg of anti-pan-acetyl histone H3 antibody (EMD Millipore) or anti-dimethyl-lysine-9 H3 antibody (Abcam) per sample, conjugated to magnetic Dynabeads (M-280 Sheep anti-Rabbit IgG; Invitrogen). IgG control antibody-conjugated beads were also used but failed to show enrichment. Beaded antibodies were incubated with the immunoprecipitated chromatin overnight (~16 h) at 4 °C and then washed eight times in ChIP buffer (0.7% Na-deoxycholate, 500 mM LiCl, 50 mM Hepes-KOH (pH 7.6), 1% NP-40, 1 mM EDTA). Bound chromatin was isolated by heating to 65 °C and shaking at 1000 r.p.m for 30 min on a Thermomixer and then removing supernatant from the beads. Chromatin in the supernatant and input samples was reverse cross-linked by heating to 65 °C overnight. DNA was then purified for quantitative PCR (qPCR) analysis with a QIAquick PCR Purification Kit (Qiagen). Levels of acetylated H3 at each promoter region were determined by qPCR (see Supplementry Figure S2).

qPCR

Human or mouse samples were stored at −80 °C until use. Prior to RNA extraction, samples were divided into portions for use in either site-directed qChIP or standard qPCR. From the qPCR portion, we isolated RNA using TriZol (Invitrogen) homogenization and chloroform layer separation. The clear RNA layer was then processed (RNAeasy MicroKit, Qiagen) and analyzed with NanoDrop. A volume of 500 ng of RNA was reverse transcribed to cDNA (qScript Kit, VWR). For qPCR, cDNA was diluted to 500 μl, and 3 μl was used for each reaction. The reaction mixture consisted of Perfecta SYBR Green (5 μl), forward and reverse primers (0.5 μl each), water (1 μl), and cDNA template. Samples were then heated to 95 °C for 2 min followed by 40 cycles of 95 °C for 15 s, 60 °C for 33 s, and 72 °C for 33 s. Analysis was carried out using the ΔΔC(t) method (Tsankova *et al*, 2006). Samples were normalized to *Gapdh*, which was not affected under the experimental conditions studied.

Mouse ChIP

qChIP was performed on pooled bilateral NAc punches from four or more mice, with each group consisting of at least 20 mice. Tissues were cross-linked, washed, and kept at −80 °C. Sheared chromatin was incubated overnight with a primary antibody (see western blotting below for a list) previously bound to magnetic beads (Dynabeads M-280, Invitrogen). Non-immune IgG was used as a control. After reverse cross-linking and DNA purification, the binding of antibodies to the CaMKIIα promoter was determined by qPCR using primers centered around the AP-1 consensus site found 447 bp upstream of the transcription start site (Supplementry Figure S1A; Forward: ACGGACTCAGGAAGAGGGATA; Reverse: CTTGCTCCTCAGAATCCATTG).

Western Blotting

Mice were decapitated without anesthesia to avoid the effects of anesthetics on neuronal protein levels. Brains were serially sliced in a 1 mm matrix (Braintree Scientific) and NAc tissue was removed in phosphate buffered saline containing protease (Roche) and phosphatase (Sigma Aldrich) inhibitors using a 14-gauge punch and immediately frozen on dry ice. Samples were homogenized by light sonication in a modified buffer: 10 mM Tris base, 150 mM sodium chloride, 1 mM EDTA, 0.1% sodium dodecyl sulfate, 1% Triton X-100, 1% sodium deoxycholate (pH 7.4), containing protease and phosphatase inhibitors as above. After addition of gel buffer, proteins were separated on 4–15% polyacrylamaide gradient gels (Criterion System, BioRad), and western blotting was performed using the Odyssey system (Li-Cor) according to manufacturer protocols.

Antibodies.

CaMKIIα (total): Upstate 05–532, 1 : 5000
CaMKII phospho-threonine 286: Promega V111A, 1 : 1000
ΔFosB (total): Cell Signaling 5G4, 1 : 250
GluA1 (total): Abcam, Ab31232, 1 : 1000
GluA1 phospho-serine 831: Millipore N453, 1 : 1000
HDAC1: Abcam, Ab46985, 1 : 1000
HDAC2: Abcam, Ab51832, 1 : 1000
HDAC4: Abcam, Ab1437, 1 : 1000
H3 (Total): Abcam, Ab1791, 1: 4000
3MeK27 H3: Abcam, Ab6002, 1 : 1000
3MeK4 H3: Abcam, Ab8580, 1 : 1000
2MeK9 H3: Abcam, Ab8898, 1 : 1000
3MeK9 H3: Abcam, Ab1220, 1 : 1000

