Published in final edited form as:

Biol Psychiatry. 2011 September 1; 70(5): 487–495. doi:10.1016/j.biopsych.2011.04.021.

FosB is essential for the enhancement of stress tolerance, and antagonizes locomotor sensitization by Δ FosB

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Abstract

Background—Molecular mechanisms underlying stress tolerance and vulnerability are incompletely understood. The *fosB* gene is an attractive candidate for regulating stress responses, because Δ FosB, an alternative splice product of the *fosB* gene, accumulates after repeated stress or antidepressant treatments. On the other hand, FosB, the other alternative splice product of the *fosB* gene, expresses more transiently than Δ FosB but exerts higher transcriptional activity. However, the functional differences of these two *fosB* products remain unclear.

Methods—We established various mouse lines carrying three different types of *fosB* allele, wild-type (*fosB*⁺), *fosB*-null (*fosB*^G) and *fosB*^d allele which encodes Δ FosB but not FosB, and analyzed them in stress-related behavioral tests.

Results—Since *fosB*^{+/d} mice show enhanced Δ FosB levels in the presence of FosB, and *fosB*^{d/d} mice show more enhanced Δ FosB levels in the absence of FosB, the function of FosB can be inferred from differences observed between these lines. *fosB*^{+/d} and *fosB*^{d/d} mice showed increased locomotor activity and elevated Akt phosphorylation, whereas only *fosB*^{+/d} mice showed antidepressive-like behaviors, and increased E-cadherin expression in striatum, compared to wild-type mice. In contrast, *fosB*-null mice showed increased depression-like behavior and lower E-cadherin expression.

Conclusions—These findings indicate that FosB is essential for stress tolerance mediated by Δ FosB. These data suggest that *fosB* gene products have a potential to regulate mood disorder-related behaviors.

Keywords

depression; bipolar disorders; mania; *fosB* gene; mood disorder; knockout mice

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Financial Disclosures

The authors report no biomedical financial interests or potential conflicts of interest.

Supplementary material cited in this article is available online.

Introduction

The *fosB* gene has an intron-like sequence in exon 4, which allows alternative splicing and the production of two distinct transcripts, *fosB* and Δ *fosB* mRNAs. The latter encodes Δ FosB protein which lack the C-terminal 101 aa containing the transactivation domain in the full -length FosB protein (1). We have previously reported that FosB and Δ FosB oppositely regulate Jun trans-activity (1, 2) and cell matrix adhesion (3) in cultured cells; the proteins also regulate cell proliferation, differentiation, and death (4–8). Interestingly, Δ FosB can act, *in vivo* and *in vitro*, as a transcriptional activator or repressor (9, 10). An example of the latter is feedback repression of the *c-fos* and *fosB* gene promoters (1, 11).

Δ FosB has been shown to be a key modulator of reward-related behaviors in response to drugs of abuse, natural reward, and coping with stress (9, 12–17). Δ FosB accumulates progressively after repeated or prolonged dopaminergic-related stimuli or convulsive seizures reflecting its high level of stability, in contrast to FosB which, like c-Fos, is highly unstable and is expressed transiently (12, 18–23). However, functional roles of Δ FosB and FosB in mood-related behaviors are incompletely understood. Induction of Δ FosB in several brain areas appears to mediate decreased sensitivity to the deleterious effects of chronic stress (13, 24), while the influence of FosB is completely unknown despite its much higher trans-activity (1).

Here, we characterized new *fosB* mutant mice: (i) *fosB^{d/d}* mice with significantly-enhanced expression of Δ FosB but no FosB, (ii) *fosB*-null (*fosB^{G/G}*) mice lacking both FosB and Δ FosB, and (iii) *fosB^{+/d}* mice with reduced expression of FosB and enhanced expression of Δ FosB. We found that *fosB^{+/d}* mice serve as a model for endogenous Δ FosB accumulating conditions, and that differences between *fosB^{d/d}* and *fosB*-null mice reflect the effects of enhanced Δ FosB expression in the absence of FosB. In addition, differences observed between *fosB^{+/d}* and *fosB^{d/d}* mice revealed the influence of FosB under Δ FosB accumulating conditions.

Methods and Materials

Generation of mutant mice

See Supplement 1.

Mice

Two to five month-old male mice were used in most experiments. *fosB^{d/d}* mice (F8-14) and *fosB^{G/G}* mice (F5-9) were obtained from heterozygous intercrosses except for experiments involving haloperidol treatment. Control wild-type mice were littermates of each mutant mouse line. No gross differences were apparent between wild-type and mutant mice. Mice were housed in plastic mouse cages with littermates with standard rodent chow and water *ad libitum*. Animals were maintained in an air-conditioned, light time-controlled, specific-pathogen-free room with a 12:12 hr light and dark cycle (light on at 8:00 A.M. and down at 8:00 P.M.). The handling and killing of all animals were carried out in accordance with the national prescribed guidelines, and ethical approval for the studies was granted by the Animal Experiment Committee of Kyushu University.

