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ΔFosB in brain reward circuits mediates resilience to stress and antidepressant responses

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Abstract

In contrast to the vast literature on stress effects on the brain, relatively little is known about the molecular mechanisms of resilience, the ability of some individuals to escape the deleterious effects of stress. Here we show that the transcription factor, ΔFosB, mediates an essential mechanism of resilience in mice. Induction of ΔFosB in the nucleus accumbens, a key brain reward region, in response to chronic social defeat stress is both necessary and sufficient for resilience. ΔFosB induction also is required for the ability of the standard antidepressant, fluoxetine, to reverse behavioral pathology induced by social defeat. ΔFosB produces these effects through the induction of the GluR2 AMPA glutamate receptor subunit, which decreases the responsiveness of nucleus accumbens neurons to glutamate, and through other synaptic proteins. Together, these findings establish a novel molecular pathway underlying both resilience and antidepressant action.

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Author Contributions V.V. and E.J.N. were responsible for overall study design. Q.L. and V.V. designed, conducted, and analyzed RNA and ChIP experiments. A.J. Robison designed, conducted, and analyzed electrophysiological studies. H.E.C. and V.V. designed and conducted the NBQX pharmacological experiments. Q.L., D.M.D., E.L.W., and V.V. performed the stereotaxic surgeries. Y.N.O. cloned the SC1 cDNA into the HSV vector. Y.H.O. performed the AP1 luciferase assay. Q.L., D.M.D., D.W., and V.V. designed and conducted the social isolation experiments. V.V., E.L.W., and A.J. Rush performed social defeat tests and immunohistochemical quantification. S.I., Q.L., B.W., and V.V. performed and analyzed rat surgery and forced swim test. E.M. and R.N. provided the viral vectors for viral-transgenesis. M.A.S., V.K., and O.B. trained V.V. in social defeat and biochemical analysis and provided quality control over the social defeat data. S.G. and C.A.T. provided the human post-mortem brain tissue. V.V. and E.J.N. wrote the paper with the help of the other authors.

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INTRODUCTION

People subjected to severe stress exhibit widely differing responses, with some able to overcome crisis while others develop severe psychopathology such as depression or post-traumatic stress disorder (PTSD). The ability to cope with stressful situations, i.e., resilience, depends on the development of adequate behavioral and psychological adaptations to chronic stress[1,2]. Psychological constructs that promote resilience include commitment, patience, optimism, and self-esteem, as well as the capacity to modulate emotions and develop adaptive social behavior. These traits implicate the brain's reward circuitry, which appears to be a critical determinant for the emergence of pathological vs. resilient phenotypes[3,4]. Neurobiological correlates of vulnerability or resistance to stress have 2 been identified in humans, but the extent to which they are the cause or consequence of susceptibility remains unknown[5].

Among current rodent models of depression and PTSD, chronic social defeat stress is an ethologically valid approach, which induces long-term physiological[6–8] and behavioral[9–11] alterations, including social avoidance, anhedonia, and anxiety-like symptoms, involving activation of several neural circuits and neurochemical systems[12–15]. The normalization of social avoidance by chronic, but not acute, antidepressant treatment makes it a valuable model for examining aspects of depression and PTSD in humans[11,16]. A significant proportion (~30%) of chronically defeated mice avoid most of the negative behavioral sequelae of defeat[10], thereby allowing for experimental investigations of resiliency. While the induction of several proteins within the nucleus accumbens (NAc), a key brain reward region, has been shown to be important for the expression of depressive-like behaviors after defeat[10,11,17,18], much less is known about the molecular basis of resiliency mediated by this brain region. Here, we addressed this question by focusing on ΔFosB, a Fos family transcription factor induced in NAc by drugs of abuse, natural rewards, and several types of stress[19–21].

RESULTS

ΔFosB in NAc promotes resilience to social defeat stress

C57BL/6J male mice were subjected to ten consecutives days of social defeat[10,11], and then separated into susceptible and resilient populations based on a measure of social avoidance (Fig. 1a), which correlates with several other depressive-like behaviors[10]. We found an increase in ΔFosB, measured by immunohistochemistry, in NAc after chronic social defeat (Fig. 1b,c), with resilient mice showing the greatest induction of ΔFosB in both core and shell NAc subregions (Fig. 1b,c). Moreover, we observed a strong ($p<0.01$) correlation between levels of ΔFosB and social interaction ($r = 0.80$, NAc shell; $r = 0.85$, NAc core; $r = 0.86$, whole NAc), suggesting that the degree of ΔFosB induction in NAc may be a critical determinant of whether an animal shows a susceptible vs. resilient phenotype. Western blot analysis of NAc dissections containing core and shell subregions confirmed ΔFosB induction in resilient mice only (see Supplementary Fig. 1).

To test the functional consequences of ΔFosB induction, we used bitransgenic mice that inducibly overexpress ΔFosB specifically in the adult NAc and dorsal striatum[22]. These mice showed a reduced propensity to develop social avoidance after four or ten days of social defeat (Fig. 1d), thereby suggesting that ΔFosB exerts a protective action against social stress. Conversely, we used bitransgenic mice that inducibly overexpress ΔcJun, a transcriptionally inactive truncated cJun mutant that antagonizes ΔFosB activity[23,24]. In contrast to mice overexpressing ΔFosB, mice overexpressing ΔcJun are more susceptible to chronic social defeat than control littermates and show maximal avoidance behavior following 4 days of defeat (Fig. 1e). The ΔcJun mice also exhibited increased immobility in a one day forced swim test, as well as reduced sucrose preference, both interpreted as increased depression-like behavior (Supplementary Fig. 2a,b). However, ΔFosB or ΔcJun overexpression did not alter

several baseline measures of locomotor activity or anxiety-like behavior (Supplementary Fig. 2c–f). Together, these findings suggest that reduced ΔFosB activity in NAc and dorsal striatum reduces positive, adaptive responses, inferred as "coping[7]", to chronic stress.

Reduced ΔFosB in NAc promotes stress susceptibility

To gain further insight into the behavioral actions of ΔFosB after chronic stress, we utilized a prolonged period of social isolation during adulthood, which induces depression-like abnormalities in mice[25] and is a major risk factor for clinical depression. We observed decreased ΔFosB levels in NAc of socially isolated mice (Fig. 2a,b). We also found that isolation renders mice dramatically more vulnerable to social defeat, and that this isolation-induced vulnerability was reversed completely by virally overexpressing ΔFosB selectively in NAc (Fig. 2c). Conversely, blockade of ΔFosB function in NAc, by viral overexpression of ΔJunD, in group-housed control mice promoted susceptibility to social defeat (Fig. 2c). ΔJunD, like ΔcJun, is an N-terminal truncated mutant that acts as a dominant-negative antagonist of ΔFosB (Supplementary Fig. 3)[23]. These findings directly implicate basal levels of ΔFosB in NAc in stress vulnerability.

To study the clinical relevance of these findings, ΔFosB levels were measured in postmortem human NAc samples obtained from depressed patients and extensively matched controls. We found a ~50% decrease in ΔFosB levels in depressed patients (Fig. 2d), supporting a role for ΔFosB in human depression. The depressed humans analyzed included individuals either on or off antidepressants at their time of death (Supplementary Table 1), and we found no correlation between ΔFosB levels and antidepressant exposure. In light of our observation that antidepressant treatment increases ΔFosB levels in mouse NAc (see below), these findings suggest that the failure to induce ΔFosB in NAc may be an important determinant for lack of antidepressant responses in humans.

