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

Vincent Vialou[1], Alfred J Robison[1,7], Quincey C LaPlant[1,7], Herbert E Covington III[1], David M Dietz[1], Yoshinori N Ohnishi[1], Ezekiell Mouzon[1], Augustus J Rush III[2], Emily L Watts[1], Deanna L Wallace[2,6], Sergio D Iñiguez[3], Yoko H Ohnishi[1], Michel A Steiner[4], Brandon L Warren[3], Vaishnav Krishnan[2], Carlos A Bolaños[3], Rachael L Neve[5], Subroto Ghose[2], Olivier Berton[2,6], Carol A Tamminga[2] & Eric J Nestler[1]

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In contrast with the many studies of 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. We found that the transcription factor ΔFosB mediates an essential mechanism of resilience in mice. Induction of ΔFosB in the nucleus accumbens, an important brain reward-associated region, in response to chronic social defeat stress was both necessary and sufficient for resilience. ΔFosB induction was also required for the standard antidepressant fluoxetine to reverse behavioral pathology induced by social defeat. ΔFosB produced these effects through induction of the GluR2 AMPA glutamate receptor subunit, which decreased the responsiveness of nucleus accumbens neurons to glutamate, and through other synaptic proteins. Together, these findings establish a previously unknown molecular pathway underlying both resilience and antidepressant action.

People subjected to severe stress show widely differing responses: some are able to overcome crisis, whereas others develop severe psychopathology such as depression or post-traumatic stress disorder (PTSD). The ability to cope with stressful situations, 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, in addition to the capacity to modulate emotions and to develop adaptive social behavior. These traits implicate the brain's reward circuitry, which seems to be a critical determinant of the emergence of pathological rather than resilient phenotypes[3,4]. Neurobiological correlates of vulnerability or resistance to stress have 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 that 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 considerable proportion (~30%) of chronically defeated mice avoid most of the negative behavioral consequences of defeat[10], thereby facilitating experimental investigations of resilience. Whereas the induction of several proteins in the nucleus accumbens (NAc), a key brain reward region, has been shown to be important for the expression of depression-like behaviors after defeat[10,11,17,18], much less is known about the molecular basis of resilience mediated by this brain region. Here we addressed this issue by focusing on ΔFosB, a Fos family transcription factor that is 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 10 consecutive days of social defeat[10,11] and then separated into susceptible and resilient populations on the basis of a measure of social avoidance (**Fig. 1a**), which correlates with several other depression-like behaviors[10]. We found an increase in ΔFosB, measured by immunohistochemistry, in NAc after chronic social defeat (**Fig. 1b**,**c**), and resilient mice showed the greatest induction of ΔFosB in both core and shell NAc subregions (**Fig. 1b**,**c**). In addition, we observed a strong ($P < 0.01$) correlation between amounts 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 a mouse shows a susceptible or a resilient phenotype. Protein blot analysis of NAc dissections containing core and shell subregions confirmed that ΔFosB was induced only in resilient mice (**Supplementary Fig. 1**).

To test the functional consequences of ΔFosB induction, we used bitransgenic mice that inducibly overexpress ΔFosB specifically in

[1]Fishberg Department of Neuroscience, Mount Sinai School of Medicine, New York, New York, USA. [2]Department of Psychiatry and Center for Basic Neuroscience, University of Texas Southwestern Medical Center, Dallas, Texas, USA. [3]Department of Psychology, Florida State University, Tallahassee, Florida, USA. [4]Max Planck Institute of Psychiatry, Munich, Germany. [5]Massachusetts Institute of Technology, Cambridge, Massachusetts, USA. [6]Present address: Helen Willis Neuroscience Institute, University of California Berkeley, Berkeley, California, USA (D.L.W.), Department of Psychiatry, University of Pennsylvania Medical School, Philadelphia, Pennsylvania, USA (O.B.). [7]These authors contributed equally to this work. Correspondence should be addressed to E.J.N. (eric.nestler@mssm.edu).

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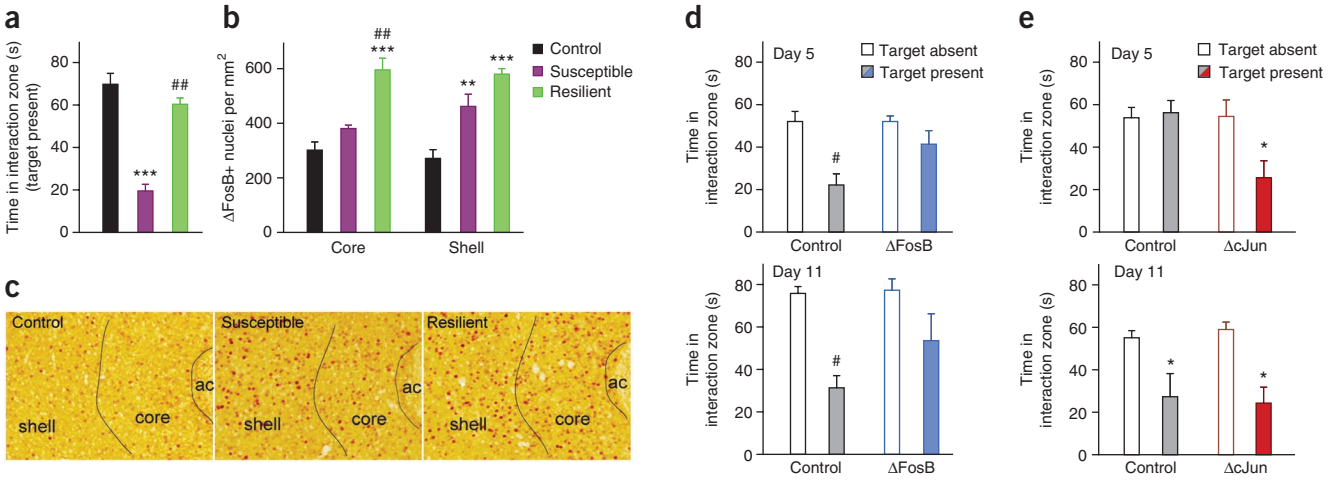