Immunohistochemistry

See Supplement 1.

Western blot analysis

See Supplement 1.

Behavior analyses

See Supplement 1.

Statistical analysis

See Supplement 1.

Results

Expression of FosB and Δ FosB in mutant mice

fosB gene expression in mutant mice was analyzed with immunohistochemistry (IHC) and Western blotting. Brain IHC of wild-type mice revealed that *fosB* gene expression is greatest in the olfactory bulb, nucleus accumbens (NAc), dorsal striatum, anterior cingulate cortex, dentate gyrus of hippocampus, and cerebral cortex. The regional pattern of *fosB* gene expression in the *fosB^{d/d}* mice is the same as wild-type mice, but the immunoreactivity (IR) is much higher (Figure 1A–C, Figure S1G, H in Supplement 1). In wild-type mice, the anti-FosB(C), which was raised against amino acids 245–315 of the C-terminus of FosB (1), detected FosB only sparsely in NAc and dorsal striatum, but substantially in cingulate cortex (Figure S1C, D in Supplement 1). This suggests that the majority of the *fosB* gene expression signal is derived from Δ FosB. FosB IR was undetectable in *fosB^{d/d}* and *fosB^{G/G}* mice, and Δ FosB IR was undetectable in *fosB^{G/G}* mice (Figure S1E, F in Supplement 1).

We next performed quantitative Western blotting of striatal nuclear extracts using anti-FosB (Figure 1D–F). Significantly enhanced expression of Δ FosB and $\Delta 2\Delta$ FosB in *fosB^{d/d}* and to a lesser extent in *fosB^{+/d}* mice was confirmed in comparison to wild-type mice. Expression levels of FosB and "variant FosB" ("vFosB") in *fosB^{+/d}* mice were apparently decreased in comparison to those in wild-type mice, and there was no detectable FosB and vFosB in *fosB^{d/d}* mice (Figure 1D, F). vFosB is thought to be $\Delta 1$ FosB or $\Delta 2$ FosB (Figure S1A in Supplement 1). In the *fosB^{+/G}* mice, the levels of vFosB and $\Delta 2\Delta$ FosB but not FosB and Δ FosB were significantly decreased in comparison to those in wild-type mice, and none of those were detected in *fosB^{G/G}* mice (Figure 1E, F).

These data suggest that *fosB^{+/d}* mice provide a model of Δ FosB accumulation over long periods of time, and that the difference between *fosB^{d/d}* and *fosB^{+/d}* mice can disclose the function of FosB under conditions of Δ FosB accumulation. Additionally, the differences observed between *fosB^{d/d}* and *fosB^{G/G}* mice, and shared traits between *fosB^{d/d}* and *fosB^{+/d}* mice, reflect the results of Δ FosB accumulation.

Higher spontaneous locomotor activity in *fosB^{d/d}* mice, and lower activity in *fosB^{G/G}* mice

To infer the function of endogenous Δ FosB and FosB in the central nervous system, we analyzed the two lines of mutant mice and their wild-type littermates, in several behavioral assays (Figure 2A–H). Spontaneous locomotor activity was followed through one week without any disturbance of individually housed mice in their homecages. The activity of all mice tended to decrease gradually due to the loss of interaction with littermates (Figure S2 in Supplement 1). By the third night, *fosB^{d/d}* mice showed significantly higher locomotor activity compared to wild-type mice and *fosB^{+/d}* mice (Figure 2A). On the other hand, *fosB^{G/G}* mice showed significantly lower locomotor activity compared to wild-type mice (Figure 2E). All mutant mice showed normal levels of locomotor activity at the start of the sleep cycle. These data suggest that Δ FosB facilitates spontaneous motor behavior during periods of wakefulness, but does not introduce abnormal motor behavior independent of the circadian cycle. Despite accumulation of comparable levels of Δ FosB in *fosB^{+/d}* mice, the mice showed lower levels of locomotion compared to that seen in *fosB^{d/d}* mice, suggesting

that FosB suppresses the effect of accumulated Δ FosB and helps to normalize basic locomotion.

We next examined the locomotor activity of mice housed independently for one month. Spontaneous locomotion was dramatically decreased in all of the genotypes studied. Nevertheless, the locomotor activity of *fosB^{d/d}* mice was still much higher; in fact, the difference between the activities of *fosB^{d/d}* and wild-type mice was larger than that seen earlier. *fosB^{+d}* mice also showed significantly higher locomotor activity than wild-type mice, suggesting that accumulated Δ FosB enhances spontaneous locomotion (Figure 2B). On the other hand, the difference between *fosB^{G/G}* and wild-type mice was abolished, suggesting that the level of locomotion after one month of isolation may settle down to basal levels without effects of social interaction. *fosB^{+G}* mice showed significantly higher locomotor activity than wild-type mice, suggesting that the higher expression ratio of Δ FosB versus FosB in *fosB^{+G}* mice may make an effect on the basal locomotor activity (Figure 2F).