ΔFosB in NAc mediates antidepressant action

Chronic antidepressant treatment reverses defeat-induced social avoidance seen in susceptible mice[11]. We therefore examined whether ΔFosB induction in NAc may be a mechanism not only for resiliency but for antidepressant action as well. Non-defeated control mice treated with fluoxetine for 20 days revealed no alterations in social behavior, but exhibited an accumulation of ΔFosB in NAc shell (Fig. 3a,b) and core (Supplementary Fig. 4). Fluoxetine treatment of susceptible mice reversed their social avoidance (Fig. 3a), as reported previously, and further enhanced ΔFosB levels in NAc (Fig. 3b, Supplementary Fig. 4).

To directly test the involvement of such ΔFosB induction in the behavioral effects of fluoxetine, we virally overexpressed ΔJunD or GFP alone (as a control) in NAc of previously defeated mice. Half of the mice in each group were then treated for three additional weeks with fluoxetine or vehicle. As expected, fluoxetine treatment of mice overexpressing GFP in NAc showed a reversal of social avoidance induced by chronic social defeat. In contrast, overexpression of ΔJunD blocked this therapeutic effect of fluoxetine (Fig. 3c), supporting the hypothesis that ΔFosB induction in NAc is required for antidepressant action. In addition, viral-mediated overexpression of ΔFosB in the rat NAc produced a significant antidepressant-like effect as measured by decreased time of immobility on day 2 of the forced swim test (Supplementary Fig. 5a). Further analysis of behavior during this test revealed ΔFosB-induced increases in both swimming and climbing (Supplementary Fig. 5b–d), features related to alterations in serotonergic and noradrenergic mechanisms[26]. Interestingly, rats overexpressing ΔFosB in NAc showed decreased immobility time on the first day of the test as well, interpreted as a pro-motivational effect (see Online Methods and Supplementary Fig. 5e–h).

AMPA receptor regulation in NAc mediates resilience

ΔFosB regulates the transcription of numerous genes in NAc[24,27]. One established target gene is the AMPA glutamate receptor subunit GluR2: mice overexpressing ΔFosB in NAc have elevated levels of GluR2, with no effects on other glutamate receptor subunits[22]. This selective upregulation of GluR2 in NAc has been linked to an enhancement of drug and natural reward[22,28]. To address the possibility that modulation of GluR2 contributes to ΔFosB's pro-resilience action as well, we studied GluR2 expression in NAc after chronic social defeat. Susceptible mice showed a significant decrease in GluR2 levels in this brain region compared to controls, while resilient mice showed increased GluR2 levels (Fig. 4a). While the mechanism underlying the suppression of GluR2 expression in susceptible mice remains unknown, the induction of GluR2 seen in resilient mice appears to reflect a direct effect of ΔFosB on the GluR2 gene, because we found increased binding of ΔFosB to the GluR2 promoter by use of chromatin immunoprecipitation (ChIP) (Fig. 4b), and quantitative PCR (qPCR) revealed sustained induction of GluR2 mRNA levels in NAc of resilient mice (Fig. 4c), which parallels the sustained induction of ΔFosB. Interestingly, GluR1 was oppositely regulated after social defeat: we observed increased expression in susceptible mice and decreased expression in resilient mice (Fig. 4a). However, no corresponding alterations were seen in GluR1 mRNA expression, suggesting post-translational mechanisms. In addition, chronic fluoxetine treatment of non-defeated mice increased GluR2 levels in NAc (Fig. 4d), and analysis of human postmortem NAc tissue from depressed patients revealed decreased GluR2 levels compared to controls (Fig. 4e). No changes in GluR1 levels were detected (Fig. 4e).

The presence of GluR2 has profound effects on AMPA receptors: GluR2-lacking AMPA receptors are Ca^{2+}-permeable, and display greater receptor conductance and strong inwardly rectifying currents, compared to GluR2-containing receptors[29]. To complement our biochemical results, we therefore performed whole-cell voltage-clamp recordings of medium spiny neurons in NAc of non-defeated mice and after social defeat in both resilient and susceptible animals. Current-voltage relationships of AMPA-mediated evoked excitatory postsynaptic currents (EPSCs) revealed significantly greater inward rectification in the susceptible mice (Fig. 5a–c) compared to controls, consistent with the increased ratio of GluR1:GluR2 seen under these conditions. Although the degree of rectification in cells recorded from susceptible mice was variable, we observed a highly significant change in rectification compared to both control and resilient groups. The consistency of this finding is indicated by the fact that the degree of rectification of all cells from susceptible mice exceeded the mean value seen for control cells. In addition, we found that the level of rectification was indirectly correlated with social avoidance (Fig. 5d), suggesting that changes in the GluR1:GluR2 ratio may partly drive this behavior. To confirm the greater prevalence of GluR2-lacking receptors in susceptible mice, we incubated slices from control and susceptible mice with 1-naphtylacetylsperimine (NASPM), a selective blocker of GluR2-lacking AMPA receptors. Evoked EPSCs in neurons recorded from susceptible mice (Fig. 5e–f) were significantly reduced by NASPM, demonstrating that GluR2-lacking AMPA receptors contribute significantly more to glutamatergic transmission in susceptible mice than controls. Of note, the effect of NASPM in susceptible mice was less than predicted considering the larger change observed in rectification. This divergence, however, is not unprecedented[30] and may result from post-translational modifications or protein-protein interactions involving GluR2 (see Discussion), or simply the extent of NASPM exposure. The stress-induced increase in inward rectification observed in susceptible mice was absent in resilient mice (Fig. 5a–d), consistent with the observed decrease in GluR1 and increase in GluR2 under these conditions. However, we did not see a decrease in inward rectification in resilient mice compared to controls (see Discussion).

Antidepressant-like effects of AMPA receptor blockade in NAc

These data suggest that increased AMPA receptor function (increased GluR1:GluR2 ratio) in NAc of susceptible mice promotes social avoidance, whereas decreased AMPA function (decreased GluR1:GluR2 ratio) contributes to resilience. To test this hypothesis, we infused the AMPA receptor antagonist NBQX directly into the NAc of defeated mice immediately prior to the social avoidance test. NBQX increased social interaction time (Fig. 4f), demonstrating that blockade of fast excitatory input to the NAc opposes the expression of this deleterious effect of chronic social stress. NBQX did not alter general locomotor activity (Supplementary Fig. 6). In addition, the antidepressant-like effect of a single infusion of NBQX on social avoidance was long-lasting as mice re-tested one week later showed further enhancement of social interaction.

We next virally overexpressed GluR2 selectively in NAc of susceptible mice. GluR2 expression completely reversed the social avoidance induced by chronic social defeat (Fig. 4g), supporting the view that GluR2 upregulation in NAc is a key mechanism of resilience. Interestingly, the effect of GluR2 overexpression persisted for at least 10 days after surgery (Fig. 4g) when viral-mediated GluR2 expression has completely dissipated. Conversely, in resilient mice, overexpression of the unedited version of GluR2, GluR2Q, which resembles GluR1 in functional studies, rendered the mice more susceptible to social defeat (Fig 4g), supporting the view that increased AMPA receptor function in NAc contributes to susceptibility.