Quantitative Immunohistochemistry

Quantification of CaMKIIα immunoreactivity specific to the NAc shell and core was performed using a Li-Cor system as described (Robison *et al*, 2013). Integrated intensities of CaMKII and GAPDH were determined with Odyssey software. Results are presented as integrated intensity values per mm^2 and are presented as means ± SEM ($n \geqslant 5$ per group). Values for GAPDH, which was unaffected by chronic social defeat stress or fluoxetine, were used to normalize CaMKII intensity for slice thickness and conditions.

Stereotaxic Surgery

Needles of 33 gauge (Hamilton) were used in all surgeries, during which 0.5 µl of purified high-titer virus was bilaterally infused over a 5 min period, followed by an additional 5 min post-infusion rest period. All distances are measured relative to Bregma: 10° angle, AP = +1.6 mm, Lat = +1.5 mm, DV = −4.4 mm (Vialou *et al*, 2010).

Agents. HSV-GFP and HSV-GFPAC3I have been previously described (Robison *et al*, 2013). For HSV-CaMKII, the cDNA for CaMKIIα was cut from the pcDNA3.1 vector (Strack *et al*, 2000b) and inserted into the p1005+ vector using the NheI and BspEI sites. The construct was validated by sequencing.

Social Defeat

Chronic (10 days) social defeat stress and social interaction tests were performed as described (Berton *et al*, 2006; Krishnan *et al*, 2007). Behavioral assays and viral surgeries were performed between 1 and 18 days after the final defeat exposure.

Sucrose Preference

Sucrose preference was tested using a two-bottle choice procedure. Mice were habituated to drink a 1% sucrose solution or water (from two drip-controlled bottles) for 2 days prior to surgery. After surgery, consumption from the two bottles was measured daily for 3 days. The position of the water and sucrose bottles (left or right) was switched each day. The preference for sucrose over water (ie, sucrose/(water + sucrose)) was used as a measure of sensitivity to natural reward.

Statistical Analyses

All statistical analyses were performed using the Prism 6 software package (GraphPad). Student's *t*-tests were used for all pairwise comparisons (indicated in Results where *t* value is given), and one-way ANOVAs were used for all multiple comparisons (indicated in results section where F-value is given). Differences were considered significant at $p < 0.05$.

RESULTS

We treated mice with fluoxetine (20 mg/kg IP) daily for 14 days, excised NAc, and determined CaMKII protein levels by western blotting (Figure 1a). Total levels of CaMKIIα protein were reduced in fluoxetine-treated animals ($p = 0.032$; $t = 2.017$; $df = 13$), whereas total levels of P-Thr^{286} CaMKII were unchanged, suggesting increased autophosphorylation of the remaining CaMKII (Figure 1b). This suggests a possible compensatory mechanism to maintain normal levels of Ca^{2+}-independent CaMKII activity, which may account for the lack of change in Ser^{831} phosphorylation of the AMPA glutamate receptor subunit GluA1, a known CaMKII substrate (Figure 1b). The fluoxetine-induced reduction in CaMKII (Figure 1c) occurred in both the shell (Figure 1d; $p = 0.019$; $t = 2.722$; $df = 12$) and core (Figure 1e; $p = 0.052$; $t = 2.178$; $df = 11$) subregions of NAc in control animals as well as in animals that underwent chronic social defeat stress prior to fluoxetine treatment. Interestingly, chronic social defeat or other forms of stress *per se* did not change total levels of NAc CaMKII (Figures 1d and e). These data suggest that downregulation of NAc CaMKII expression does not contribute to the onset of a stress-related phenotype but may be a mechanism of fluoxetine action.

On the basis of recent evidence that ΔFosB binds to the CaMKIIα promoter and induces CaMKIIα expression in NAc during cocaine treatment (Robison *et al*, 2013), we considered a role for ΔFosB in fluoxetine's regulation of this kinase. In support of a transcriptional mechanism for CaMKIIα suppression, we found that fluoxetine decreased mRNA levels of CaMKIIα but not CaMKII*β* in NAc (Figure 2a; $p = 0.026$; $t = 2.458$; $df = 15$). As reported previously (Vialou *et al*, 2010), fluoxetine treatment increased ΔFosB levels in NAc (Figure 1b; $p = 0.038$; $t = 1.930$; $df = 13$). Surprisingly, however, qChIP revealed that ΔFosB binding to the CaMKIIα promoter in NAc was reduced by fluoxetine (Figure 2b; $p = 0.002$; $t = 4.111$; $df = 10$). In contrast, such binding was increased after chronic social defeat ($p = 0.022$; $t = 2.837$; $df = 8$), when ΔFosB levels are also known to be elevated (Vialou *et al*, 2010).