Higher exploratory activity in both *fosB^{d/d}* and *fosB^{G/G}* mice, but opposite behavioral responses to fear-eliciting stimuli

On the first day in the open field test, which provides a measure of anxiety-like behavior, both *fosB^{d/d}* and *fosB^{G/G}* mice showed significantly higher exploratory activity than wild-type mice (Figure 2C, G), suggesting that FosB may increase anxiety-like responses or suppress intrinsic curiosity. Though all of the mice showed habituation after the second day of the test, *fosB^{+d}* and *fosB^{d/d}* mice on the second day, and *fosB^{+G}* mice on the third and fourth days, respectively, exhibited significantly higher locomotor activity than did wild-type mice, suggesting that the difference reflects spontaneous locomotor activity (Figure 2C, G).

In the elevated plus maze, another test of anxiety-like behavior, *fosB^{d/d}* mice stayed significantly longer in the open arms than wild-type mice, and *fosB^{G/G}* mice spent significantly less time in the open arms (Figure 2D, H), suggesting that Δ FosB might suppress anxiety-like behavior, and that the presence of FosB may help to normalize it. There was no significant difference in the number of entries into the open and closed arms, but *fosB^{d/d}* mice showed a trend for increased transitions (Figure S3A, C in Supplement 1). In contrast to the open-field and elevated plus maze tests, mutant mice showed no differences in the light-dark test (Figure S3B, D in Supplement 1).

Repeated forced swimming in the Morris water maze

Next, we used the Morris water maze to evaluate spatial learning ability and found some differences among the mouse lines tested (Figure 3A, B). The swimming speed of the mutant mice showed no difference through the training days (Figure S4C, D in Supplement 1). Interestingly, though the actual swimming time (Figure 3C, D) and distance traveled (Figure S4A, B in Supplement 1) for arrival to the platform were grossly normal, the time to reach the platform was shorter in *fosB^{+d}* mice, and longer in *fosB^{G/G}* mice especially around the middle of the training days (Figure 3A, B, and Table S1 in Supplement 1), due to immobility in the water during training (Figure 3E, F, and Table S1 in Supplement 1). In the probe test, all of the genotypes spent the longest time in the quadrant where the platform had previously existed (Figure 3G, H and Table S1 in Supplement 1). However, in *fosB^{+G}* and *fosB^{G/G}* mice, the time was shorter than that in wild-type mice due to immobility in the first quadrant (white boxes in Figure 3G, H).

The forced swim test is used widely as a measure of stress responsiveness, with immobility reflecting greater stress vulnerability. Not surprisingly, the Morris water maze was reported

recently to provide useful measures of swim stress responses (25, 26). Together, these data suggest that *fosB* gene products do not affect learning ability, however, accumulated Δ FosB decreases stress vulnerability, and such activity requires the presence of FosB.

Repeated forced swim test in inescapable condition

Next, to further assess the behavioral responses of *fosB* mutant mice to inescapable stress, we used a conventional forced swim test. We found a trend for decreased swimming time over repeated days of the test in wild-type, *fosB^{d/d}*, and *fosB^{G/G}* mice, but not in *fosB^{+/-}* mice (Figure 4). *fosB^{d/d}* and *fosB^{G/G}* mice showed significantly decreased swimming time on the second and third day, respectively. Additionally, we injected the mice with paroxetine (10 mg/kg), a standard antidepressant, 30 min before the swim test on the fourth day. Paroxetine increased the swimming time well beyond levels seen on the first day in all type of mice except *fosB^{G/G}* mice. In *fosB^{G/G}* mice, the swimming time recovered but did not reach the level of the first day, and this “recovery ratio” was significantly lower than that seen in wild-type mice.