SC1, another ΔFosB target, is also a mediator of resilience

To identify additional ΔFosB target genes that contribute to resilience, we compared gene expression array datasets that were obtained from the NAc of bitransgenic mice overexpressing ΔFosB and from C57Bl/6J mice 48 hours after chronic social defeat that displayed a resilient vs. susceptible phenotype[10,24]. Fig. 6a shows the considerable (>75%) overlap between genes induced in NAc both by ΔFosB and by resilience. Among these genes (listed in Supplementary Table 2), we selected for further analysis SC1, based on the magnitude of its induction in both resiliency and upon ΔFosB overexpression. SC1, also known as Sparc (secreted protein, acidic, rich in cysteine)-like 1 or hevin, is an anti-adhesive matrix molecule that is highly expressed in the adult brain, where it localizes in the postsynaptic density and is implicated in synaptic plasticity[31]. To assess directly the potential role of SC1 in resilience, we virally overexpressed SC1 in NAc of susceptible mice. SC1 significantly reversed the social avoidance induced by chronic social defeat (Fig. 6b). SC1 overexpression also exerted an antidepressant-like effect on day 2 of the rat forced swim test (Fig. 6c and Supplementary Fig. 7a–c), but had no effect on basal locomotor activity and anxiety-related behaviors (Supplementary Fig. 7d–g). In addition, we found a strong trend for decreased SC1 levels in human postmortem NAc tissue from depressed patients (Fig. 6d).

DISCUSSION

The results of the present study provide the first evidence of molecular adaptations occurring in medium spiny neurons of NAc that underlie resilient responses to chronic stress and that contribute to the therapeutic effects of chronic antidepressant treatment. We show that basal levels of ΔFosB in NAc determine an individual's initial vulnerability to social defeat stress, and that the degree of ΔFosB induction in response to chronic stress determines susceptible vs. resilient responses to that stress. We show further that the successful reversal of behavioral abnormalities induced in susceptible animals by chronic fluoxetine administration requires the drug's induction of ΔFosB in this brain region. These findings demonstrate that ΔFosB induction in NAc is both a necessary and sufficient mechanism of resiliency and of antidepressant responses. The finding of lower levels of ΔFosB in NAc of depressed humans

supports the relevance of these observations in mouse models to clinical depression. ΔFosB regulates NAc function by inducing or repressing numerous target genes[24,27]. We identify two of its target genes, the AMPA receptor subunit GluR2 and SC1, an extracellular matrix protein, and directly implicate them in mediating resilience to social defeat stress.

Such a pro-resiliency role for ΔFosB in the context of chronic stress is interesting in light of a wealth of evidence for ΔFosB's involvement in regulating responses to drugs of abuse and natural rewards such as food, sex, and exercise[19]. ΔFosB is induced in NAc by drug and natural rewards, and increases rewarding responses to these stimuli. It is thus implicated as a mediator of certain aspects of drug addiction. The present findings in stress models provide fundamentally new insight into the role of this protein in the regulation of complex emotional behavior. Under normal conditions, ΔFosB is expressed at highest levels in NAc compared to all other brain regions[19]. We hypothesize that levels of ΔFosB in NAc play an important role in setting the level of an individual's motivation and in orienting motivated behaviors toward prominent rewarding stimuli. The removal of environmental stimulation during prolonged isolation reduces basal levels of ΔFosB in the mouse NAc, impairing their motivation and increasing their vulnerability to chronic social stress, as we show here. The observed decrease in ΔFosB levels in postmortem NAc of depressed patients is in line with this hypothesis, and suggests a role of ΔFosB in the impaired motivation and reward seen in many people with depression. Conversely, the ability to induce ΔFosB in NAc in response to chronic stress enables an individual to enhance motivation and natural reward despite the ongoing stress, a hypothesis consistent with current views of resilience in humans[1,2]. We hypothesize further that induction of ΔFosB in NAc by chronic exposure to drugs of abuse, which is much greater in magnitude than that seen with stress or natural rewards[19], results in a pathological degree of enhanced motivation in a way that corrupts the reward circuitry toward the stronger drug stimuli.

Clearly, specific features of this hypothesis require further investigation. The induction of ΔFosB in NAc by chronic stress or by fluoxetine might be expected to increase drug reward. Indeed, comorbidity of depression and addiction is well established in humans, and cross-sensitization between drugs of abuse and stress has been demonstrated in rodents[32–34]. On the other hand, depression and addiction are both highly complex, heterogeneous syndromes and most people with depression do not have addiction and *vice versa*. Moreover, fluoxetine does not exert clear effects on drug responses in animals, nor is it an effective treatment of addiction in addicts who are not also depressed. Consistent with this complexity, we have found that susceptible mice, not resilient mice, in the social defeat paradigm show enhanced responses to drugs of abuse[10]. This would suggest that the enhanced vulnerability of susceptible mice to drugs of abuse is mediated via many other adaptations induced in NAc and elsewhere, as just one example, BDNF, which is induced in susceptible, not resilient, mice in NAc and enhances drug reward mechanisms[see 10].

The interpretation that ΔFosB promotes aspects of addiction, while promoting resilience to stress, is not surprising given the complex relationships observed between the role of a given protein in NAc in addiction vs. depression models. Some proteins (e.g., BDNF) promote responses to drugs of abuse and to stress, while many other proteins exert opposite effects under these two conditions: e.g., CREB in NAc produces a pro-depression phenotype, yet blunts responses to drugs of abuse [see 4,10]. These findings emphasize the need for further research in delineating the molecular underpinnings of complex emotional behavior, and the importance of employing the widest possible range of behavioral tests in such investigations. The results also indicate that, as would be expected, ΔFosB alone cannot explain the full phenomena of depression and addiction, rather, it is a key regulator of NAc-dependent reward mechanisms and thereby is important in mediating certain aspects of both conditions. However, a major caveat of this discussion is the different cell types in NAc in which ΔFosB is induced

in stress and addiction models. Drugs of abuse and natural rewards induce ΔFosB primarily in the subclass of medium spiny neurons in NAc that express D_1 dopamine receptors[19,22], while stress induces ΔFosB roughly equally within D_1 and D_2 receptor-containing medium spiny neurons[20]. This differential induction could have dramatic functional consequences, since the ability of ΔFosB to enhance reward has been shown for D_1 class neurons only[19].

The identification of GluR2 as a target gene involved in mediating ΔFosB's pro-resilience effect sheds some light on these considerations. We show that susceptibility in mice, and human depression, are associated with an increase in the GluR1:GluR2 ratio in NAc, which suggests increased medium spiny neuron excitability in response to glutamate. The NAc receives glutamatergic inputs from several brain regions, in particular, prefrontal cortex, amygdala, and hippocampus[35]. Such glutamatergic input modulates the valence and saliency of rewarding and aversive stimuli and thereby controls motivated behavior[36–38]. Recent studies are consistent with our hypothesis that enhanced NAc excitability may promote stress vulnerability. Forced swim stress increases synaptic strength and AMPA receptor function in NAc[39], while glutamate infusion into NAc reduces swimming behavior in the forced swim test, a pro-depression-like effect[40]. More generally, increased NAc firing encodes aversive states in several animal models[41]. Alterations in NAc activity have been observed in patients with major depression[42] and in special forces soldiers pre-selected and trained to be resilient in the face of severe trauma[43]. Likewise, deep brain stimulation of subgenual cingulate cortex or of NAc (a major target of subgenual cingulate cortex), an intervention thought to reduce excitability of the stimulated brain region, alleviates depressive symptoms in treatment-refractory patients[3,44].