To understand the mechanism underlying this paradox, we used qChIP to examine fluoxetine effects on the chromatin state of the CaMKIIα promoter, and found that pan-acetylation of H3, a mark of transcriptional activation, was reduced in NAc by chronic fluoxetine ($p = 0.049$; $t = 1.749$; $df = 18$), whereas dimethylation of lysine 9 of H3, a mark of transcriptional repression, was increased under these conditions (Figure 2c; $p = 0.043$; $t = 1.866$; $df = 12$). In contrast, trimethylation of lysine 4 or 27 of H3, associated with gene activation or repression, respectively, was unaffected. Partly different results were seen for CaMKIIβ: chronic social defeat stress increased ΔFosB binding to the CaMKIIβ gene promoter in NAc ($p = 0.019$; $t = 2.915$; $df = 8$; Figure 2d) as seen for CaMKIIα, but chronic fluoxetine had no effect. Furthermore, ΔFosB binding to an unrelated target gene, Sparc-like 1 (also known as hevin) (Vialou *et al*, 2010), was increased in response to chronic fluoxetine (Figure 2e; $p = 0.0063$; $t = 3.540$; $df = 9$), indicating that the induction of ΔFosB by fluoxetine can drive increased DNA binding to certain gene targets presumably because of their unique epigenetic state. In addition, we found that chronic fluoxetine does not alter global levels of several forms of histone modifications or of histone deacetylases in NAc (Supplementry Figure S1). Together, these results indicate some gene specificity of fluoxetine action.