Responsivity to dopamine signals in mutant mice

fosB^{d/d} mice showed hyperlocomotion in the active phase with a normal circadian pattern (Figure S2 and Figure S4A in Supplement 1), but appeared less anxious or fearful in the elevated plus maze (Figure 2D). These behavioral phenotypes are somewhat reminiscent of the symptoms of attention deficit hyperactivity disorder (ADHD) (27). ADHD patients can be treated successfully with methylphenidate (MPH). MPH inhibits dopamine reuptake and increases the dopamine concentration in synaptic clefts, which leads to hyperlocomotion in healthy individuals. However, ADHD patients calm down in paradoxical reactions to MPH. Therefore, we examined whether MPH exerted therapeutic-like effects in *fosB^{d/d}* mice compared with effects of other dopaminergic drugs (Figure 5A–D, Figure S5 in Supplement 1). We found that MPH induced transient hyperlocomotion (Figure 5A, B), which settled down to baseline within 4 hr after intraperitoneal injection in all types of *fosB* mutant mice and their wild-type littermates (Figure S5C, D in Supplement 1). In particular, *fosB^{+/-}* and *fosB^{d/d}* mice exhibited more hyperactivity in response to MPH than wild-type mice. The transient hyper-locomotion exhibited by *fosB^{+/-}* and *fosB^{d/d}* mice were similar, but the locomotor activity exhibited in the dark phase after MPH treatment was decreased in *fosB^{+/-}* mice (Figure 5A, C). These data suggest that accumulated Δ FosB drives locomotion during the dark phase, while the existence of FosB prevents it (Figure S5C in Supplement 1). *fosB^{+/-}* and *fosB^{G/G}* mice exhibited equivalent reactions to MPH as wild-type mice, suggesting that normal low levels of Δ FosB do not contribute to basal dopamine sensitivity, and that only accumulated Δ FosB can facilitate it (Figure 5B, Figure S5B, D in Supplement 1).

Basal ganglia circuitry regulates motor function via two pathways, termed the direct and indirect pathways. More than 90% of striatal neurons are medium spiny GABAergic projection neurons. About half of them project directly to the midbrain, and express dynorphin and the D1 dopamine receptor; activation of these neurons increases locomotion and induces *fosB* gene expression. The other half indirectly project to the midbrain via the globus pallidus and subthalamic nucleus and express enkephalin and the D2 dopamine receptor; activation of these neurons decreases locomotion (28). D2 receptor signaling in these neurons inhibits such down regulation of locomotion, and suppresses *fosB* gene expression (29).

Werme *et al.* reported that Δ FosB overexpression selectively in direct pathway neurons increased daily running compared with control littermates, whereas Δ FosB overexpression predominantly in indirect pathway neurons decreased it (30). At first, we hypothesized that

spontaneous hyperlocomotion and higher responsivity to MPH in *fosB*^{+/-d} and *fosB*^{d/d} mice are dependent on increased D1 receptor signaling sensitivity. However, locomotor responses to a D1 receptor agonist (2 mg/kg SKF81297) showed no differences among all of the *fosB* mutant and wild-type mice (Figure 5A, B, and Figure S5E, F in Supplement 1). Next, we administered a D2 receptor antagonist (1 mg/kg haloperidol), which is reported to induce *fosB* gene expression in D2 containing neurons as well as hypolocomotion (29, 31). The total locomotor activity over 4 hr after haloperidol treatment was significantly higher in *fosB*^{d/d} mice, and lower in *fosB*^{G/G} mice, compared to wild-type mice (Figure 5A, B and Figure S5G, H in Supplement 1). This finding, along with the pattern of results in the elevated plus maze (Figure 2D, H) and spontaneous locomotion test on the second and third day after isolation from littermates (Figure S2 in Supplement 1), suggests that accumulated Δ FosB suppresses the D2 receptor signal. This in turn may contribute to the increased locomotion and reduction in anxiety-like behavior induced by Δ FosB, with FosB negatively regulating the effects of accumulated Δ FosB.

We detected no behavioral differences in the effects of a D2 agonist (1 mg/kg LY 171555) among the *fosB* mutant and wild-type mice (Figure 5A, B, and Figure S5I, J in Supplement 1). These data suggest that, in the mutant mice, negative feedback via D2 receptors on dopamine neurons in the midbrain may function normally (32).

E-cadherin expression levels parallel stress responses and *fosB* gene expression

To further elucidate how *fosB* gene products regulate complex behavior, we selectively picked several postulated target proteins from previous reports (3, 14, 33), and then performed Western blotting with striatum samples (Figure S6A in Supplement 1). E-cadherin was significantly up-regulated in *fosB*^{+/-d} mice, and down-regulated in *fosB*^{+/-G} and *fosB*^{G/G} mice, in other words, the expression level of E-cadherin paralleled the behavioral phenotype of stress tolerance seen with forced swimming. Similar tendencies were detected in hippocampus (data not shown). In contrast, there was no difference in the expression levels of Cdk5, GluR2, α -catenin, or β -catenin (the first two are putative Δ FosB targets identified in bitransgenic mice, and the latter two are binding partners of E-cadherin) among *fosB* mutant and wild-type mice (Figure S6A in Supplement 1). The level of E-cadherin mRNA in NAc also showed no difference among mutant and wild-type mice (data not shown), suggesting that the up-regulation of E-cadherin is regulated by post-translational mechanisms.