Like stress models, increased glutamatergic responsiveness in NAc has also been implicated in drug addiction[30,45–47]. This includes an increase in GluR2-lacking AMPA receptors in this brain region[30,47], similar to what we report here for stress susceptibility. Together, these observations raise the interesting possibility that enhanced glutamatergic transmission in NAc promotes vulnerability to both addiction and depression. The opposite change, that is, a reduced GluR1:GluR2 ratio, shown here in NAc of resilient mice, suggests that reduced glutamatergic function may be protective against the deleterious effects of chronic stress. This is consistent with observations that increased GluR2 activity, or reduced GluR1 activity, in NAc enhances reward and motivation[28,37,48]. The ability of fluoxetine to similarly induce GluR2 expression in NAc raises the possibility that reduced glutamate innervation of this brain region may also contribute to antidepressant responses. Indeed, we show here that inhibition of AMPA receptor function within the NAc produces a potent and long-lived antidepressant-like response.

While the changes we demonstrate in AMPA receptor expression in NAc of susceptible mice are consistent with our electrophysiological observations, the changes observed in resiliency are more complex. We did not obtain electrophysiological evidence for decreased GluR2-lacking AMPA receptors in NAc of resilient mice compared to controls. We hypothesize that ΔFosB-mediated induction of GluR2 in resilience is just one of many adaptations that occur in NAc that affect glutamatergic transmission and that, while this adaptation is sufficient to reverse the excessive AMPA receptor function seen in susceptibility, it does not induce net changes in the opposite direction. Indeed, our data reveal complex regulation of glutamatergic transmission in NAc after chronic social defeat stress. The opposite changes in GluR1 expression in this brain region in susceptibility vs. resilience are not seen at the mRNA level, nor are the decreased levels of GluR2 in susceptibility seen at the mRNA level. This is consistent with post-translational modifications, including alterations in AMPA receptor trafficking, also playing an important role, as has been observed in drug abuse models[30,47].

The complex regulation of glutamatergic transmission in NAc by chronic stress is highlighted by our discovery of SC1 as another target gene for ΔFosB, which, like induction of GluR2,

mediates resiliency. SC1 is known to regulate synaptic plasticity[31]. As a result of its anti-adhesive properties, SC1 induction in NAc might result in a more permissive environment for the structural changes that accompany the plasticity at glutamatergic synapses that appear crucial for resilience. For example, recent evidence shows that the removal of the extracellular matrix allows for the diffusion of AMPA receptors and thereby promotes synaptic plasticity[49].

In summary, our results support a scheme whereby ΔFosB in NAc mediates resilience in the face of chronic stress in part by inducing a form of synaptic plasticity that counteracts the strong negative associative learning occurring in susceptible mice. For example, increases in GluR2-lacking AMPA receptors in NAc, which we see in susceptible mice, have been shown to exacerbate responses to cocaine-associated cues that promote craving and relapse in addiction models[30,47]. In contrast, the dampening of glutamatergic tone in resilient mice, through enhancement of GluR2 and perhaps induction of SC1, might render a salient stimulus, such as a novel mouse in the social defeat paradigm, less able to activate NAc neurons, and thereby enable goal-directed behavior to continue despite the stress. Our gene arrays suggest the likely involvement of many additional targets of ΔFosB contributing to resilience as well. The dominant role played by ΔFosB and its targets in an individual's ability to adapt positively to chronic stress raises fundamentally new avenues for the development of novel antidepressant treatments.

METHODS

Methods and associated references are available in the online version of the paper at http://www.nature.com/natureneuroscience/.

Supplementary Material

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References

1. McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev 2007;87:873–904. [PubMed: 17615391]
2. Feder A, Nestler EJ, Charney DS. Psychobiology and molecular genetics of resilience. Nat Rev Neurosci 2009;10:446–457. [PubMed: 19455174]
3. Ressler KJ, Mayberg HS. Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. Nat Neurosci 2007;10:1116–1124. [PubMed: 17726478]
4. Krishnan V, Nestler EJ. The molecular neurobiology of depression. Nature 2008;455:894–902. [PubMed: 18923511]
5. Yehuda R, Flory JD, Southwick S, Charney DS. Developing an agenda for translational studies of resilience and vulnerability following trauma exposure. Ann N Y Acad Sci 2006;1071:379–396. [PubMed: 16891584]
6. Tornatzky W, Miczek KA. Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. Physiol Behav 1993;53:983–993. [PubMed: 8511216]
7. Koolhaas JM, Meerlo P, De Boer SF, Strubbe JH, Bohus B. The temporal dynamics of the stress response. Neurosci Biobehav Rev 1997;21:775–782. [PubMed: 9415902]

8. De Kloet ER. Hormones and the stressed brain. Ann N Y Acad Sci 2004;1018:1–15. [PubMed: 15240347]
9. Rygula R, et al. Anhedonia and motivational deficits in rats: impact of chronic social stress. Behav Brain Res 2005;162:127–134. [PubMed: 15922073]
10. Krishnan V, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 2007;131:391–404. [PubMed: 17956738]
11. Berton O, et al. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 2006;311:864–868. [PubMed: 16469931]
12. Tidey JW, Miczek KA. Acquisition of cocaine self-administration after social stress: role of accumbens dopamine. Psychopharmacology (Berl) 1997;130:203–212. [PubMed: 9151353]
13. Martinez M, Calvo-Torrent A, Herbert J. Mapping brain response to social stress in rodents with c-fos expression: a review. Stress 2002;5:3–13. [PubMed: 12171762]
14. Kollack-Walker S, Don C, Watson SJ, Akil H. Differential expression of c-fos mRNA within neurocircuits of male hamsters exposed to acute or chronic defeat. J Neuroendocrinol 1999;11:547–559. [PubMed: 10444312]
15. Becker C, et al. Enhanced cortical extracellular levels of cholecystokinin-like material in a model of anticipation of social defeat in the rat. J Neurosci 2001;21:262–269. [PubMed: 11150343]
16. Rygula R, Abumaria N, Domenici E, Hiemke C, Fuchs E. Effects of fluoxetine on behavioral deficits evoked by chronic social stress in rats. Behav Brain Res 2006;174:188–192. [PubMed: 16949682]
17. Wilkinson MB, et al. Imipramine treatment and resiliency exhibit similar chromatin regulation in the mouse nucleus accumbens in depression models. J Neurosci 2009;29:7820–7832. [PubMed: 19535594]
18. Covington HE 3rd, et al. Antidepressant actions of histone deacetylase inhibitors. J Neurosci 2009;22:11451–11460. [PubMed: 19759294]
19. Nestler EJ. Review. Transcriptional mechanisms of addiction: role of DeltaFosB. Philos Trans R Soc Lond B Biol Sci 2008;363:3245–3255. [PubMed: 18640924]
20. Perrotti LI, et al. Induction of deltaFosB in reward-related brain structures after chronic stress. J Neurosci 2004;24:10594–10602. [PubMed: 15564575]
21. Nikulina EM, Arrillaga-Romany I, Miczek KA, Hammer RP Jr. Long-lasting alteration in mesocorticolimbic structures after repeated social defeat stress in rats: time course of mu-opioid receptor mRNA and FosB/DeltaFosB immunoreactivity. Eur J Neurosci 2008;27:2272–2284. [PubMed: 18445218]
22. Kelz MB, et al. Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. Nature 1999;401:272–276. [PubMed: 10499584]
23. Peakman MC, et al. Inducible, brain region-specific expression of a dominant negative mutant of c-Jun in transgenic mice decreases sensitivity to cocaine. Brain Res 2003;970:73–86. [PubMed: 12706249]
24. McClung CA, Nestler EJ. Regulation of gene expression and cocaine reward by CREB and DeltaFosB. Nat Neurosci 2003;6:1208–1215. [PubMed: 14566342]
25. Wallace DL, et al. CREB regulation of nucleus accumbens excitability mediates social isolation-induced behavioral deficits. Nat Neurosci 2009;12:200–209. [PubMed: 19151710]
26. Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology (Berl) 1995;121:66–72. [PubMed: 8539342]
27. Renthal W, et al. Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins. Neuron 2009;62:335–348. [PubMed: 19447090]
28. Todtenkopf MS, et al. Brain reward regulated by AMPA receptor subunits in nucleus accumbens shell. J Neurosci 2006;26:11665–11669. [PubMed: 17093088]
29. Bredt DS, Nicoll RA. AMPA receptor trafficking at excitatory synapses. Neuron 2003;40:361–379. [PubMed: 14556714]
30. Conrad KL, et al. Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. Nature 2008;454:118–121. [PubMed: 18500330]

