Hippocampal subgranular zone *FosB* expression is critical for neurogenesis and learning

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Abstract

Neural proliferation in the dentate gyrus (DG) is closely linked with learning and memory, but the transcriptional programming that drives adult proliferation remains incompletely understood. Our lab previously elucidated the critical role of the transcription factor FosB in the dorsal hippocampus (dHPC) in learning and memory, and the *FosB* gene has been suggested to play a role in neuronal proliferation. However, the subregion-specific and potentially cell-autonomous role of dHPC FosB in neurogenesis-dependent learning has not been studied. Here, we crossed neurotensin receptor-2 (NtsR2) Cre mice, which express Cre within the subgranular zone (SGZ) of dHPC DG, with floxed *FosB* mice to show that knockout of FosB in hippocampal SGZ neurons reduces antidepressant-induced neurogenesis and impedes hippocampus-dependent learning in the novel object recognition task. Taken together, these data indicate that *FosB* gene expression in SGZ is necessary for both hippocampal neurogenesis and memory formation.

Keywords

FosB; NtsR2; Hippocampus; Neurogenesis; Learning; Memory

Introduction:

As learning and memory are thought to develop through repeated activation of discrete networks of neurons, activity-dependent immediate early genes (IEGs) are critical mediators of learning and memory processes. Indeed, many studies have linked various IEGs to the

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creation and expression of memories (Liu X et al., 2014;Minatohara K et al., 2015;Ramirez S, 2018;Tonegawa S et al., 2015), including IEG transcription factors that orchestrate the sweeping changes in gene expression underlying the formation of stable engrams central to learning. As the process of learning and memory consolidation can take place over timescales of up to days or weeks, mechanisms controlling gene expression over these timescales, including epigenetics (Duke CG et al., 2017;Kyrke-Smith M and Williams JM, 2018;Leighton LJ et al., 2018), have become a key area of study in the learning field. The IEG FosB, a transcription factor produced by the *FosB* gene, is unique in its stability, with a half-life *in vivo* of around eight days (Carle TL et al., 2007;Nishijima T et al., 2013;Ulery-Reynolds PG et al., 2009), allowing it to accumulate in neurons and regulate gene expression after repeated stimulation (Nestler EJ, 2012). Moreover, hippocampal FosB is critical for learning (Eagle AL et al., 2015), though its mechanism(s) of action in hippocampal function are not fully understood.

FosB function has been implicated in learned rewarding and social behaviors. For example, transcriptional silencing of FosB in the nucleus accumbens (NAc) impairs experience-induced facilitation of sexual behavior (Pitchers KK et al., 2010) and NAc FosB is necessary for learning association of spatial context with positive reinforcers, like cocaine (Robison AJ et al., 2013). FosB is induced by the antidepressant fluoxetine in the NAc (Vialou V et al., 2010;Vialou V et al., 2015) and its function there is critical for antidepressant effects on social interaction (Vialou V et al., 2010). Fluoxetine also induces FosB in the dentate gyrus (DG) of the dHPC (Vialou V et al., 2015), and its antidepressant efficacy is dependent on neurogenesis in the DG (Malberg JE et al., 2000;Santarelli L et al., 2003), so probing the link between FosB and neurogenesis may provide new insights into learning and antidepressant function.