Akt phosphorylation is increased by Δ FosB accumulation

Finally, we checked the level of Akt and its phosphorylation in the striatum of *fosB* mutant mice (Figure S6B in Supplement 1). Total Akt protein levels showed no difference among mutant and wild-type mice, but the phosphorylation state of Akt (T308) was significantly elevated in *fosB*^{+/-d} and *fosB*^{d/d} mice.

Discussion

In this study, we provide evidence that accumulated Δ FosB increases locomotor activity, and that FosB antagonizes this effect similar to findings from *in vitro* cellular assays (1). On the other hand, stress tolerance appears to be the sum of Δ FosB and FosB. These effects may be partly mediated via E-cadherin, an indirect target of *fosB* gene products. Together, these data suggest distinct patterns of behavioral abnormalities among the mutant mouse lines examined (Figure 6). We propose that *fosB*^{G/G} mice exhibit behaviors that resemble depression, including decreased locomotion, increased immobility during forced swimming, and increased anxiety-like responses. In contrast, *fosB*^{+/-d} mice exhibit behaviors that in some ways resemble manic-like symptoms, including hyperlocomotion, increased stress

tolerance, and reduced anxiety-like responses. Interestingly, *fosB^{d/d}* mice exhibit significantly higher dopamine sensitivity, and most of the altered behaviors seen in *fosB^{+/-}* mice except for increased stress tolerance. These mice thus present a picture of blended responses perhaps reminiscent of certain aspects of bipolar disorder. Clearly, these interpretations are based on initial analyses in rodent models and require much further work for validation, including studying these molecular findings in the human disorders.

The similar results obtained from *fosB^{G/G}* mice in the water maze (Figure 3) and repeated forced swim test (Figure 4) indicate that the water maze has the potential to reveal responses to stress. Whereas the repeated forced swim test revealed the stress vulnerability of *fosB^{d/d}* mice more clearly than the water maze, the difference between wild-type and *fosB^{+/-}* mice was more apparent in the water maze, suggesting that the repeated forced swim test may be better to detect initial stress vulnerability, while the water maze may be better to detect stress tolerance that develops over time. Data with paroxetine suggest that *fosB* gene products are partly required for antidepressant responses to the drug, as reported recently (24).

In *fosB^{+/-}* mice, the antagonistic relationship between FosB and accumulated Δ FosB, first seen *in vitro* (1), was captured in measures of spontaneous locomotor activity (Figure 2A, Figure S2A in Supplement 1), elevated plus maze (Figure 2D), and sensitivity to a D2 antagonist (Figure 5A, Figure S5G in Supplement 1). The expression level of Δ FosB in *fosB^{+/-}* mice is much higher than that of FosB, but FosB has much higher trans-activity than Δ FosB (1, 34). These data suggest that lower expression levels of FosB may be sufficient to suppress the activity of accumulated Δ FosB, and thereby normalize some of the consequences of its accumulation (Figure S7 in Supplement 1). Such antagonism is not seen in all cases, perhaps reflecting the different cell types or target genes involved. In Δ FosB bitransgenic mice, where Δ FosB expression is inducible and relatively restricted to D1 neurons in NAc and dorsal striatum, Kelz *et al.* reported higher locomotor activity in a novel test chamber not on the first day but on the second day (14). This finding corresponds to observations in *fosB^{+/-}* mice, which exhibited higher locomotor activity with a similar pattern (Figure 2C), thus suggesting that Δ FosB accumulation in striatum plays an important role in mediating locomotor activation via dopamine signaling.

We know that the *fosB* gene is induced by dopaminergic signals (23). Since Akt phosphorylation is also induced by dopamine (35), it is possible that accumulated Δ FosB enhances Akt phosphorylation, and that the extent of spontaneous dopaminergic activation reflects levels of Akt phosphorylation. Perrotti *et al.* reported that morphine induces Δ FosB accumulation in NAc (23), and Russo *et al.* reported that overexpression of a constitutively active form of Akt in NAc enhances locomotor sensitization induced by morphine (36). Locomotor sensitization by morphine is regulated by indirect dopaminergic mechanisms such as increased dopamine release in NAc (37, 38). Cocaine directly increases dopamine release in NAc by inhibiting dopamine reuptake. Overexpression of Δ FosB enhanced cocaine locomotor sensitization (39), suggesting that enhanced Akt phosphorylation by accumulated Δ FosB might facilitate locomotor sensitization by cocaine and by morphine. This is consistent with the significant up-regulation of Akt phosphorylation and enhanced locomotor sensitization by MPH observed in *fosB^{d/d}* mice (Figures 5A, S6C in Supplement 1). The mechanism by which Δ FosB accumulation leads to increased Akt phosphorylation is not known, since Δ FosB could affect the expression of any of several regulators of Akt phosphorylation (40). One possibility is that accumulated Δ FosB may repress the expression of a protein phosphatase, such as PP2A, since Akt phosphorylation is known to be suppressed by D2 signaling in striatum via the activation this phosphatase (41). These and other possibilities now require direct investigation.