Figure 1 ΔFosB induction in NAc by social defeat mediates resilience. (**a**) Interaction zone times show social avoidance only in susceptible mice ($n = 4$). $F_{2,11} = 34.91$, $P < 0.001$; *post hoc* test, $^{***}P < 0.001$ versus control, $^{\#\#}P < 0.01$ versus susceptible. (**b**) Chronic social defeat induces ΔFosB in NAc as quantified on day 11. Resilient mice show greater ΔFosB induction in both the 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, $^{**}P < 0.01$ versus control. (**c**) Representative photomicrographs of ΔFosB immunohistochemistry in NAc 24 h after the last defeat. ac, anterior commissure. (**d**) Inducible bitransgenic mice overexpressing ΔFosB (day 5, $n = 29$–32; day 11, $n = 6$–15) do not 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, $t = 4.190$. (**e**) Conversely, overexpression of ΔcJun increases susceptibility to social defeat with increased social aversion seen after 4 d 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. Although control littermates of the ΔFosB line show social aversion after four defeat episodes, control littermates of the ΔcJun line do not owing to differences in genetic background. Such baseline differences between the lines are also seen in measures of anxiety- and depression-related behavior (Online Methods and **Supplementary Fig. 2**).

the adult NAc and dorsal striatum[22]. These mice showed a reduced propensity to develop social avoidance after 4 or 10 d 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 were more susceptible to chronic social defeat than control littermates and showed maximal avoidance behavior after 4 d of defeat (**Fig. 1e**). The ΔcJun mice also showed increased immobility in a 1-day forced swim test and reduced sucrose preference, both of which can be interpreted as increased depression-like behavior (**Supplementary Fig. 2**). However, ΔFosB or ΔcJun overexpression did not alter several baseline measures of locomotor activity or anxiety-like behavior (**Supplementary Fig. 2**). 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 used a prolonged period of social isolation during adulthood, which induces depression-like abnormalities in mice[25] and is a chief risk factor for clinical depression. We observed lower ΔFosB concentrations in NAc of socially isolated mice (**Fig. 2a**,**b**). Isolation also rendered the mice markedly more vulnerable to social defeat, and this isolation-induced vulnerability was reversed completely by virally overexpressing ΔFosB selectively in NAc (**Fig. 2c**). Conversely, blockade of ΔFosB function in NAc, by means of viral overexpression of ΔJunD, in group-housed control mice promoted susceptibility to social defeat (**Fig. 2c**). ΔJunD, similar to ΔcJun, is an N-terminally truncated mutant that functions as a dominant-negative antagonist of ΔFosB[23] (**Supplementary Fig. 3**). These findings directly implicate basal concentrations of ΔFosB in NAc in stress vulnerability.

To study the clinical relevance of these findings, ΔFosB concentrations were measured in postmortem human NAc samples obtained from depressed individuals and extensively matched controls. We found ~50% lower ΔFosB concentrations in depressed individuals (**Fig. 2d**), supporting the idea that ΔFosB has a role 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 and antidepressant exposure. In light of our observation that antidepressant treatment increases ΔFosB in mouse NAc (see below), these findings suggest that the failure to induce ΔFosB in NAc may be an important determinant of lack of antidepressant responses in humans.

ΔFosB in NAc mediates antidepressant action

Chronic antidepressant treatment reverses the defeat-induced social avoidance seen in susceptible mice[11]. We therefore examined whether ΔFosB induction in NAc may be a mechanism not only of resilience but also of antidepressant action. Non-defeated control mice treated with fluoxetine for 20 d demonstrated no alterations in social behavior, but showed an accumulation of ΔFosB in the 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 concentrations in NAc (**Fig. 3b** and **Supplementary Fig. 4**).

To test directly the involvement of such ΔFosB induction in the behavioral effects of fluoxetine, we virally overexpressed ΔJunD or green fluorescent protein (GFP) alone (as a control) in NAc of previously defeated mice. Half of the mice in each group were then treated

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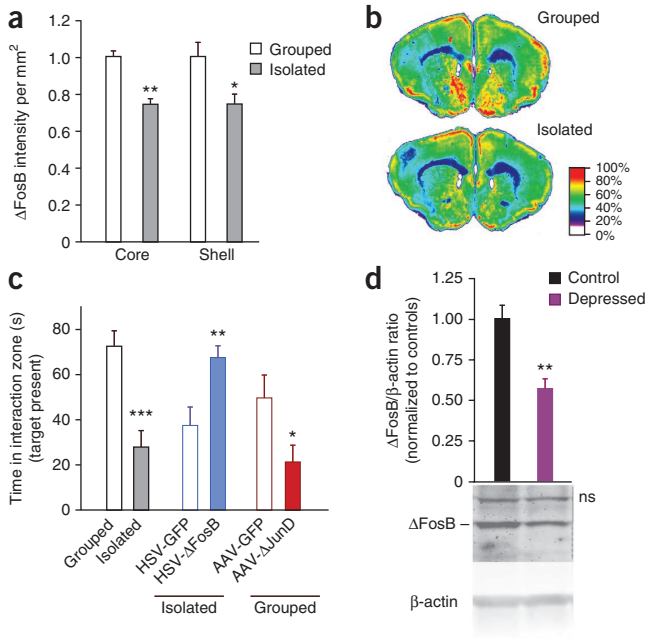

Figure 2 Effect of social isolation on ΔFosB and on susceptibility to social defeat. (**a**) Long-term social isolation ($n = 4$) lowers basal concentrations of ΔFosB in the NAc shell ($t = 2.882$, d.f. = 6, $^{*}P < 0.05$) and core ($t = 6.338$, d.f. = 6, $^{**}P < 0.01$). (**b**) Representative brain sections showing ΔFosB in NAc of group-housed and isolated mice. (**c**) Social isolation triggers vulnerability to an acute social defeat (Online Methods) on the basis of social avoidance measured on the following day ($n = 8$–10), an effect rescued by virus-mediated overexpression of ΔFosB ($n = 12$) in NAc of isolated mice. Isolation, $t = 4.351$, d.f. = 16, $^{***}P < 0.001$; HSV-ΔFosB, $t = 3.030$, d.f. = 22. Overexpression of ΔJunD ($n = 8$–12) in NAc mimics social isolation by causing social avoidance after short-term social defeat ($t = 2.251$, d.f. = 18). (**d**) Postmortem human NAc show smaller amounts of ΔFosB in depressed individuals as compared with matched controls ($n = 8$; $t = 3.416$, d.f. = 14). ns, nonspecific band unrelated to ΔFosB.