Mice lacking expression of the *FosB* gene in all tissues from conception (*FosB* knockout mice) have a variety of hippocampal malformations, including thinning of the DG granular cell layer, and display reduced hippocampal neurogenesis (Yutsudo N et al., 2013). However, such global, germ-line *FosB* knockout cannot indicate the specific contribution of hippocampal neurogenesis to behavior. Our group has shown that FosB regulation of transcription in dorsal hippocampus (dHPC) is necessary for spatial memory (Eagle AL et al., 2015). However, these studies used a viral method that inhibited FosB activity in fully differentiated CA1 and DG neurons of dHPC, and thus did not address a role for FosB-driven neurogenesis in learning. Moreover, it is critical to consider how specific hippocampal subregions may be involved in learning and memory. Of particular note is the subgranular zone (SGZ) of the DG, an important site of neurogenesis in the brain. SGZ neurogenesis has been tied to learning and memory in the context of spatial learning tasks (Epp JR et al., 2016), stress-induced behaviors (Hill AS et al., 2015;Lagace DC et al., 2010), and fear (Seo D-o et al., 2015). We therefore used *NtsR2-Cre* mice, in which hippocampal Cre expression is confined to the SGZ, to test the hypothesis that knockout of *FosB* gene products specifically in the SGZ of mice inhibits neurogenesis and impairs performance in learning and memory-related tasks.

Experimental Procedures:

Animals and Genotyping: 76 adult male and female mice (>8 weeks) were included in these studies. Neurotensin receptor-2 IRES-Cre (NtsR2$^{cre/+}$) mice lacking the frt-flanked blocking cassette were crossed with Cre-inducible Rosa$^{eGFP-L10a}$ mice, so that any cells expressing NtsR2/Cre are permanently marked with GFP (Woodworth HL et al., 2018). NtsR2$^{cre/+}$ mice were crossed with floxed *FosB* (FosB$^{lox/lox}$) mice (Ohnishi YN et al., 2017) to generate progeny with intact *FosB* (NtsR2$^{+/+}$;FosB$^{lox/lox}$) and those lacking *FosB* in NtsR2 cells (NtsR2$^{Cre/+}$;FosB$^{lox/lox}$). Mice were genotyped using standard PCR with the following primers:

> IRES-Cre forward: 5' – GGACGTGGTTTTCCTTTGAA – 3'
>
> IRES-Cre reverse: 5' – AGGCAAATTTTGGTGTACGG – 3'
>
> Rosa26EGFP-L10a:
>
>> mutant forward: 5' – TCTACAAATGTGGTAGATCCAGGC – 3'
>>
>> wild type forward: 5' – GAGGGGAGTGTTGCAATACC – 3'
>>
>> common reverse: 5' – CAGATGACTACCTATCCTCCC – 3'
>
> FB loxPu sequence: 5' – GCT GAA GGA GAT GGG TAA CAG – 3'
>
> LIPz sequence: 5' – AAG CCT GGT GTG ATG GTG A – 3'
>
> LNEo1 sequence: 5' – AGA GCG AGG GAA GCG TCT ACC TA – 3'.

Adult mice were group housed 4–5 per cage in a 12 h light/dark cycle and provided *ad libitum* food and water. In some cases, a wheel was provided for *ad libitum* wheel running (see results below). All experiments were approved by the Institutional Animal Care and Use Committee at Michigan State University and performed in accordance with AAALAC and NIH guidelines.

Immunocytochemistry: 12 mice were transcardially perfused with cold PBS followed by 10% formalin. Brains were post-fixed for 24 hours in 10% formalin, cryopreserved in 30% sucrose, and sliced into 35μm sections. Immunofluorescence was performed using the following primary antibodies: Anti-FosB (FosB 5G4, 1:1000, rb, #2251S, Cell Signaling Technologies), Anti-NeuN (1:1000, ms, MAB377 Millipore), Anti-GFP (1:1000, gt, ab5450, Abcam or ms, A11120, Invitrogen), Anti-BrdU (1:1000, rat, MCA2060, Bio-Rad), and Anti-Doublecortin (1:1000, gt, sc-8066, Santa Cruz). The following corresponding secondary antibodies were then used: Donkey anti-rabbit Cy3 (1:200, 711-165-152, Jackson Immunoresearch), Donkey anti-goat Ig bitotin (1:200, 705-065-147 Jackson Immunoresearch), Donkey anti-goat Alexa Fluor 488 (1:200, 705-545-147 Jackson Immunoresearch), Donkey anti-goat Alexa Fluor 568 (1:200, A11057 Invitrogen), Donkey anti-mouse Cy3 (1:200, 715-165-150 Jackson Immunoresearch), Donkey anti-mouse Alexa Fluor 488 (1:200, 715-545-150 Jackson Immunoresearch), Donkey anti-rat Cy3 (1:200, 712-165-150 Jackson Immunoresearch). BrdU-positive cells were quantified on an Olympus FluoView 1000 filter-based laser scanning confocal microscope by a blinded experimenter. Pseduo-3D image was generated by compiling a z-stack of 40 0.5 micrometer slices using the Olympus FluoView 1000 software.