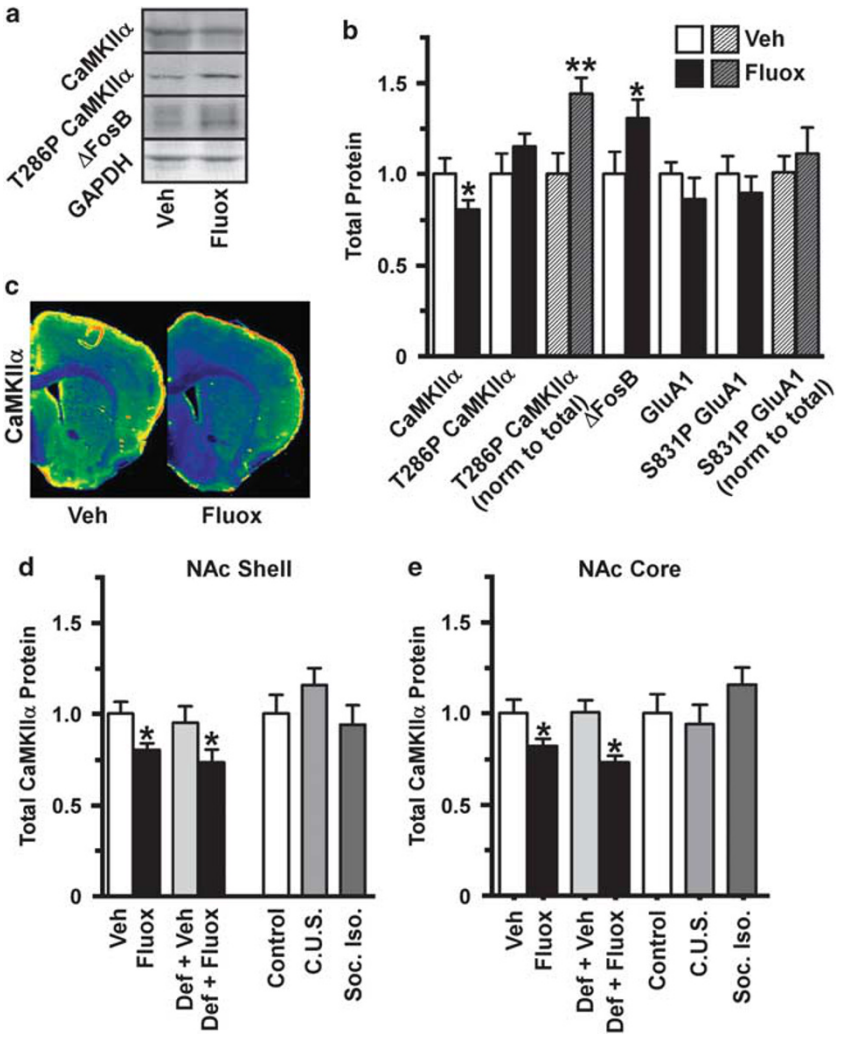

Figure 1 (a) Western blotting of mouse NAc after 14 daily fluoxetine injections (20 mg/kg IP), analyzed 24 h after the last dose. (b) Quantitation of (a) reveals decreased total CaMKIIα, no change in total Thr^{286} autophosphorylation but an inferred increase in relative phosphorylation, and an increase in total ΔFosB ($n = 7$–9 mice). (c) Immunohistochemical analysis of CaMKIIα expression in brains of chronic fluoxetine- or vehicle-treated mice. Quantitation of immunohistochemical signal in both NAc shell (d) and core (e) demonstrates fluoxetine-induced decrease in CaMKIIα expression in both control and socially defeated mice (Def), but no change resulting from several forms of chronic stress alone (Def.; C.U.S.: chronic unpredictable stress; or Soc. Iso.: adult social isolation; $n = 7$–9 mice).

To determine whether these effects of fluoxetine are clinically relevant, we analyzed postmortem NAc from control and depressed patients who were taking antidepressants at their time of death (Table 1). We found that these medicated depressed individuals displayed reduced H3 pan-acetylation (Figure 3b; $p = 0.011$; $t = 2.709$; df = 30; primer pair at −360 position) as well as increased dimethylation of H3 lysine 9 (Figure 3c; $p = 0.018$; $t = 2.509$; df = 30; primer pair at −38 position) at adjacent regions of the CaMKIIα promoter, exactly mimicking our findings in the mouse model. This same set of samples showed reduced expression of CaMKIIα mRNA (Figure 3d; $p = 0.012$; $t = 2.709$; df = 27) in the NAc of medicated depressed individuals. Furthermore, H3 acetylation and mRNA expression levels in NAc were significantly correlated (Figure 3e; $p = 0.041$; F = 4.622; DFd = 26), further suggesting that antidepressant treatment regulates CaMKIIα levels in human NAc by reducing acetylation at its promoter. In contrast, dimethylation of H3 lysine 9 did not correlate with CaMKIIα mRNA expression (Supplementry Figure S2). Importantly, we replicated reduced expression of CaMKIIα in the NAc of a second human cohort (Figure 3d; $p = 0.018$; $t = 2.456$; df = 43).

To determine whether the reduction in CaMKIIα was sufficient for the antidepressant effects of chronic fluoxetine, we used HSV to transiently express AC3I (a peptide inhibitor of CaMKII) fused to GFP (Klug *et al*, 2012; Robison *et al*, 2013), or GFP alone, in NAc of mice that underwent chronic social defeat stress (Supplementry Figure S3). Mice expressing AC3I mimicked chronic fluoxetine-treated animals (Berton *et al*, 2006; Klug *et al*, 2012) by displaying increased social interaction (Figure 4a; $p = 0.035$; $t = 1.937$; df = 16), decreased time spent in the corner (Figure 4b; $p = 0.017$; $t = 2.329$; df = 16), and increased sucrose preference (Figure 4c; $p = 0.015$; $t = 2.713$; df = 16), all antidepressant-like effects. We next determined whether reduced CaMKIIα expression in NAc is also required for the behavioral effects of chronic fluoxetine