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. By 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, virus-mediated overexpression of ΔFosB in rat NAc produced a significant antidepressant-like effect, as measured by a decrease in the time of immobility on day 2 of the forced swim test in rats ($P < 0.01$; **Supplementary Fig. 5**). Further analysis of behavior during this test showed that ΔFosB induced increases in both swimming and climbing (**Supplementary Fig. 5**), features that are related to alterations in serotonergic and noradrenergic mechanisms[26]. Notably, rats overexpressing ΔFosB in NAc also showed a decrease in immobility time on the first day of the test, which we interpreted as a pro-motivational effect (Online Methods and **Supplementary Fig. 5**).

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 larger amounts of GluR2, but show no differences in 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 the pro-resilience action of ΔFosB, we studied GluR2 expression in NAc after chronic social defeat (**Fig. 4**). Susceptible mice had significantly lower GluR2 concentrations in this brain region as compared with controls ($P < 0.001$), whereas resilient mice showed higher GluR2 concentrations (**Fig. 4a**). Although the mechanism underlying the suppression of GluR2 expression in susceptible mice remains unknown, the induction of GluR2 seen in resilient mice seems to reflect a direct effect of ΔFosB on the gene encoding GluR2, because we found increased binding of ΔFosB to the GluR2 promoter by using chromatin immunoprecipitation (ChIP; **Fig. 4b**); in addition, quantitative PCR (qPCR) revealed sustained induction of GluR2 mRNA in NAc of resilient mice (**Fig. 4c**), which paralleled the sustained induction of ΔFosB. Notably, GluR1 was oppositely regulated after social defeat: we observed higher expression in susceptible mice and lower expression in resilient mice (**Fig. 4a**). However, no corresponding alterations were seen in GluR1 mRNA expression, suggesting the involvement of post-translational mechanisms. In addition, chronic fluoxetine treatment of non-defeated mice led to larger amounts of GluR2 in NAc (**Fig. 4d**), whereas analysis of human postmortem NAc tissue from depressed individuals revealed smaller GluR2 amounts as compared with controls (**Fig. 4e**). No changes in GluR1 concentrations were detected (**Fig. 4e**).

The presence of GluR2 has profound effects on AMPA receptors: GluR2-lacking AMPA receptors are Ca^{2+}-permeable and show greater receptor conductance and strong inwardly rectifying currents, as compared with GluR2-containing receptors[29]. To complement our biochemical results, we therefore performed whole-cell voltage-clamp recordings of medium spiny neurons

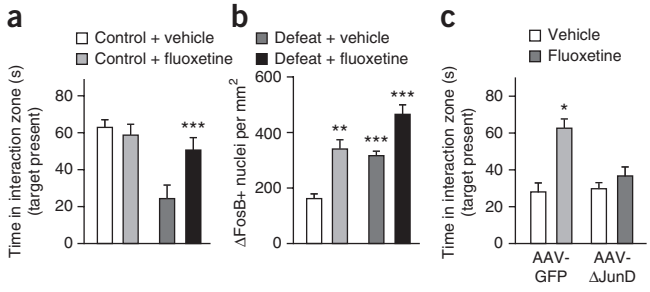

Figure 3 ΔFosB induction in NAc mediates the antidepressant effect of fluoxetine. (**a**) Chronic treatment with fluoxetine completely reverses the social avoidance, measured on day 11, induced by chronic (10 d, $n = 7$) social defeat. Interaction, $F_{1,24} = 5.325$, $P < 0.05$; *post hoc* test, $^{***}P < 0.001$ versus defeat with vehicle. (**b**) ΔFosB concentrations in the NAc shell measured by immunohistochemistry are increased after chronic fluoxetine treatment of control mice. ΔFosB is also increased in susceptible mice after chronic social defeat, and fluoxetine induces a further increase ($n = 4$). No interaction effect, $F_{1,12} = 0.2122$; significant effect of social defeat and antidepressant treatment, *a posteriori t* test, $t = 8.417$, d.f. = 6 (defeat fluoxetine); $t = 4.516$, d.f. = 6 (control fluoxetine); $t = 6.063$, d.f. = 6 (defeat vehicle); $^{**}P < 0.001$ versus control vehicle. Similar results were obtained in the NAc core (**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.