Open Field: Open field was performed essentially as previously described (Eagle AL et al., 2015). 64 mice underwent 60 minutes of habituation and were placed into an empty arena for one hour under red light conditions. Activity was recorded with a digital CCD camera connected to a computer running automated video tracking software package (Clever Sys). Time spent within the center of the box (the center starts approximately 9.5cm from the edge of the wall) and distance moved were measured as anxiety-like and locomotor behaviors.

Elevated Plus Maze (EPM): EPM was performed essentially as previously described (Eagle AL et al., 2015). In brief, 64 mice underwent 60 minutes of habituation, were placed in the center of the maze, and allowed to roam for 5 minutes under red light conditions. The amount of time spent in the open arms was assessed as a measure of anxiety. Mice that fell from the arena were not excluded from analysis.

Novel Object Recognition (NOR): NOR was assessed using a 3-day paradigm as described previously (Eagle AL et al., 2015). Briefly, 64 mice were habituated for 60 minutes under red light every day, and then placed into the open-field (OF) apparatus for one hour (Day 1). 24 hours later, mice were exposed to two identical objects placed in opposite corners of the open-field (OF) box and allowed to explore the apparatus for 30 min (Day 2). Twenty-four hours later, mice were tested for NOR. One object was removed and replaced with a dissimilar object, and mice were allowed to freely explore the apparatus for 5 min (Day 3).

Statistics: Analyses of one independent variable were performed using PRISM 8.0 (GraphPad Software) using fixed-effect models and treating all samples as independent. Assumptions of normality and equal variance were tested using a D'Agostino & Pearson test and variance ratio test, respectively. If the dependent variable met these criteria, comparisons were tested using unpaired student's t-tests, or were otherwise tested with the nonparametric Mann-Whitney U test. Analyses of two independent variables, including Immunohistochemistry quantifications and NOR data, were analyzed with Levene's and Shapiro-Wilks tests for homogeneity of variance and normality, respectively, in in SPSS 25.0, followed by 2×2 factorial ANOVA (IHCs) or Repeated Measures 2×2 ANOVA (NOR) if appropriate; otherwise, an extension of the nonparametric Kruskal-Wallis test was applied. In cases of interaction between variables, multiple comparisons were tested using Sidak's post-hoc tests. A cutoff of alpha=0.05 was used in all analyses.

Results:

NtsR2-Cre;GFP mice identify cells in the dentate gyrus subgranular zone

Previous reports indicate that NtsR2 is sparsely expressed in adult mouse brain, and this expression is predominantly in glia (Mazella J et al., 1996;Nouel D et al., 1999;Sarret P et al., 1998;Woodworth HL et al., 2018). Additionally, adult NtsR2$^{Cre/+,GFP}$ mice exhibit robust GFP-expression in cells of the SGZ of the DG and in the pyramidal layer of CA3 in the adult dHPC (Fig 1). However,the NtsR$^{Cre/+,GFP}$ transgenic line permanently expresses GFP independent of current NtsR2 expression and NtsR2 expression is thought to be phasic in new cells, peaking in P5–P15 (Lepee-Lorgeoux I et al., 1999). As the SGZ is a region enriched in neuroprogenitor cells, we sought to determine the developmental status of the GFP-positive cells. We performed immunohistochemistry for NeuN and Doublecortin

(DCX), markers of mature and immature neurons, respectively in two animals (Fig 1A and B). We noted that in the CA3 pyramidal neurons, there was extensive colabeling with NeuN but not DCX, indicating that many of these GFP-positive cells were mature neurons. However, in the SGZ, there was significant overlap between DCX- and GFP-positive cells (Fig 1B), indicating that $NtsR2^{Cre/+,GFP}$ expresses in neuroprogenitor cells in this region.