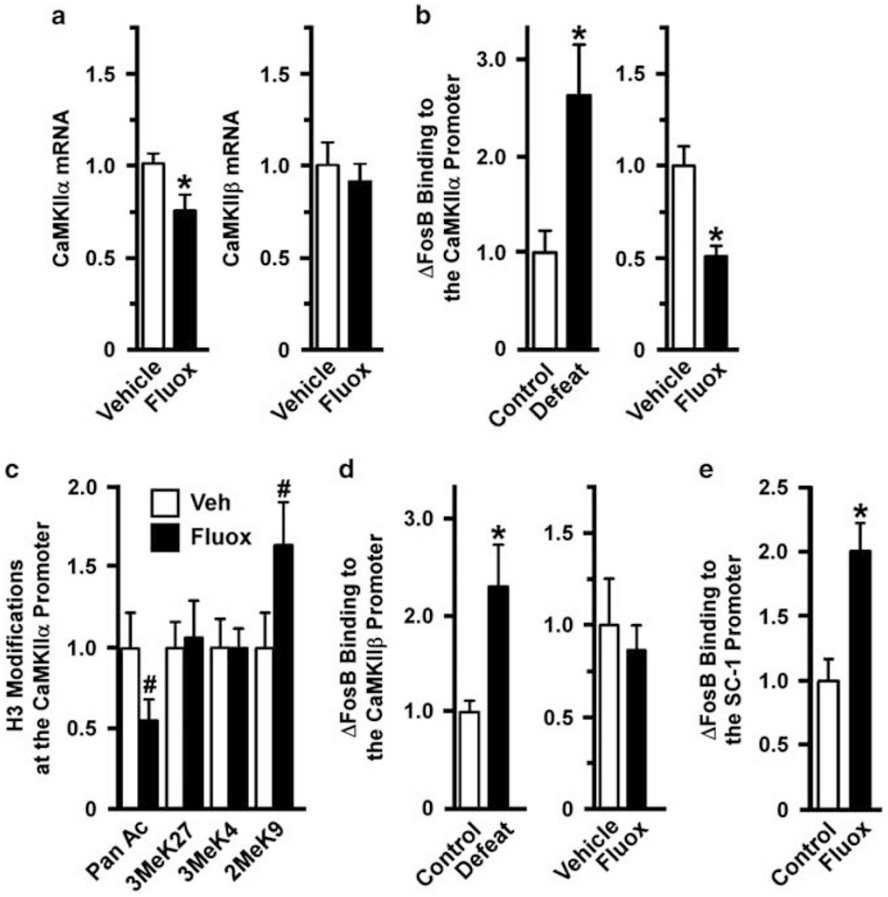

Figure 2 (a) qPCR reveals a significant decrease in NAc CaMKIIα, but not in CaMKII*β*, mRNA in response to chronic fluoxetine. (b) qChIP of mouse NAc reveals increased ΔFosB binding to the CaMKIIα promoter after chronic social defeat (Def), but a paradoxical decrease in binding in response to chronic fluoxetine ($n = 5$–6 groups of mice). (c) qChIP shows reduced H3 pan-acetylation and increased H3 lysine 9 dimethylation (2MeK9) at the CaMKIIα promoter in NAc after chronic fluoxetine, with no change in 3MeK27 or 3MeK4 ($n = 10$ groups of mice). (d) A similar analysis reveals increased ΔFosB binding to the CaMKIIβ promoter after Def, but no change in response to chronic fluoxetine ($n = 5$–6 groups of mice). (e) qChIP of mouse NAc reveals increased ΔFosB binding to the Sparc-like 1 (SC-1) promoter after chronic fluoxetine ($n = 5$–6 groups of mice) (*$p \leqslant 0.05$, two-tailed *t*-test; $^{\#}p \leqslant 0.05$, one-tailed *t*-test).

Table 1 Human Medicated-depressed Patients and Matched Controls

Group	Age	Sex (% male)	pH	Refrigeration delay (hr)
Control ($n = 16$)	49.8 ± 4.5	67%	8.2 ± 1.0	17.0 ± 1.2
Medicated-depressed ($n = 15$)	45.8 ± 4.4	87%	7.8 ± 1.4	15.5 ± 1.5
P value	0.53		0.32	0.42

All values are mean ± standard error. *P* values were calculated using two-tailed student's *t*-test.

by virally overexpressing CaMKII in the NAc (Supplementry Figure S3). Fluoxetine reversed the behavioral deficits caused by social defeat in mice expressing GFP alone ($p = 0.036$; $t = 1.924$; $df = 16$), but not in mice overexpressing CaMKIIα in NAc (Figures 4d and e).

DISCUSSION

The data presented here uncover fluoxetine-induced epigenetic modifications at the CaMKIIα gene promoter in NAc, which are accompanied by a reduction in CaMKIIα expression and are conserved in human patients on antidepressants. Our findings also establish a causal role for such CaMKIIα repression in NAc in the therapeutic-like behavioral actions of fluoxetine. In contrast, we found no evidence for altered CaMKIIα expression in this brain region in mouse depression models *per se*, suggesting that this phenomenon is a mechanism of antidepressant drug action rather than of disease phenotype. It will be interesting in future studies to determine whether other classes of antidepressant medications also work in part through suppression of CaMKIIα expression in this brain region.