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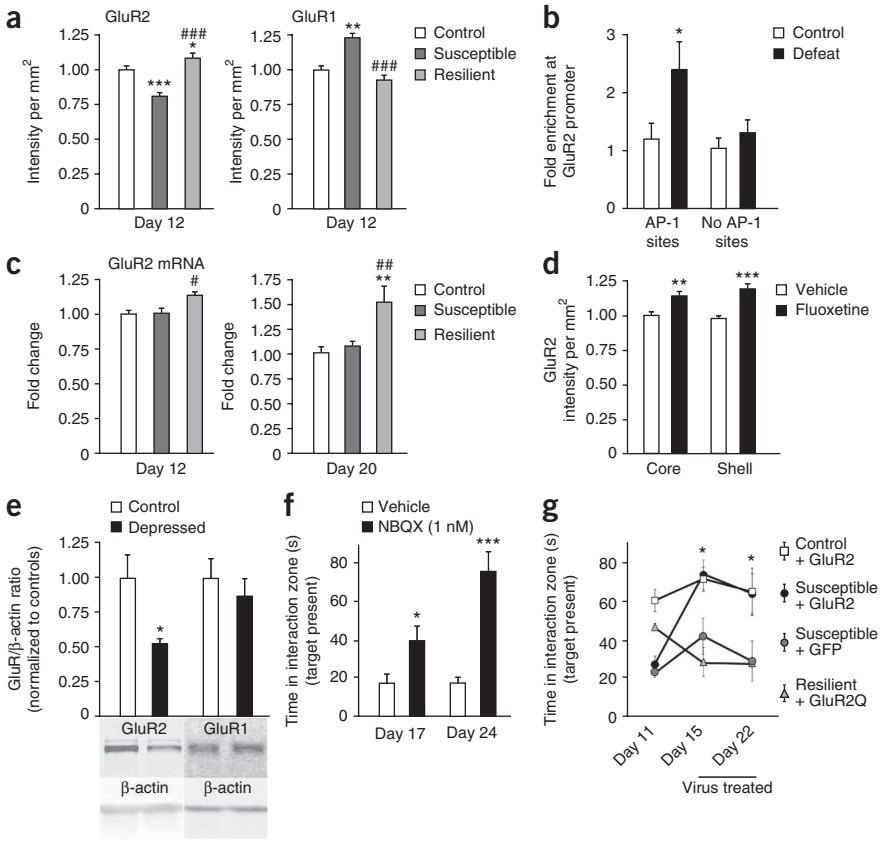

Figure 4 GluR2 has a pro-resilience, antidepressant-like effect in NAc. (**a**) Resilient mice show higher GluR2 concentrations and lower GluR1 concentrations, versus control and susceptible mice ($n = 4$). Conversely, susceptible mice show opposite changes. GluR2: $F_{2,11} = 69{,}89$, $P < 0.001$; *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. (**b**) Social defeat increases ΔFosB binding to the GluR2 promoter ($n = 5$, $t = 2.158$, d.f. = 8, $P < 0.05$). This effect was seen only for a region of the promoter that contains AP1 sites. (**c**) qPCR performed 2 or 10 d after the last defeat shows higher GluR2 mRNA concentrations in resilient mice ($n = 6$–8). Day 12: $P > 0.05$; *a posteriori t* test, $t = 2.838$, d.f. = 13, $^{\#}P < 0.05$. Day 20: group, $F_{2,20} = 8.739$, $P < 0.05$; *post hoc* test, $^{\#\#}P < 0.01$ versus defeat. (**d**) Fluoxetine treatment increases GluR2 in NAc ($n = 4$). Core, $t = 3.778$, d.f. = 6, $P < 0.01$; shell, $t = 6.602$, d.f. = 6, $P < 0.01$. (**e**) Protein blotting revealed lower GluR2 concentrations in NAc of depressed humans ($n = 8$; $t = 2.381$, d.f. = 14, $P < 0.05$). (**f**) Intra-NAc infusion of NBQX ($n = 8$) has an immediate and persistent (1-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, $t = 2.156$, d.f. = 15. (**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 d, 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, $t = 2.702$, d.f. = 12, $P < 0.05$; day 22, $t = 2.008$, d.f. = 12, $P < 0.05$.

in NAc of non-defeated mice and after social defeat in both resilient and susceptible mice. Current-voltage relationships of AMPA-mediated evoked excitatory postsynaptic currents (EPSCs) revealed greater inward rectification in susceptible mice (**Fig. 5a–c**) as compared with controls, consistent with the higher 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 as compared with 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 reduced by NASPM, demonstrating that GluR2-lacking AMPA receptors contribute more to glutamatergic transmission in susceptible mice than in controls. Notably, the effect of NASPM in susceptible mice was less than would be predicted from the large change in rectification. This divergence, however, is not unprecedented[30]; it may result from post-translational modifications or protein-protein interactions involving GluR2 (see Discussion), or simply from 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 as compared with controls (see Discussion).

Antidepressant-like effects of AMPA receptor blockade in NAc

These data suggest that increased AMPA receptor function (higher GluR1:GluR2 ratio) in NAc of susceptible mice promotes social avoidance, whereas decreased AMPA function (lower GluR1:GluR2 ratio) contributes to resilience. To test this hypothesis, we infused the AMPA receptor antagonist NBQX directly into NAc of defeated mice immediately before the social avoidance test. NBQX increased social interaction time (**Fig. 4f**), demonstrating that blockade of fast excitatory input to 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 because mice retested 1 week later showed further enhancement of social interaction.

Next, we 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. Notably, the effect of GluR2 overexpression persisted for at least 10 d after surgery (**Fig. 4g**), a stage when virus-mediated GluR2 expression has completely dissipated. In resilient mice, conversely, overexpression of the unedited version

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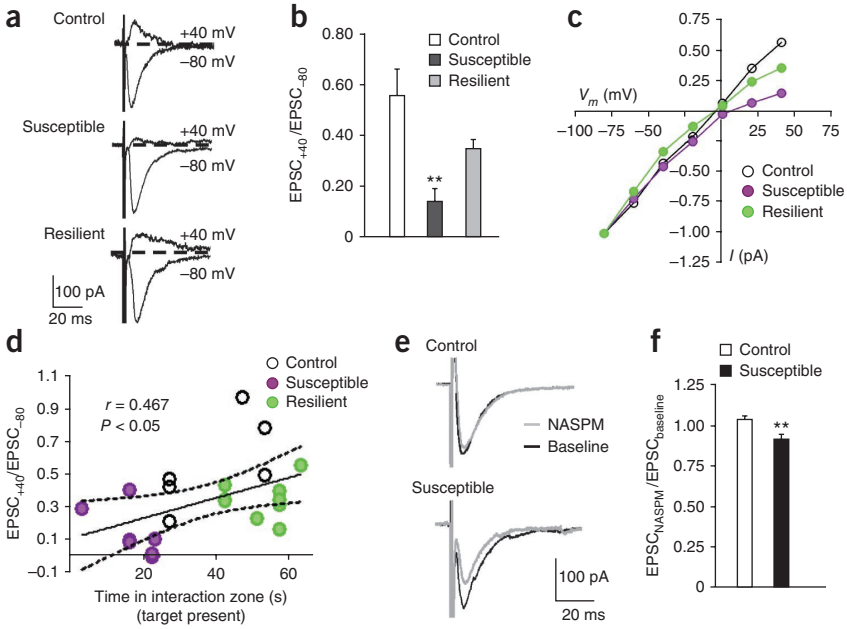