fosB-knockout (KO) mice had been established by Brown *et al.* (42), and actually have a potential to express $\Delta 3\Delta$ FosB, an alternative-translation initiation product similar to $\Delta 2\Delta$ FosB (Figure S1 in Supplement 1). Hiroi *et al.* reported that *fosB*-KO mice showed slightly higher locomotor activity when they were introduced into a novel test chamber (12, 43). This corresponds to our observation that *fosB^{G/G}* mice exhibit significantly higher locomotor activity in the open field on the first day (Figure 2G), suggesting that FosB may suppress exploratory behavior in the novel environment independent of the presence of Δ FosB. On the other hand, Zhu *et al.* (43) reported that the *fosB*-KO mice exhibit lower sensitivity to other types of stress paradigms (tail suspension test), and Brown *et al.* (42) had reported that the *fosB*-KO mice are defect in nurturing, and no difference in the Morris water maze test. These findings are not consistent with our results, since *fosB^{G/G}* mice exhibited higher stress vulnerability and no defect in nurturing (data not shown). These differences could reflect the potential influence of $\Delta 3\Delta$ FosB or the different genetic backgrounds (BALB-C in the prior studies by Brown *et al.* and C57BL6/J in the present investigations).

Interestingly, the expression level of E-cadherin and the stress tolerance observed upon repeated forced swimming paralleled the combined expression of FosB and Δ FosB. Only with regard to the particular expression pattern and behavioral phenotype does FosB not antagonize but coordinates with Δ FosB. Δ FosB has been reported to accumulate after chronic stress in several brain regions (22, 24), and accumulated Δ FosB in the ventrolateral periaqueductal gray promotes active coping responses against stress, such as forced swimming (13). More recently, overexpression of Δ FosB in the NAc suppresses the deleterious effects of social defeat (24). Findings from the present study suggest that such antidepressant-like effects of Δ FosB require the presence of FosB.

E-cadherin is an important cell-cell adhesion molecule, and influences synapse formation in neuronal cells (44). In rodent models of depression, the number of mature synapse buttons or dendritic spines is decreased for several neuronal cell types, and antidepressant treatments reverse these abnormalities (45–47). Here, we found that the expression level of E-cadherin in *fosB* mutant and wild-type mice parallels stress tolerance, suggesting that E-cadherin may be one mechanism underlying the stress tolerance induced by *fosB* gene products. FosB might inhibit E-cadherin degradation via transactivation of an inhibitory binding protein against Hakai, which is an E3-ligase of E-cadherin, or directly inhibit the transcription of Hakai mRNA (48). Accumulated Δ FosB might increase E-cadherin translation via TGF- β signaling, which is increased in *fosB^{+/d}* and *fosB^{d/d}* ES cells (3). Future studies are needed to explore these possibilities directly.

Cdk5 and GluR2, putative Δ FosB targets identified in bitransgenic mouse models (14, 33, 49), are expressed at similar levels among *fosB* mutants and wild type. McClung and Nestler reported that the expression levels of various Δ FosB targets in these mice switched from up- to down-regulated, or vice versa, between two and eight weeks after turning on Δ FosB expression (9). The constitutive changes in *fosB* gene expression in our mutant mice might not result in altered expression of Cdk5 and GluR2.

In summary, the *fosB^{G/G}* mutant is a definitive *fosB*-null mouse, and *fosB^{+/d}* and *fosB^{d/d}* mutants differentially express aberrant levels of Δ FosB and FosB from the endogenous *fosB* gene. These three mouse lines exhibit behavioral abnormalities mimicking different types of mood disorders. Further studies of these mice will shed light on the mechanisms controlling mood disorder-related behaviors, and thus contributing to the development of improved treatments for these disorders.

Brief summary

fosB gene encodes FosB and Δ FosB proteins. Δ FosB is highly stable and accumulated in specific regions of brain after stress or antidepressant treatments. Using two types of new *fosB* mutant mice, we found that accumulated Δ FosB increases locomotor activity and elevates Akt phosphorylation independent of FosB, while it acts as anti-depressant cooperatively with FosB and the effect is paralleled with E-cadherin expression in striatum. These data suggests the importance of *fosB* gene in mood disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. M. Katsuki (National Institutes of Natural Sciences, Tokyo, Japan) for CCE ES cells, M. Otsu in the Laboratory for Technical Support, Medical Institute of Bioregulation for the DNA sequence analyses, A. Matsuyama and K. Nakabeppu for animal care, and S. Kitamura for tissue processing. We also thank all members of our laboratory for their helpful discussions. This work was supported by grants from CREST, Japan Science and Technology Agency, the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Grant number: 16012248), and the Japan Society for the Promotion of Science (Grants 16390119 and 18300124, 22221004).

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Figure 1.