31. Lively S, Brown IR. The extracellular matrix protein SC1/hevin localizes to excitatory synapses following status epilepticus in the rat lithium-pilocarpine seizure model. J Neurosci Res 2008;86:2895–2905. [PubMed: 18488994]
32. Nikulina EM, Covington HE 3rd, Ganschow L, Hammer RP Jr, Miczek KA. Long-term behavioral and neuronal cross-sensitization to amphetamine induced by repeated brief social defeat stress: Fos in the ventral tegmental area and amygdala. Neuroscience 2004;123:857–865. [PubMed: 14751279]
33. Koob GF. A role for brain stress systems in addiction. Neuron 2008;59:11–34. [PubMed: 18614026]
34. Haney M, Maccari S, Le Moal M, Simon H, Piazza PV. Social stress increases the acquisition of cocaine self-administration in male and female rats. Brain Res 1995;698:46–52. [PubMed: 8581502]
35. Sesack SR, Grace AA. Cortico-Basal Ganglia reward network: microcircuitry. Neuropsychopharmacology 2010;35:27–47. [PubMed: 19675534]
36. Kalivas PW, Volkow N, Seamans J. Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. Neuron 2005;45:647–650. [PubMed: 15748840]
37. Reynolds SM, Berridge KC. Glutamate motivational ensembles in nucleus accumbens: rostrocaudal shell gradients of fear and feeding. Eur J Neurosci 2003;17:2187–2200. [PubMed: 12786986]
38. Grace AA, Floresco SB, Goto Y, Lodge DJ. Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. Trends Neurosci 2007;30:220–227. [PubMed: 17400299]
39. Campioni M, Xu M, McGehee DS. Stress-Induced Changes in Nucleus Accumbens Glutamate Synaptic Plasticity. J Neurophysiol. 2009
40. Rada P, et al. Glutamate release in the nucleus accumbens is involved in behavioral depression during the PORSOLT swim test. Neuroscience 2003;119:557–565. [PubMed: 12770568]
41. Roitman MF, Wheeler RA, Carelli RM. Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. Neuron 2005;45:587–597. [PubMed: 15721244]
42. Tremblay LK, et al. Functional neuroanatomical substrates of altered reward processing in major depressive disorder revealed by a dopaminergic probe. Arch Gen Psychiatry 2005;62:1228–1236. [PubMed: 16275810]
43. Vythilingam M, et al. Reward circuitry in resilience to severe trauma: An fMRI investigation of resilient special forces soldiers. Psychiatry Res 2009;172:75–77. [PubMed: 19243926]
44. Schlaepfer TE, et al. Deep brain stimulation to reward circuitry alleviates anhedonia in refractory major depression. Neuropsychopharmacology 2008;33:368–377. [PubMed: 17429407]
45. Churchill L, Swanson CJ, Urbina M, Kalivas PW. Repeated cocaine alters glutamate receptor subunit levels in the nucleus accumbens and ventral tegmental area of rats that develop behavioral sensitization. J Neurochem 1999;72:2397–2403. [PubMed: 10349849]
46. Boudreau AC, Reimers JM, Milovanovic M, Wolf ME. Cell surface AMPA receptors in the rat nucleus accumbens increase during cocaine withdrawal but internalize after cocaine challenge in association with altered activation of mitogen-activated protein kinases. J Neurosci 2007;27:10621–10635. [PubMed: 17898233]
47. Anderson SM, et al. CaMKII: a biochemical bridge linking accumbens dopamine and glutamate systems in cocaine seeking. Nat Neurosci 2008;11:344–353. [PubMed: 18278040]
48. Taha SA, Fields HL. Inhibitions of nucleus accumbens neurons encode a gating signal for reward-directed behavior. J Neurosci 2006;26:217–222. [PubMed: 16399690]
49. Frischknecht R, et al. Brain extracellular matrix affects AMPA receptor lateral mobility and short-term synaptic plasticity. Nat Neurosci 2009;12:897–904. [PubMed: 19483686]
50. Chen J, et al. Transgenic animals with inducible, targeted gene expression in brain. Mol Pharmacol 1998;54:495–503. [PubMed: 9730908]