Figure 5 AMPA receptor composition is differentially regulated in susceptible and resilient mice. (**a**,**b**) Evoked EPSCs recorded after social defeat (between 2 and 28 d after the last defeat episode). (**a**) Sample traces of AMPAR EPSCs at two resting potentials. (**b**) Measure of rectification ($EPSC_{+40mV}$/$EPSC_{-80mV}$, $n = 6–10$) 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 showed a significant difference between susceptible and both control and resilient ($t = 3.482$, d.f. = 11, $P < 0.01$ versus controls; $t = 3.146$, d.f. = 14, $P < 0.01$ versus resilient), but not between resilient and control mice. 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 revealing a significant correlation between rectification value and time interacting with the target ($r = 0.467$, $^{*}P < 0.05$). (**e**,**f**) NASPM (200 μM, 5 min) decreases the AMPAR EPSC amplitude evoked in susceptible mice. (**e**) Representative traces showing the effect of NASPM after 10 min of bath application. (**f**) EPSC amplitude evoked with NASPM normalized to baseline amplitude ($t = 2.689$, d.f. = 7).

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 data sets obtained from NAc of both bitransgenic mice overexpressing ΔFosB and C57Bl/6J mice 48 h after chronic social defeat that showed a resilient rather than a susceptible phenotype[10,24]. There was considerable (>75%) overlap between the genes induced in NAc by ΔFosB and those induced by resilience (**Fig. 6a**). Among these genes (**Supplementary Table 2**), we selected for further analysis SC1 (also known as Sparc-like 1 or hevin) on the basis of the magnitude of its induction both in resilience and on ΔFosB overexpression. SC1 is an anti-adhesive matrix molecule that is highly expressed in 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 reversed the social avoidance induced by chronic social defeat (**Fig. 6b**). Overexpression of SC1 also exerted an antidepressant-like effect on day 2 of the rat forced swim test (**Fig. 6c** and **Supplementary Fig. 7**), but had no effect on basal locomotor activity or anxiety-related behaviors (**Supplementary Fig. 7**). In addition, we found a strong trend for smaller amounts of SC1 in human postmortem NAc tissue from depressed individuals (**Fig. 6d**).

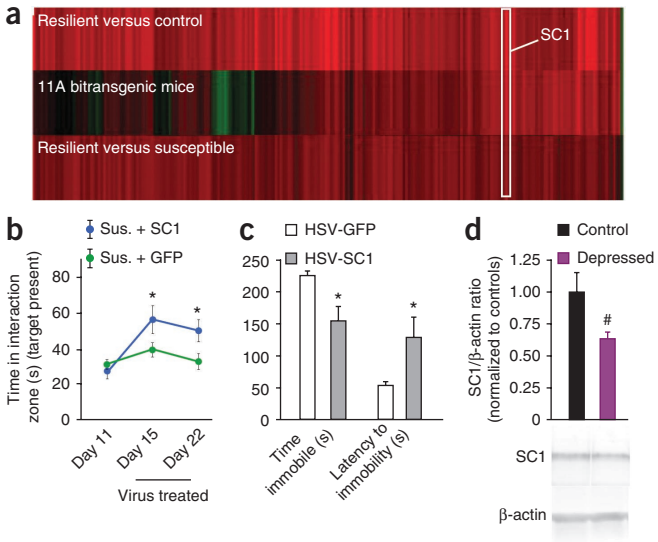

Figure 6 SC1 has pro-resilience, antidepressant-like effects in NAc. (**a**) Changes in gene expression observed in NAc during resilience overlap with those observed on overexpression of ΔFosB (comparison of data sets in refs. 10,24). Shown are 106 genes that are significantly regulated (>1.5 fold; $^{*}P < 0.05$) in NAc by social defeat in resilient mice as compared with controls (upper heat map) and how these genes are regulated both in resilient mice versus susceptible mice (lower heat map) and by overexpression of ΔFosB in 11A mice (middle heat map). The position of SC1 on the heat maps is indicated. (**b**) Viral overexpression of SC1 in NAc reverses the social avoidance induced by chronic (10 d, $n = 17–24$) 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, $t = 1.875$, d.f. = 43, $^{*}P < 0.05$; day 22, $t = 2.138$, d.f. = 39, $P < 0.05$. Sus, susceptible. (**c**) Overexpression of SC1 has an antidepressant-like effect as measured by a decrease in time spent immobile ($t = 2.384$, d.f. = 14, $P < 0.05$) and an increase in latency to immobility ($t = 2.606$, d.f. = 16, $P < 0.05$) in the forced swim test. Rats were injected with HSV-GFP or HSV-SC1 into NAc before the test ($n = 8$). (**d**) Human NAc samples from depressed individuals show a strong trend for lower SC1 concentrations as compared with matched controls ($n = 8$; $t = 1.922$, d.f. = 14, $^{\#}P = 0.068$).

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DISCUSSION

Our results provide evidence of molecular adaptations occurring in the medium spiny neurons of NAc that underlie resilient responses to chronic stress and that contribute to the therapeutic effects of chronic antidepressant treatment. We have shown that basal concentrations 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 versus resilient responses to that stress. We have shown further that the successful reversal of behavioral abnormalities induced in susceptible mice by chronic fluoxetine administration requires the induction of ΔFosB in this brain region by the drug. These findings demonstrate that ΔFosB induction in NAc is both a necessary and sufficient mechanism of resilience and antidepressant response. The finding of lower concentrations 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 have identified two of its target genes, the AMPA receptor subunit GluR2 and the extracellular matrix protein SC1, and have implicated them directly in mediating resilience to social defeat stress.