We demonstrate histone changes consistent with gene repression—reduced levels of H3 acetylation and increased levels of H3 lysine 9 dimethylation—at the CaMKIIα promoter in NAc in response to chronic fluoxetine administration. These histone modifications are associated with the reduced ability of ΔFosB to bind to and activate the CaMKIIα promoter despite the fact that ΔFosB is itself induced under these conditions. We know that ΔFosB induction in NAc during chronic social defeat stress is a mechanism of natural resilience, whereas its induction is required for fluoxetine's antidepressant-like effects as well (Vialou *et al*, 2010). We have identified numerous target genes in NAc through which ΔFosB produces these effects,

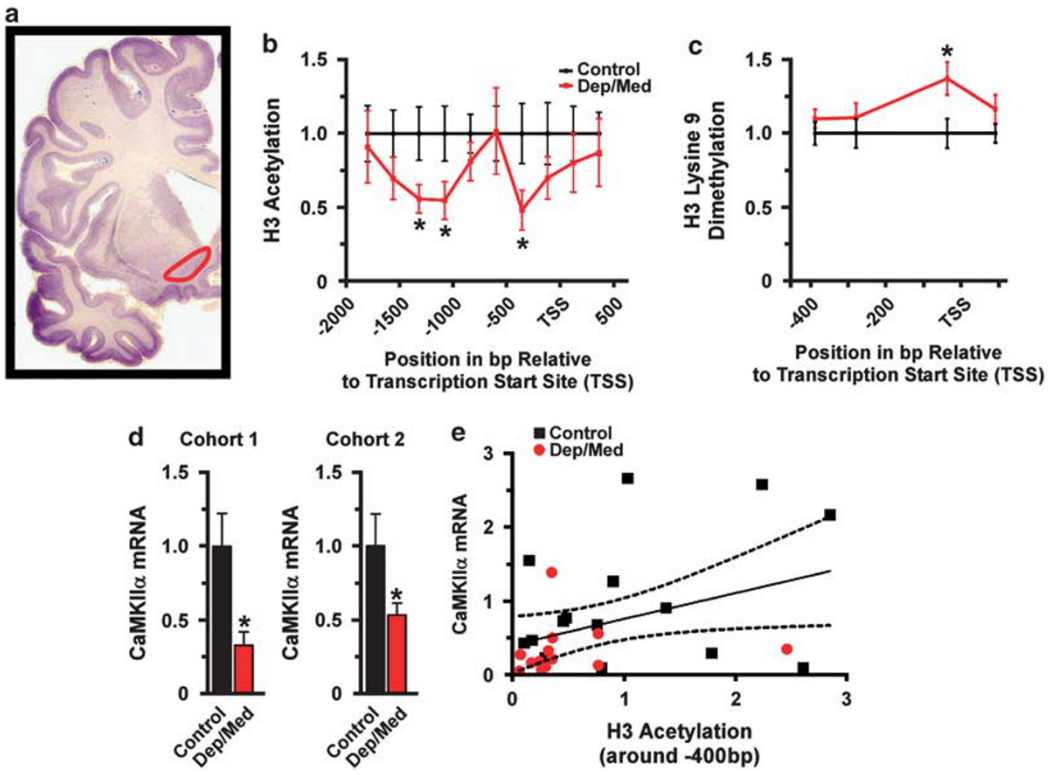

Figure 3 (a) Coronal slice of human brain highlighting NAc (red circle; courtesy MSU Human Brain Atlas). (b) qChIP for H3 pan-acetylation across the human CaMKIIα promoter in NAc from control and medicated-depressed patients ($n = 13$–16). (c) Similar analysis of H3 lysine 9 dimethylation ($n = 13$–16). (d) qPCR from two separate human cohorts reveals reduced CaMKIIα mRNA expression in the NAc of medicated-depressed patients ($n = 13$–16). (e) Correlation between CaMKIIα mRNA and H3 acetylation at the CaMKIIα promoter ($r^2 = 0.1509$; $p = 0.04$ slope deviation from 0) (*$p \leqslant 0.05$, two-tailed *t*-test).