Expression of FosB and ΔFosB in the brain of *fosB* mutant mice. (A–C)

Immunohistochemical detection of *fosB* gene products in mouse brain. Coronal sections of brain prepared from wild-type, *fosB*^{d/d}, and *fosB*^{G/G} mice were subjected to immunohistochemistry with anti-FosB(N). The FosB(N) antibody was raised against amino acids 79–131 of the N-terminus common to FosB and ΔFosB. Representative sections are shown. St, striatum; HP, hippocampus (HP). The regional distribution of *fosB* gene expression is not different between wild-type and *fosB*^{d/d} mice, and there is no expression of *fosB* gene products in *fosB*^{G/G} mice. (D, E) Western blotting analysis of *fosB* gene products in striatal nuclear extracts. Blotting membranes were reacted with a rabbit monoclonal anti-FosB (5G4, Cell Signaling). The black arrow indicates p43 (FosB), the open arrowheads indicate p32/36 (ΔFosB), the red arrow indicates p31 (vFosB), and the closed arrowhead indicates p24 (Δ2ΔFosB). e(+/+) is enhanced image of wild type result. The blots were stained with Ponceau S (lower panels in D and E). (F) Quantification of FosB, ΔFosB, vFosB, and Δ2ΔFosB bands in striatal samples. Relative values of integrated densities to that of ΔFosB in wild-type mice are shown (n=3–5, mean ± S.E.M.). Expression levels of ΔFosB and Δ2ΔFosB were increased in *fosB*^{+/d} and *fosB*^{d/d} mice compared to wild-type mice [ΔFosB: F(2, 12) = 21.47, p = 0.0002; Δ2ΔFosB; F(2, 12) = 24.92, p = 0.0001], while those of FosB and vFosB in *fosB*^{+/d} mice were apparently decreased [FosB: F(2, 12) = 3.54, p = 0.0689; vFosB: F(2, 12) = 2.6531, p = 0.119]. In the *fosB*^{+/G} mice, the levels of vFosB and Δ2ΔFosB were significantly decreased, and no *fosB* gene product was detected in *fosB*^{G/G} mice [FosB: F(2, 11) = 10.366, p = 0.0046; ΔFosB: F(2, 11) = 16.052, p = 0.0011;

vFosB: $F(2, 11) = 37.565, p < 0.0001$; $\Delta 2\Delta$ FosB: $F(2, 11) = 39.063, p < 0.0001$]. *, $p < 0.05$ in Dunnett's multiple comparison test. #, $p < 0.05$ in t-test.

Figure 2.

Spontaneous locomotor activity, open field, and elevated plus maze tests in *fosB* mutant mice. Littermates obtained from mating of *fosB*^{+d} × *fosB*^{+d} (upper panels: A to D), and *fosB*^{+G} × *fosB*^{+G} (lower panels: E to H), mice were used for the experiments. Upper panels represent data of line d (Δ FosB accumulation type). Lower panels represent data of line G (*fosB*-null type). (A, B, E, F) Spontaneous locomotor activity during a 12 hr period in the night. Data in panels A and E were obtained in the night of the third day after isolation, those in B and F after 1 month of isolation. Bars represent locomotor counts per 12 hr (mean ± S.E.M.). On the third night, *fosB*^{d/d} mice showed significantly higher locomotor activity compared to wild-type and *fosB*^{+d} mice [$F(2, 51) = 8.82$; $p < 0.001$], while *fosB*^{G/G} mice showed significantly lower locomotor activity compared to wild-type mice [$F(2, 60) = 3.87$; $p = 0.026$]. *fosB*^{+d} mice also showed significantly higher locomotor activity than wild-type mice [$H = 45.0$, $p < 0.001$, Kruskal-Wallis test]. (C, G) Results of the open field test. Bars represent travel distance (cm) for 10 min (mean ± S.E.M.). On the first day, both *fosB*^{d/d} and *fosB*^{G/G} mice showed significantly higher locomotor activity than wild-type mice [$F(2, 51) = 3.56$; $p < 0.05$ and $F(2, 50) = 3.79$; $p < 0.05$, respectively]. *fosB*^{+d} and *fosB*^{d/d} mice [$F(2, 51) = 4.48$; $p < 0.05$] on the second day, and *fosB*^{+G} mice [$F(2, 50) = 5.96$; $p < 0.01$, and $F(2, 50) = 4.02$; $p < 0.05$] on the third and fourth days, respectively, exhibited significantly higher locomotor activity than did wild-type mice. (D, H) Results of the elevated plus maze test. Bars represent time in open arm (sec) (mean ± S.E.M.). *fosB*^{d/d} mice stayed significantly longer in the open arms than wild-type mice [$H = 9.79$, $p = 0.007$, Kruskal-Wallis test], and *fosB*^{G/G} mice spent significantly less time in the open arms [$F(2, 45) = 4.91$; $p < 0.01$]. In A to H, #, $p < 0.05$; ##, $p < 0.01$; statistical difference between homozygous mice and wild-type mice. *, $p < 0.05$; **, $p < 0.01$; statistical difference

between heterozygous and homozygous mice. §, $p < 0.05$; statistical difference between heterozygous and homozygous mice, using ANOVA (Dunnett 's posthoc test)

Figure 3.