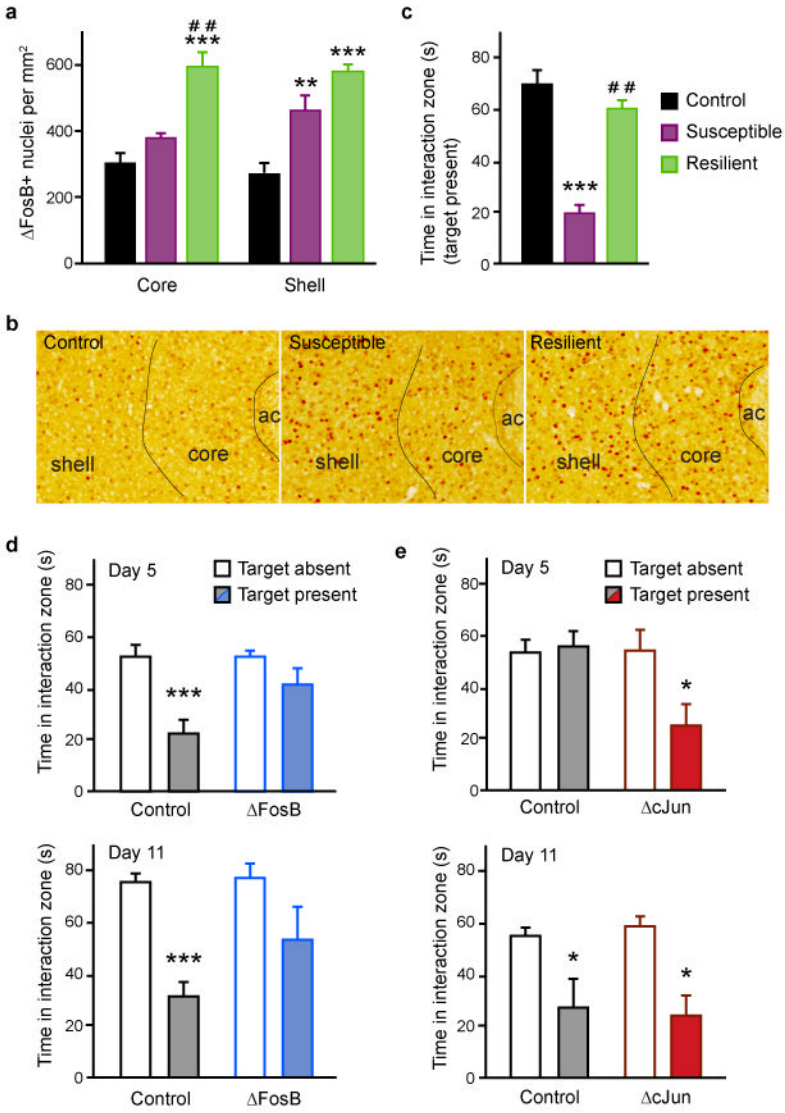

Figure 1. ΔFosB induction in NAc by social defeat mediates resilience

(a) Interaction zone times showing social avoidance in the susceptible mice only (n=4). [$F(2,11)=34.91$, $P<0.001$; Post-hoc test: ***$P<0.001$, versus "control"; $^{\#\#}P<0.01$ versus "susceptible".] **(b)** Chronic social defeat induces ΔFosB in NAc as quantified on day 11. Resilient mice show greater ΔFosB induction in both core and shell of NAc versus control mice (n=3–4). [Core, $F(2,11)=16.81$, $P<0.001$; Shell, $F(2,11)=39.9$, $P<0.001$. Post-hoc test: ***$P<0.001$, **$P<0.01$ versus "control"; $^{\#\#}P<0.01$ versus "susceptible"]. **(c)** Representative photomicrographs of ΔFosB immunohistochemistry in NAc 24 hr after the last defeat. ac, anterior commissure. **(d)** Inducible bitransgenic mice overexpressing ΔFosB (Day 5: n=29–32; Day 11: n=6–15) don't develop social aversion. ["Day 5": Interaction $F(1,118)=5.908$, $P<0.05$; Post-hoc test: ***$P<0.001$ versus "no target"; "Day 11": Significant effect of "target", $F(1,38)=13.20$; A posteriori *t*-test, $t=4.190$, ***$P<0.01$ versus "no target".] **(e)** Conversely, overexpression of ΔcJun increases susceptibility to social defeat with increased social aversion seen after 4 days of defeat (Day 5: n=15–23; Day 11: n=6–7). ["Day 5": Interaction $F(1,72)=4.198$, $P<0.05$; Post-hoc test: *$P<0.05$ versus control "no target"; "Day 11": Significant effect of "target" $F(1,20)=13.16$; A posteriori *t*-test, $t=2.313$, *$P<0.05$, $t=3.801$, **$P<0.01$ versus "no

target”]. Whereas control littermate mice of the ΔFosB line show social aversion after 4 defeat episodes, control littermates of the ΔcJun line do not due to differences in genetic background. Such baseline differences between the lines are also seen in measures of anxiety- and depression-related behavior (see Online Methods and Supplementary Fig. 2).

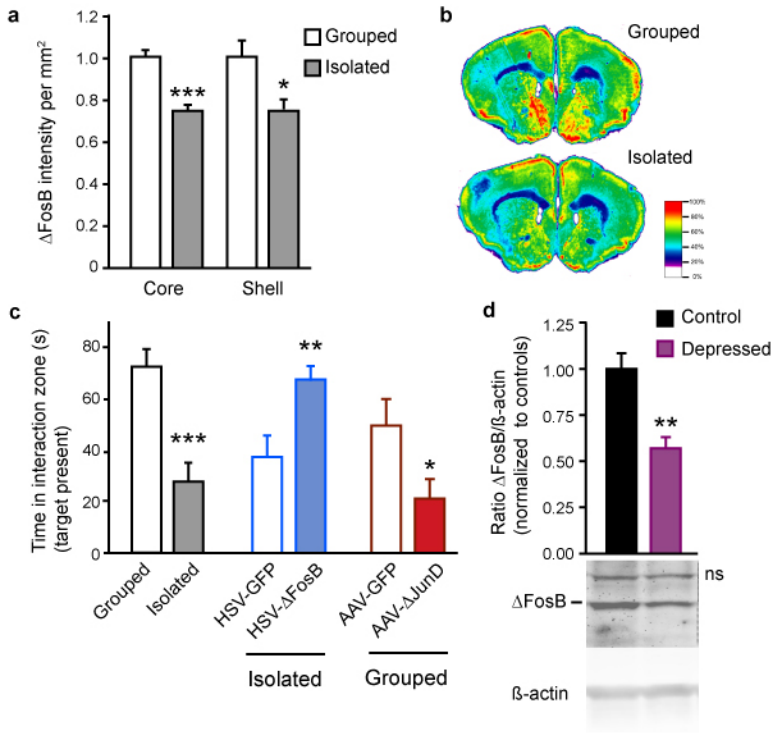

Figure 2. Effect of social isolation on ΔFosB and on susceptibility to social defeat

(a) Long-term social isolation (n=4) decreases basal levels of ΔFosB in NAc shell [t=2.882, df=6, *P<0.05] and core [t=6.338, df=6, ***P<0.01]. **(b)** Representative brain sections showing ΔFosB levels in NAc of grouped-housed and isolated mice. **(c)** Social isolation triggers vulnerability to an acute social defeat (see Online Methods for details) based on the social avoidance measured on the following day (n=8–10), an effect rescued by viral-mediated overexpression of ΔFosB (n=12) in NAc of isolated mice. [Isolation: t=4.351, df=16, ***P<0.001, HSV-ΔFosB: t=3.030, df=22, **P<0.01.] Overexpression of ΔJunD (n=8–12) in NAc mimics social isolation by causing social avoidance after short-term social defeat [t=2.251, df=18, *P<0.05]. **(d)** Postmortem human NAc show decreased ΔFosB levels in depressed patients compared to matched controls (n=8) [t=3.416, df=14, **P<0.01]. *ns*, non-specific band unrelated to ΔFosB.

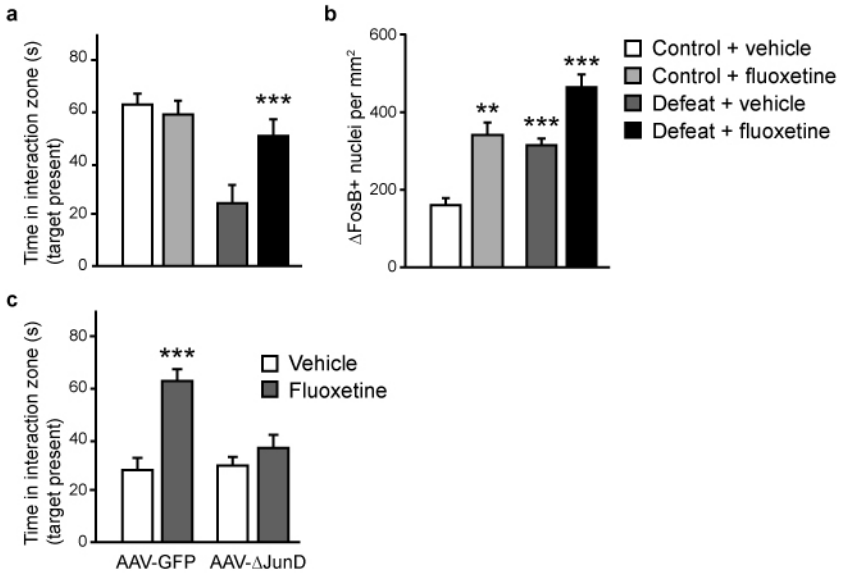

Figure 3. ΔFosB induction in NAc mediates the antidepressant effect of fluoxetine

(a) Chronic treatment with fluoxetine completely reverses the social avoidance, measured on day 11, induced by chronic (10 days, n=7) social defeat. [Interaction, F(1,24)=5.325, $P<0.05$, Post-hoc test: ***$P<0.001$ versus "defeat with vehicle".] **(b)**ΔFosB levels in NAc shell measured by immunohistochemistry are increased after chronic fluoxetine treatment of control mice. Such levels are also increased in susceptible mice after chronic social defeat, with fluoxetine inducing a still further increase (n=4). [No interaction effect, F(1,12)=0.2122, significant effect of social defeat and antidepressant treatment; *A posteriori t*-test $t=8.417$, df=6 ("defeat fluoxetine"), $t=4.516$, df=6 ("control fluoxetine"), $t=6.063$, df=6 ("defeat vehicle"), ***$P<0.001$, **$P<0.001$ versus "control vehicle".] Similar results were obtained in NAc core (See Supplementary Fig. 4). **(c)** Overexpression of ΔJunD in NAc blocks the antidepressant-like effect of chronic fluoxetine treatment (n=8). [Interaction, F(1,28)=6.121, $P<0.05$, Post-hoc test: ***$P<0.001$ versus "GFP vehicle".]