including the AMPA glutamate receptor subunit GluA2 and the anti-adhesive matrix protein Sparc-like 1, both of which display increased ΔFosB binding to their promoters after fluoxetine treatment (Vialou *et al*, 2010). Results of the present study suggest that fluoxetine's mechanism of action also involves some adaptations beyond those of natural resilience—namely, epigenetic changes at the CaMKIIα promoter that might prevent ΔFosB binding. Thus, under conditions of social defeat, and in the absence of fluoxetine treatment, ΔFosB binds to the CaMKIIα promoter, but is repelled from the promoter only after fluoxetine administration. These findings are an important illustration of the complexity of chromatin adaptations in the brain and provide a mechanism for how a given transcription factor like ΔFosB controls a partly distinct subset of target genes under different treatment conditions. Nevertheless, it is crucial to emphasize that the evidence relating epigenetic modifications to one another and to suppression of CaMKIIα expression remains correlative. For example, it is possible that chronic fluoxetine suppresses ΔFosB binding to the CaMKIIα promoter through other chromatin mechanisms. Likewise, it is conceivable that other conditions, like cocaine and stress, which increase ΔFosB binding to the CaMKIIα promoter in NAc, enable such ΔFosB binding through the induction of some mechanism not shared by fluoxetine. Ultimately, novel tools, which make it possible to experimentally induce a specific epigenetic modification at a single gene within NAc *in vivo*, will be required to test these and many alternative hypotheses.

We show that fluoxetine's repression of CaMKII—associated with repressive epigenetic modifications and blockade of ΔFosB binding to the CaMKIIα promoter—is necessary and sufficient for fluoxetine's behavioral effects. A peptide inhibitor of CaMKII, AC3I, has antidepressant effects when expressed in the NAc, whereas overexpression of CaMKIIα in this brain region blocks fluoxetine action. Although AC3I mimics the autoinhibitory domain of CaMKII, and thus inhibits enzyme catalytic activity, it also blocks multiple protein–protein interactions that are important for CaMKII's translocation and for synaptic physiology and behavior (Halt *et al*, 2012; Robison *et al*, 2005; Strack *et al*, 2000a). Unfortunately, it is not currently feasible to obtain an accurate, direct measure of CaMKII activity within a discrete brain region like the NAc, let alone within specific subcellular compartments, of behaving animals. Thus, understanding which aspect of CaMKII function in NAc is important for antidepressant effects is a future goal for the field. As CaMKII isoforms are expressed ubiquitously and are essential for the function of multiple organs, systemic inhibition of this enzyme is not an appealing target for novel antidepressants. However, because CaMKIIα forms unique complexes important for synaptic function, future studies may reveal novel routes of therapeutic action based on disruption of CaMKIIα activity or intracellular targeting.

Although they increase serotonin signaling rapidly *in vivo*, fluoxetine and other selective serotonin reuptake inhibitors typically require weeks of treatment before