Repeated forced swimming in the Morris water maze in *fosB* mutant mice. Left panels represent data of line d (Δ FosB accumulation type). Right panels represent data of line G (*fosB*-null type). (A, B) Escape latency in the hidden platform test. (C, D) Actual swimming time exhibited no gross differences in the mutant mice. (E, F) The difference in time to reach to the hidden platform is dependent on immobility in the water maze. To further study initial stress vulnerability and tolerance over time in this assay, we repeated training for several days. In A–F, all points are shown as mean $\pm$ S.E.M. #, $p < 0.05$; ##, $p < 0.01$; statistical difference between homozygous mice and wild-type mice. *, $p < 0.05$; **, $p < 0.01$; statistical difference between heterozygous and homozygous §, $p < 0.05$; §§, $p < 0.01$;

statistical difference between heterozygous and homozygous mice, using ANOVA (Dunnett's posthoc test) (G, H) Time in each quadrant in probe test at 11th day after 10 days training. The white box in the opposite quadrant reflects the time until moving to the other quadrant from the first quadrant. *, $p < 0.01$; comparison with the training quadrant, using ANOVA (Dunnett's posthoc test).

Figure 4.

Repeated forced swim test of *fosB* mutant mice in inescapable condition. Conventional repeated forced swim test was performed once a day for 4 consecutive days. Each trial was performed for 6 min, and relative ratio of swimming time during last 4 min on a given day to that in the day 1 is shown in a bar graph (mean $\pm$ S.E.M., $n = 12-16$). Paroxetine (10 mg/kg) was injected 30 min before the fourth day trial. In *fosB^{G/G}* mice, the recovery ratio of the swimming time after administration of paroxetine was significantly lower than that seen in wild-type mice [$F(3, 56) = 3.11$; $p < 0.05$]. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; statistical difference between the day 1 and a given day in each mouse line. #, $p < 0.05$;

statistical difference between *fosB^{GG}* and wild-type mice using ANOVA (Dunnett's posthoc test).

Figure 5.

Locomotor activity induced by dopamine receptor agonists and antagonists in *fosB* mutant mice. Left panels represent data of line d (Δ FosB accumulation type). Right panels represent data of line G (*fosB*-null type). Naïve mice, without prior behavioral assays, were housed individually for two weeks, and then placed in a cage with an infrared automatic monitor system for 3 days. On the second day at 4:00 pm, the mice were injected with saline, and on the third day at 4:00 pm the mice were injected with 30 mg/kg methylphenidate (MPH), 2 mg/kg SKF81297 (SKF), 1 mg/kg haloperidol (HAL), or 1 mg/kg LY 171555 (QNP) as shown in Figure S5 in Supplement 1. (A, B) Immediate effects on locomotor activity during the first 4 hr after treatment. Bars represent locomotor counts observed during the first 4 hr from 4:00 pm to 8:00 pm (mean $\pm$ S.E.M.). *fosB*^{+/d} and *fosB*^{d/d} mice exhibited more hyperactivity in response to MPH than wild-type mice [$F(2, 23) = 4.14$; $p < 0.05$]. The total locomotor activity immediately after haloperidol treatment was significantly higher in *fosB*^{d/d} mice [$F(2, 29) = 4.95$; $p < 0.05$], and lower in *fosB*^{G/G} mice [$F(2, 16) = 7.43$; $p < 0.01$], compared to wild-type mice. (C, D) Delayed effects on locomotor activity. Bars represent locomotor counts observed during the last 12 hr from 8:00 pm to 8:00 am in the next day (mean $\pm$ S.E.M.). The locomotor activity exhibited in the dark phase after MPH treatment was decreased in *fosB*^{+/d} mice [$F(2, 23) = 3.78$; $p < 0.05$]. In A to D, #, $p < 0.05$; statistical difference between homozygous mice and wild-type mice, §, $p < 0.05$; statistical difference between heterozygous and homozygous mice, using ANOVA (Dunnett's posthoc test). The number of mice is provided in Figure S5 in Supplement 1.

Figure 6. Summary of the effects of different levels of *fosB* gene products on locomotor activity and stress tolerance. FosB and accumulated Δ FosB amplify stress tolerance and E-cadherin expression cooperatively. Accumulated Δ FosB facilitates Akt phosphorylation and locomotor activity through its dopaminergic sensitization, while FosB antagonizes the effects.