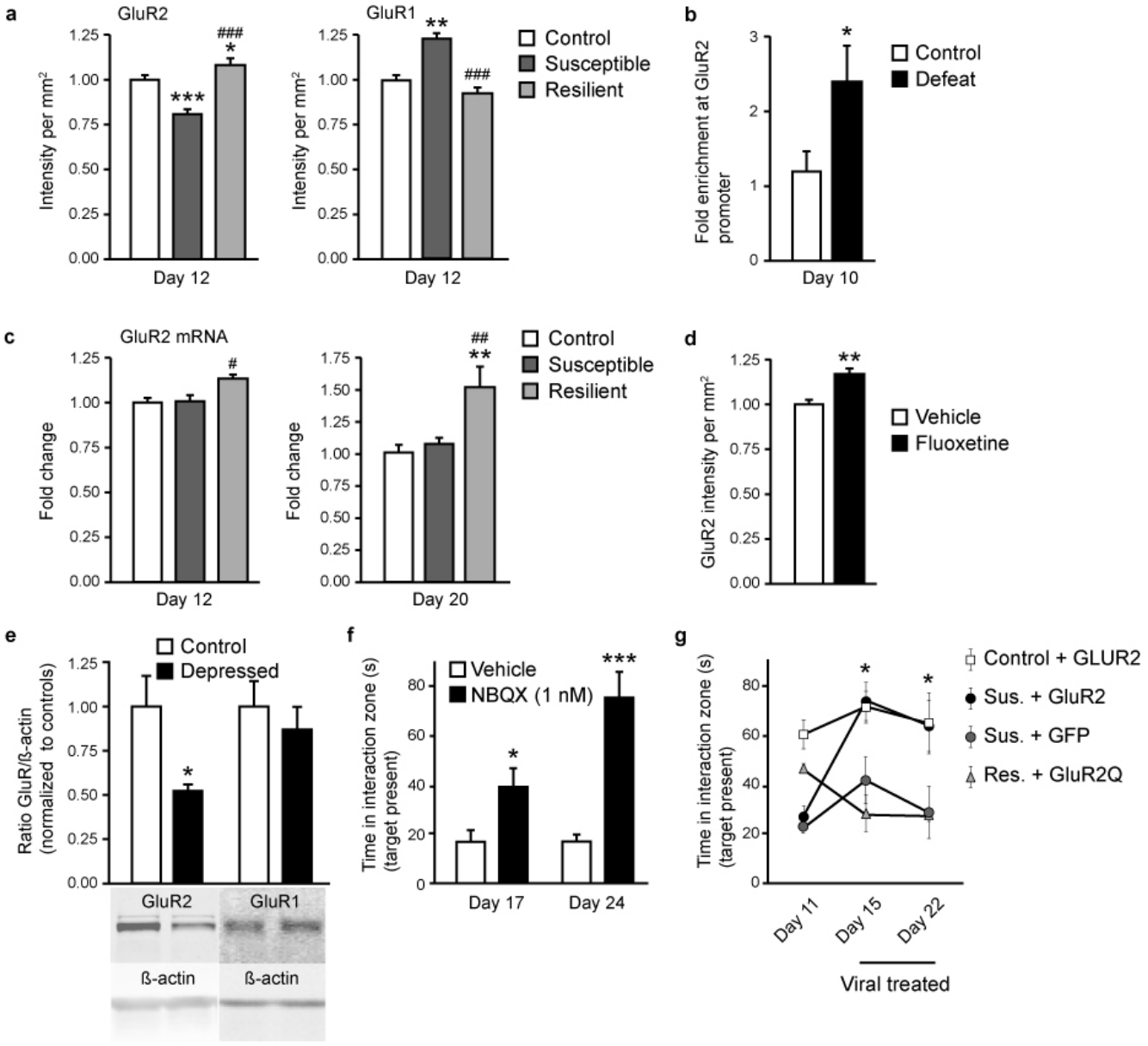

Figure 4. Pro-resilience, antidepressant-like effect of GluR2 in NAc

(a) Resilient mice show increased GluR2 levels, and decreased GluR1 levels, versus control and susceptible mice (n=4). Conversely, susceptible mice show opposite changes. [GluR2: $F(2,11)=69,89$, $P<0.001$; Post-hoc test: ***$P<0.001$, *$P<0.05$ versus "control", $^{###}P<0.001$ versus "susceptible"; GluR1: $F(2,11)=27.58$, $P<0.001$; Post-hoc test: **$P<0.01$, versus "control", $^{###}P<0.001$ versus "susceptible".] **(b)** Social defeat increases ΔFosB binding to the GluR2 promoter (n=5) [$t=2.158$, df=8, *$P<0.05$]. This effect was seen only for a region of the promoter that contains AP1 sites. **(c)** qPCR performed two or ten days after the last defeat revealed increased GluR2 mRNA levels in resilient mice (n=6–8) [Day 12, $P>0.05$, *A posteriori* *t*-test: Day 12, $t=2.838$, df=13, $^{#}P<0.05$; Day 20, Group $F(2,20)=8.739$, $P<0.05$; Post-hoc test: **$P<0.01$ versus "control", $^{##}P<0.01$ versus defeat]. **(d)** Fluoxetine treatment increases GluR2 levels in NAc (n=4) [Core: $t=3.778$, df=6, **$P<0.01$; Shell: $t=6.602$, df=6, ***$P<0.01$] **(e)** Western-blotting revealed decreased GluR2 levels in NAc of depressed humans (n=8)

[*t*=2.381, df=14, **P*<0.05]. **(f)** Intra-NAc infusion of NBQX (n=8) had an immediate and persistent (one week) antidepressant effect in susceptible mice [Interaction, F(1,28)=6.128, *P*<0.05; Drug: ****P*<0.001; Day 7: ****P*<0.001 versus "vehicle"; *A posteriori t*-test: Day 1: *t*=2.156, df=15, **P*<0.05 versus "vehicle".] **(g)** Overexpression of GluR2 in NAc of susceptible mice reverses defeat-induced social avoidance (n=7–8), an effect that persists for at least 10 days, when viral expression has dissipated [Virus F(1,41)=9.553, *P*<0.01, Days F(2,41)=7.248, *P*<0.01, no effect on Interaction. *A posteriori t*-test: Day 15: *t*=2.702, df=12, **P*<0.05; Day 22: *t*=2.008, df=12, **P*<0.05.]