Such a pro-resilience role for ΔFosB in the context of chronic stress is notable given the involvement of ΔFosB in regulating responses to both 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 some aspects of drug addiction. Our findings in stress models provide insight into the role of this protein in the regulation of complex emotional behavior. Under normal conditions, ΔFosB is expressed at highest concentrations in NAc as compared with all other brain regions[19]. We propose that concentrations of ΔFosB in NAc are important 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 amounts of ΔFosB in mouse NAc, impairing the motivation of the mice and increasing their vulnerability to chronic social stress. The smaller amounts of ΔFosB observed in postmortem NAc of depressed individuals is consistent with this hypothesis, and suggests that ΔFosB has a role 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 the induction of ΔFosB in NAc by chronic exposure to drugs of abuse, an induction that 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. On the one hand, the induction of ΔFosB in NAc by either chronic stress or 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 or vice versa. In addition, 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, susceptible mice, not resilient mice, in the social defeat model show enhanced responses to drugs of abuse[10]. This observation suggests that the enhanced vulnerability of susceptible mice to drugs of abuse is mediated through many other adaptations induced in NAc and elsewhere; for example, BDNF, is induced in susceptible, not resilient, mice in NAc and enhances drug reward mechanisms[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 and its role in depression models. Some proteins (for example, BDNF) promote responses to drugs of abuse and to stress, whereas others exert opposite effects under these two conditions: for example, CREB in NAc produces a pro-depression phenotype, yet blunts responses to drugs of abuse[4,10]. These findings emphasize the need for further research in delineating the molecular underpinnings of complex emotional behavior, and the importance of using the widest possible range of behavioral tests in such investigations. The results also indicate that, as 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 aspects of both conditions. A chief caveat of this discussion, however, 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], whereas stress induces ΔFosB roughly equally within D_1 and D_2 receptor–containing medium spiny neurons[20]. This differential induction could have marked functional consequences because the ability of ΔFosB to enhance reward has been shown only for D_1 class neurons[19].

The identification of GluR2 as a target gene involved in mediating the pro-resilience effect of ΔFosB sheds light on these considerations. We have shown that both susceptibility in mice and human depression are associated with a higher GluR1:GluR2 ratio in NAc, which suggests increased medium spiny neuron excitability in response to glutamate. 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]. Other 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], and 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 individuals 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 either subgenual cingulate cortex or NAc (a chief target of subgenual cingulate cortex), an intervention that is thought to reduce excitability of the stimulated brain region, alleviates depressive symptoms in treatment-refractory patients[3,44].

As in stress models, increased glutamatergic responsiveness in NAc has been implicated in drug addiction[30,45–47], and includes an increase in GluR2-lacking AMPA receptors in this brain region[30,47], similar to what we have found for stress susceptibility. Together, these observations raise the possibility that enhanced glutamatergic transmission in NAc promotes vulnerability to both addiction and depression. The opposite change, that is, the reduced GluR1:GluR2 ratio observed in NAc of resilient mice, suggests that reduced glutamatergic function may be protective against the deleterious effects of chronic stress. This idea 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

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innervation of this brain region may also contribute to antidepressant responses. Indeed, we have shown here that inhibition of AMPA receptor function within NAc produces a potent and long-lived antidepressant-like response.

Whereas the changes in AMPA receptor expression in NAc of susceptible mice are consistent with our electrophysiological observations, the changes observed in resilience are more complex. We did not obtain electrophysiological evidence of a decrease in GluR2-lacking AMPA receptors in NAc of resilient mice as compared with controls. We hypothesize that ΔFosB-mediated induction of GluR2 in resilience is one of many adaptations that occur in NAc that affect glutamatergic transmission and that, although 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 versus resilience are not seen at the mRNA level, nor are the decreased concentrations of GluR2 in susceptibility seen at the mRNA level. This observation is consistent with the view that post-translational modifications, including alterations in AMPA receptor trafficking, have an important role, as 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 of ΔFosB that, similar to GluR2, mediates resilience. SC1 is known to regulate synaptic plasticity[31]. As a result of its anti-adhesive properties, SC1 in NAc might result in a more permissive environment for the structural changes that accompany the plasticity at glutamatergic synapses that seems crucial for resilience. For example, removal of the extracellular matrix facilitates the diffusion of AMPA receptors and thereby promotes synaptic plasticity[49].

In summary, our results support a scheme whereby ΔFosB in NAc mediates resilience during chronic stress, in part, by inducing a form of synaptic plasticity that counteracts the strong negative associative learning that occurs in susceptible mice. For example, increases in GluR2-lacking AMPA receptors in NAc, which we have seen in susceptible mice, exacerbate responses to cocaine-associated cues that promote craving and relapse in addiction models[30,47]. By 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 model—less able to activate NAc neurons and thereby might enable goal-directed behavior to continue despite the stress. Our gene arrays suggest the likely involvement of many additional targets of ΔFosB in resilience. The dominant role of ΔFosB and its targets in an individual's ability to adapt positively to chronic stress opens new avenues for the development of antidepressant treatments.