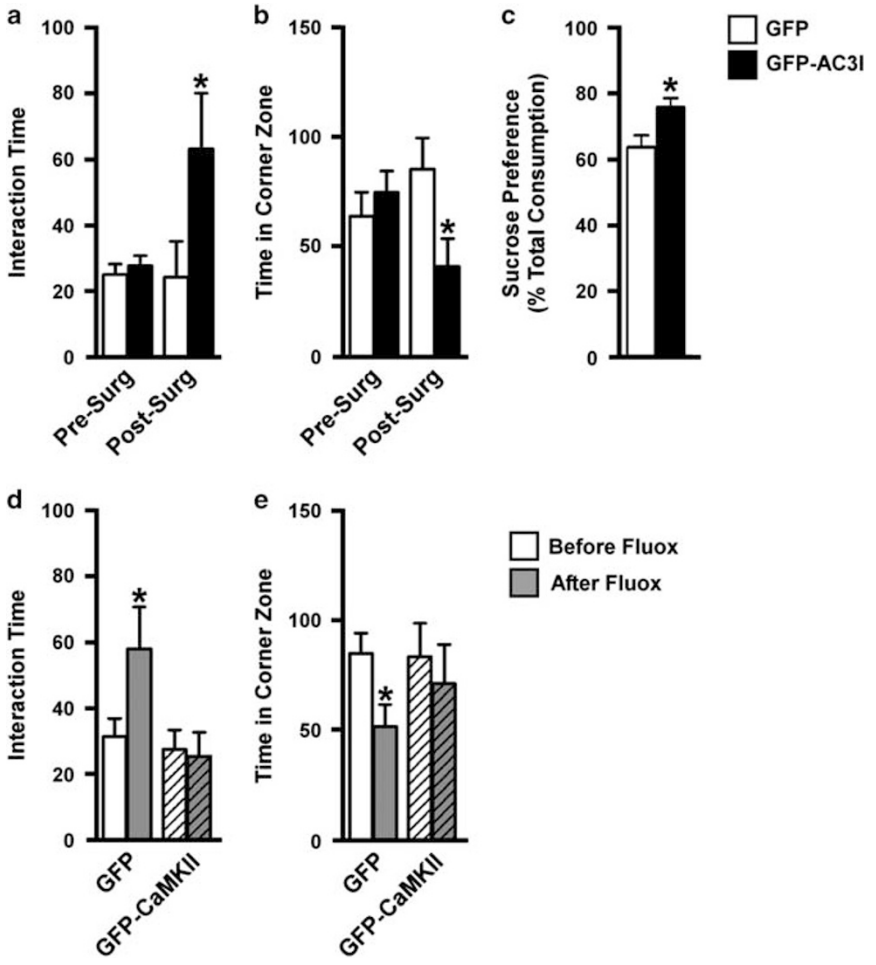

Figure 4 Social interaction time (a), time spent in the corner zones (b), and sucrose preference (c) from socially defeated mice before and after surgery for NAc expression of GFP alone or GFP-AC3I indicate that local inhibition of CaMKII activity reverses stress-induced behavioral abnormalities ($n = 9$). (d) Social interaction time and (e) time spent in the corner zones in defeated animals before and after chronic fluoxetine in mice with NAc expression of GFP alone or GFP and CaMKIIα demonstrate that CaMKII overexpression blocks the behavioral effects of fluoxetine ($n = 8$–9) (*$p \leqslant 0.05$, two-tailed *t*-test).

having antidepressant effects in humans. This suggests that the antidepressant effects of these compounds are, at least in part, due to long-term cellular adaptations downstream, and perhaps far removed, from increased serotonin signaling. Multiple subtypes of serotonin receptors are expressed in NAc, including 5-$HT_{1/7}$, 5-HT_{2C}, and 5-HT_6 receptors, and NAc-specific pharmacological manipulation of these individual receptor types alters multiple behaviors, including feeding (Pratt *et al*, 2012) and alcohol action (Andrade *et al*, 2011). Although the mechanisms that link increased serotonin signaling to epigenetic modifications at the CaMKIIα promoter remain virtually completely unknown, we have demonstrated previously that chronic, systemic administration of fluoxetine or chronic NAc-specific inhibition of histone deacetylases—both of which produce antidepressant-like behavioral actions—have strikingly similar effects on global patterns of gene expression in NAc (Covington *et al*, 2009). These results are consistent with our hypothesis that one important long-term consequence of SSRI action is to exert epigenetic modifications in the NAc.

A related question is: how does suppression of CaMKII in NAc lead to antidepressant-like behavioral effects? Acute application of imipramine, an older SSRI, potentiates hippocampal synapses (Cai *et al*, 2013), a process long known to involve CaMKIIα function (Colbran and Brown, 2004); a similar process occurs in the cerebral cortex upon acute 5-$HT_{2A/2C}$ activation (Jitsuki *et al*, 2011). It is thus interesting to speculate that long-term SSRI treatment might decrease CaMKIIα expression as a compensatory mechanism to prevent over-strengthening of synaptic connections in several brain regions. Chronic stress induces NMDAR-dependent synaptic plasticity in the NAc (Belujon and Grace, 2011; Jiang *et al*, 2013) as well as plasticity of NAc synaptic structures (Christoffel *et al*, 2011), and both of these processes have been shown to involve CaMKII in various contexts (Colbran and Brown, 2004; Huang and Hsu, 2012; Robison *et al*, 2013). However, as CaMKIIα is present throughout the cell and is known to regulate histone modification machinery itself (Linseman *et al*, 2003), speculation for a specifically synaptic role for the fluoxetine-mediated reduction in CaMKIIα expression is premature.

Exposure to chronic stress and treatment with antidepressants are both associated with a variety of epigenetic adaptations, including changes at specific genes as well as changes in global levels of certain histone modifications

(Vialou *et al*, 2013). For example, pharmacological or viral manipulation of the machinery regulating histone acetylation (Covington *et al*, 2009) or methylation (Covington *et al*, 2011) in NAc potently regulates stress responses and produces antidepressant-like effects. The present study suggests that these actions may be mediated in part by epigenetic regulation of transcription factor binding to specific genes. As the epigenetic state of each gene may vary widely between individuals (Rakyan *et al*, 2004), this may explain some of the variation in resilience to stress and the large number of patients with treatment-refractory depression. It is our expectation that the present study and others like it will, when combined with technology allowing the high-throughput determination of an individual's epigenetic state at specific genes, provide the basis for more specific diagnoses of depressive syndromes and more effective, individually tailored treatments for depression.

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The authors declare no conflict of interest.

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