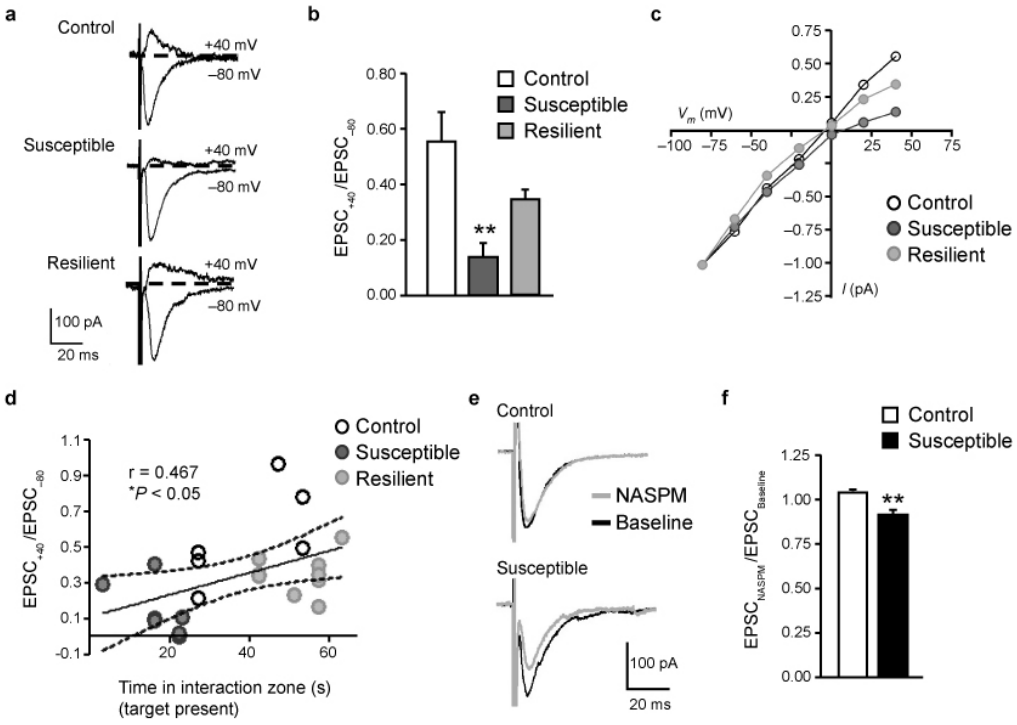

Figure 5. AMPA receptor composition is differentially regulated in susceptible and resilient mice

(a–b) Evoked EPSCs recorded after social defeat (between 2 and 28 days after the last defeat episode). **(a)** Sample traces of AMPAR EPSCs at two resting potentials. **(b)** A measure of rectification ($EPSC_{+40mV}/EPSC_{-80mV}$, n=6–10) from NAc neurons of control, susceptible, and resilient mice. [$F(2,21)=8.773$, $P<0.01$. Post-hoc test: $^{**}P<0.01$, versus "control"; *A posteriori t*-test show a significant difference between susceptible and both control and resilient, $t=3.482$, df=11, $^{\#\#}P<0.01$ versus "controls" and $t=3.146$, df=14, $^{\#\#}P<0.01$ versus "resilient", but not between resilient and control]. A decrease in the $EPSC_{+40mV}/EPSC_{-80mV}$ ratio corresponds to an increase in inward rectification. **(c)** Current-voltage relationships for neurons recorded from control, susceptible, and resilient mice demonstrate changes in rectification at positive potentials only. **(d)** Linear regression analysis reveal significant correlation between rectification value and time interacting with the target (r=0.467, $^{*}P<0.05$). **(e–f)** NASPM (200 μM, 5 min) decreased evoked AMPAR EPSC amplitude in susceptible mice. **(e)** Representative traces illustrating the effect of NASPM after 10 min of bath application. **(f)** Evoked EPSC amplitude with NASPM normalized to baseline ($t=2.689$, df=7, $^{**}P<0.01$).

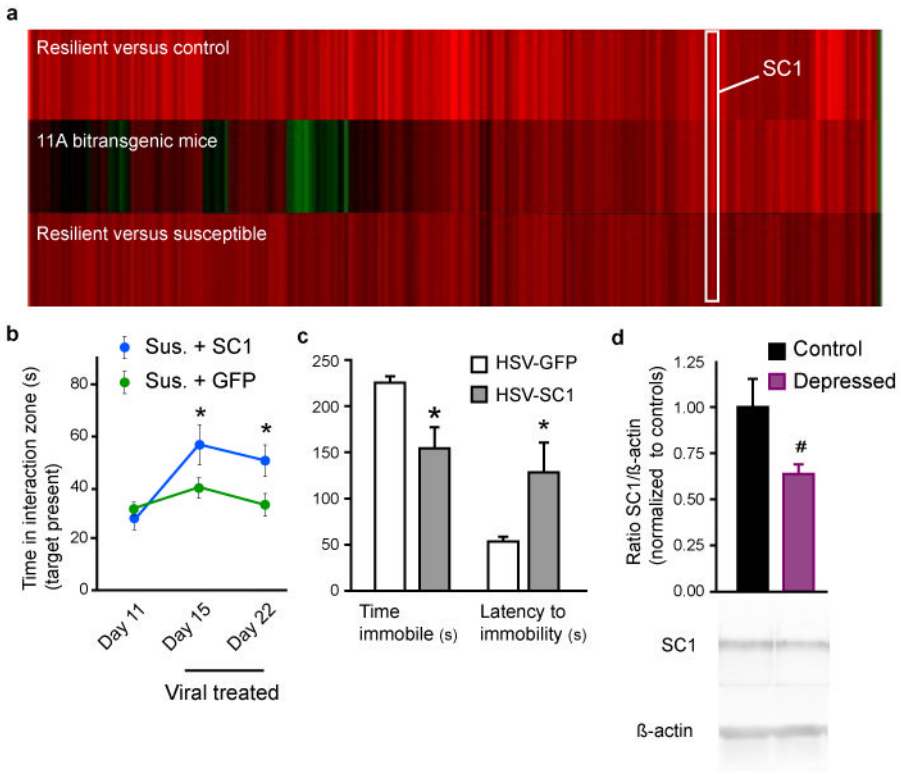

Figure 6. Pro-resilience, antidepressant-like effects of SC1 in NAc

(a) Changes in gene expression observed in NAc during resiliency overlap with those observed upon overexpression of ΔFosB (comparing datasets in refs [10] and [24]). The upper heatmap shows 106 genes significantly regulated (>1.5 fold; **P*<0.05) in NAc by social defeat in resilient mice compared to control, and how those same genes are regulated in resilient mice vs. susceptible mice (lower heatmap) and by overexpression of ΔFosB in 11A mice (middle heatmap). Position of SC1 on the heatmaps is indicated. **(b)** Viral overexpression of SC1 in NAc reversed the social avoidance induced by chronic (10 days, n=17–24) social defeat. [Virus F(1,125)=7.002, *P*<0.01, Days F(2,125)=6.908, *P*<0.01, no effect on Interaction; *A posteriori t*-test: Day 15: *t*=1.875, df=43, **P*<0.05, and on Day 22: *t*=2.138, df=39, **P*<0.05.] Sus, susceptible. **(c)** Rats injected with HSV-GFP or HSV-SC1 into NAc were subjected to the forced swim test (n=8). SC1 overexpression had an antidepressant-like effect as measured by a decrease in time spent immobile [*t*=2.384, df=14 **P*<0.05] and an increase latency to immobility [*t*=2.606, df=16, **P*<0.05]. **(d)** Human NAc samples from depressed patients show a strong trend for decreased SC1 levels compared to matched controls (n=8) [*t*=1.922, df=14, $^{\#}P$=0.068].