METHODS

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

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

V.V. and E.J.N. were responsible for overall study design. Q.C.L. and V.V. designed, conducted and analyzed the RNA and ChIP experiments. A.J. Robison designed, conducted and analyzed the electrophysiological studies. H.E.C. and V.V. designed and conducted the NBQX pharmacological experiments. Q.C.L., D.M.D., E.L.W. and V.V. performed the stereotaxic surgeries. Y.N.O. cloned SC1 cDNA into the HSV vector. Y.H.O. performed the AP1 luciferase assay. Q.C.L., D.M.D., D.L.W. and V.V. designed and conducted the social isolation experiments. V.V., E.L.W. and A.J. Rush performed the social defeat tests and immunohistochemical quantification. S.D.I., Q.C.L., B.L.W., C.A.B. and V.V. performed and analyzed rat surgery and the forced swim test. E.M. and R.L.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 postmortem brain tissue. V.V. and E.J.N. wrote the paper with the help of the other authors.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Mice. Male bitransgenic mice derived from crosses of *NSE-tTA* (line A) and *TetOp-ΔfosB* (line 11), and *NSE-tTA* (line A) and *TetOp-FLAG-ΔcJun* (line E) mice[23,50] were conceived and raised on 100 μg ml^{-1} of doxycycline to suppress ΔFosB or ΔcJun expression during development. Line 11A is fully backcrossed on a C57BL/6J background. Line EA is a roughly 50:50 mixture of FVB and 129 backgrounds. This background difference explains some of the variations in baseline behavior observed between the controls of the two bitransgenic lines. For every experiment, however, littermate controls were used to limit any effects of genetic background on these studies. Thus, littermates were divided at weaning: half remained on doxycycline and half were switched to water, and the mice were used 8–11 weeks later when transcriptional effects of ΔFosB and ΔcJun are maximal[22,24]. For chronic social isolation stress, 8-week-old C57Bl/6J mice were housed individually for a period of 8 weeks before testing as described[25]. Chronic fluoxetine was administered by means of subcutaneous pellets (Innovative Research) implanted in the dorsal interscapular region under brief isoflurane anesthesia (Henry Schein). Pellets were designed to administer 20 mg per kg per day of fluoxetine (or placebo) over a 20-d interval and to result in clinically relevant blood concentrations[18]. Mouse procedures were performed in accordance with the Institutional Animal Care and Use guidelines of the University of Texas Southwestern and Mount Sinai School of Medicine.

Social defeat stress and social interaction experiment. In most experiments, 8-week-old C57Bl/6J mice were submitted to social defeat stress for 4 or 10 consecutive days as described[10,11]. Social interaction tests were performed 1 d after the last day of defeat unless specified otherwise. Day 1 refers to the beginning of the social defeat procedure such that day 11, for example, would refer to a social avoidance test performed 24 h after the last day of 10 d of social defeat.

Bitransgenic mice overexpressing ΔFosB or ΔcJun, or their control littermates, were tested at 16 weeks of age and subjected to 4 or 10 d of social defeat. To examine the vulnerability of isolated mice to social defeat, the isolated mice were subjected to three consecutive defeats on the same day, and then tested for social interaction the following day. This acute defeat model has been validated previously to reveal pro-susceptibility factors[10]. The segregation of defeated mice into susceptible and resilient subpopulations was performed as described[10]. Because most of the control mice spent more time interacting with a social target than an empty target enclosure, an interaction ratio of 100 (equal time spent in the interaction zone in the presence versus absence of a social target) was set as a cutoff: mice with scores of <100 were labeled 'susceptible' and those with scores of >100 were labeled 'resilient'. Extensive behavioral, biochemical and electrophysiological analyses support the validity of these distinct susceptible and resilient subpopulations[10,17].

Virus-mediated gene transfer. Herpes simplex virus (HSV) vectors encoding GFP, ΔFosB, ΔJunD, GluR2 or GluR2Q (unedited version) have been previously used and validated[10,22]. SC1, contained within a pSPORT vector, was inserted into the HSV amplicon HSV PrpUC and packaged into virus with helper 5dl1.2, as described[22]. Adeno-associated virus (AAV) overexpressing ΔJunD or enhanced GFP have been used according to previous studies[11,22]. Expression of the HSV transgene is maximal between 1 and 4 d after infusion, and dissipates completely by day 7 (refs. 10,25). By contrast, the AAV transgene is relatively long-lived and persists for at least several months. Importantly, we have shown that both HSV and AAV vectors express their encoded transgenes only within neurons[22]. Within NAc, expression predominates in medium spiny neurons with relatively sparse expression in GABAergic and cholinergic interneurons, reflecting the neuronal makeup of this brain region. Viruses were injected into NAc by using established stereotaxic coordinates[10,11,22,25]. Viral injection sites were confirmed for all mice by using standard histological methods (for examples, see **Supplementary Fig. 8**).

Immunohistochemistry and protein blotting. Brain sections from mice subjected to social defeat were processed for immunohistochemistry as described[20]. Brains were perfused 18–24 h after the last exposure to the last defeat or the last injection of fluoxetine, resulting in the degradation of any residual full-length FosB protein such that all remaining immunoreactivity reflects ΔFosB. This degradation was confirmed by protein blotting, which showed no significant staining with an antibody directed against the C terminus of full-length FosB that does not recognize ΔFosB (data not shown). The number of ΔFosB immunopositive cells was quantified in several sections through the NAc of each mouse, and mean values were then calculated for each mouse. Each mouse was considered an individual observation for statistical analysis.

Quantification of GluR2 and GluR1 immunoreactivity after social defeat, and of ΔFosB after social isolation, was performed on NAc sections and revealed by using a Licor system as described[18]. Integrated intensities of the protein of interest, for example, GluR2, GluR1, ΔFosB and H1, were determined with Odyssey software. Results are presented as integrated intensity values per mm^2 and are presented as means ± s.e.m. (n = 8–12 per group). Values for H1 were used as reference. Ratios of GluR2, GluR1 and ΔFosB over total H1 were analyzed, and Student's t-test was used to compare means for each brain region. Differences were considered significant at value of $P < 0.05$. Statistical analyses were performed with GraphPad Prism.

Microdissected NAc punches from mice subjected to social defeat were processed for protein blotting as described[10,18]. Protein blots were probed with an antibody to ΔFosB (Cell Signaling) and GAPDH (Abcam), and then scanned and quantified with an Odyssey imaging system (Licor).

Human postmortem brain tissue. Human postmortem brain tissue was obtained from the Dallas Brain Collection (DBC), where tissue is collected from the Dallas Medical Examiner's Office and the UT Southwestern Tissue Transplant Program after consent from the next of kin. Tissue was analyzed only from males, and 8 depressed and 8 normal samples were matched for age, postmortem interval, RNA integrity number and pH (see **Supplementary Table 1** for case demographics). Outstanding tissue quality was confirmed by a high RNA integrity number. Samples were subjected to a standard dissection before being snap-frozen in −40 °C isopentane and stored at −80 °C; later dissection of NAc was performed on the frozen tissue. The UT Southwestern IRB reviewed and approved the collection of this tissue for research use. A direct informant interview was carried out for each depression sample at a later date, where information regarding the individual's illness was documented; a consensus diagnosis of major depressive disorder was made by two research psychiatrists using DSM-IV criteria. None of the samples included in the study had blood toxicology screens positive for drugs of abuse, alcohol or prescription drugs other than antidepressants. A few samples were positive for antidepressants (**Supplementary Table 1**). Tissue samples were dispensed in a blind manner for analysis and processed for protein blotting as described[10,18]. Protein blots were probed with an antibody to ΔFosB (rabbit anti-FosB polyclonal antibody generated against amino acids 1–16 of FosB/ΔFosB), GluR2 (Millipore), GluR1 (Millipore) or SC1 (R&D), and β-actin (Cell Signaling), and then scanned and quantified with an Odyssey imaging system.

Other behavioral tests. Bitransgenic mice (lines 11A and EA) were tested in open-field, elevated plus maze, light-dark, sucrose preference and forced swim tests on the basis of published protocols[10,11,18,25]. We used a 1-day forced swim test in mice. We also routinely confirmed the ability of anxiolytic benzodiazepine drugs to reduce anxiety-like measures in the open-field, elevated plus maze and light-dark tests. The antidepressant effects of HSV-mediated overexpression of ΔFosB or SC1 were tested in rats using a 2-day forced swim test. Rats were used for this experiment because a larger range of behaviors can be measured in the forced swim test in rats as compared with mice[26]. The 2-day forced swim test in rats was designed to test antidepressant efficacy: immobility time is increased on day 2 of the test and reduced by antidepressant administration. Time of immobility on day 1 has also been proposed to reflect a stress-sensitive measure[18]; therefore, we also analyzed immobility time on the first day of testing.

RNA isolation and qPCR. Two or ten days after the last defeat episode, mice were rapidly decapitated, and brains were removed and placed on ice. Dissections of NAc were taken with a 14-gauge needle punch and quickly frozen on dry ice until RNA was extracted. RNA isolation, qPCR and data analysis were performed as described[18]. Data were analyzed by comparing $C(t)$ values of the treatment condition (control versus susceptible or resilient) with the $\Delta\Delta C(t)$ method.

Chromatin immunoprecipitation. ChIP was performed on pooled bilateral NAc punches from four mice 1 h after their last defeat. A total of 20 mice per group were used. Tissues were cross-linked, washed and kept at −80 °C. Sheared chromatin was incubated overnight with anti-FosB antibody (SC048, Santa

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Cruz) 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 ΔFosB to the GluR2 promoter was determined by qPCR using primers spanning the portion −500 to −320 nucleotides from the start codon, a region containing AP-1 sites. qPCR performed on a region with no AP1 sites, with primers amplifying a product from −240 to −100 from the start codon, showed no induction in defeated tissue as compared with control tissue.

Electrophysiology. All electrophysiological experiments were performed on control mice, or those subjected to chronic (10 d) social defeat, 2–28 d after the last defeat episode. Coronal NAc slices (250-μm thick) were cut in ice-cold sucrose ACSF containing 254 mM sucrose, 3 mM KCl, 1.25 mM NaH_2PO4, 10 mM D-glucose, 24 mM $NaHCO_3$, 2 mM $CaCl_2$ and 2 mM $MgSO_4$, and oxygenated with 95% O_2 and 5% CO_2 (pH 7.35, 295–305 mOsm). After a 1-h recovery at 37 °C in ACSF (254 mM sucrose replaced by 128 mM NaCl), electrophysiological recordings were performed at 30–32 °C in ACSF containing 50 μM 2-amino-phosphonovaleric acid (APV) and 50 μM picrotoxin. Patch pipettes (3–5 MΩ resistance) for whole-cell recordings were filled with an internal solution containing 115 mM potassium gluconate, 20 mM KCl, 1.5 mM $MgCl_2$, 10 mM HEPES, 10 mM phosphocreatine, 2 mM ATP-Mg, 0.5 mM GTP, 1 mM QX-314 (a Na^+ channel blocker) and 100 μM spermine (pH 7.2, 280–290 mOsm). Medium spiny neurons from the shell of NAc were identified under visual guidance by using infrared-differential interference contrast (IR-DIC) video microscopy with a 40× objective (Olympus BX51-WI). Whole-cell patch-clamp recordings were performed with a computer-controlled amplifier (MultiClamp 700B), digitized (Digidata 1440) and acquired with an Axoscope 10.1 instrument (all from Axon Instruments) at a sampling rate of 10 kHz. Electrode potentials were adjusted to zero before obtaining the whole-cell configuration, and only cells with a resting membrane potential of −72 to −82 mV were used. Synaptic responses were elicited by local electrical stimulation of 0.01–0.25-mA square pulses, 100 μsec in duration, delivered every 10 s using a tungsten bipolar stereotrode (1.0 MΩ) and DS-8000 digital stimulator (World Precision Instruments). Evoked AMPA excitatory postsynaptic currents (EPSCs) were recorded in voltage-clamp configuration and amplitudes were measured at 0.033 ms after stimulus. An average of at least six EPSCs at −80 mV and +40 mV were used to determine the ratio in each cell. For NASPM experiments, AMPAR EPSCs were recorded at −80 mV before (baseline) and 10 min after bath application of 100 μM NASPM. An average of at least six EPSCs in each condition for each cell were used to determine the ratio reported.

Statistical analyses. Data displayed are expressed as means ± s.e.m. (represented as error bars). One-way ANOVA was used to compare means between control, susceptible and resilient mice in all immunohistochemical, biochemical and behavioral analyses. Two-way ANOVA was used to compare the effect of overexpression of the transgene in the bitransgenic lines or with the virus, the effect of fluoxetine, or the effect of NBQX on the social interaction test. Student's *t* test was used to compare means in the social isolation experiment and between groups in the human post-mortem tissue study. Differences between experimental conditions were considered statistically significant at a value of $P < 0.05$.

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