To determine whether these NtsR2-GFP labeled cells of the SGZ are indeed neuroprogenitor cells undergoing division, we used BrdU labeling to mark newly divided cells. We injected adult male $NtsR2^{Cre/+,GFP}$ mice with 50mg/kg BrdU daily for five days, during which they had *ad libitum* access to a running wheel, as wheel running promotes hippocampal neurogenesis in rodents (van Praag H et al., 1999). Subsequent immunohistochemistry revealed distinct BrdU-staining in nuclei of some NtsR2-GFP cells of the SGZ (Fig 2A), indicating that some of the $NtsR2^{Cre/+,GFP}$ cells had undergone mitosis in the previous five days. Additional immunostaining revealed that ΔFosB was present in some $NtsR2^{Cre/+,GFP}$ cells. However, $NtsR2^{Cre/+,GFP};FosB^{lox/lox}$ mice did not exhibit ΔFosB immunoreactivity in GFP-positive SGZ cells (Fig 2B). Taken collectively, these data indicate that $NtsR2^{Cre/+,GFP}$ marks multiple cell types in the dHPC, including newly dividing cells in the SGZ, and that some of these cells express *FosB* gene products that can be knocked out using our Cre/lox approach.

FosB in the SGZ is critical for induced neurogenesis

It has been previously suggested that the *FosB* gene is critical for adult hippocampal neurogenesis, as germline *FosB* knockout mice show reduced BrdU staining in response to kainic acid (Yutsudo N et al., 2013). Moreover, multiple antidepressants induce hippocampal neurogenesis, and *FosB* gene products are critical for the behavioral effects of antidepressants like fluoxetine (Robison AJ et al., 2014;Vialou V et al., 2010). To examine if *FosB* gene expression in the hippocampal SGZ is necessary for fluoxetine-induced hippocampal neurogenesis, we studied $NtsR2^{Cre/+,GFP}$ mice crossed onto the $FosB^{lox/lox}$ line, providing developmental *FosB* knockout in a subset of hippocampal cells including the SGZ. 4 $NtsR2^{Cre/+,GFP};FosB^{lox/lox}$ mice (referred to as Cre-positive) and 5 littermates lacking Cre (referred to as WT) were injected i.p with fluoxetine (2mg/kg) daily and BrdU (50mg/kg) every 3rd day for 18 days to both induce neurogenesis and mark dividing cells, respectively (Fig 3A). Mice were sacrificed 24 hours after the last injection (Fig 3A) and brains were then immunolabeled for BrdU and DCX, (Fig 3B and C). Cre-positive animals showed reduced BrdU staining compared to WT animals (Fig 3D), with main effect of genotype on BrdU labeled cells and dorsoventral axis of the brain (dorsal vs ventral HPC) ($H_{(1)}$=4.218; p=0.039 and $H_{(1)}$=7.25; p=0.007, respectively), without interaction between the two variables ($H_{(1)}$=0.206; p=0.649). DCX staining was significantly reduced in the vHPC compared to dHPC (Fig 3E). We found a main effect of the dorsoventral axis, but not genotype ($F_{(1,13)}$=29.64, p=0.0001 and $F_{(1,13)}$=2.832, p=0.1162, respectively), with a trend for an interaction between the genotype and dorsoventral axis ($F_{(1,13)}$=4.634, p=0.0507). These indicate that mitotic division, but not differentiation into a neuronal lineage, are reduced with *FosB* SGZ knockout, and that these effects are exaggerated in the dHPC.

FosB KO in the SGZ does not alter basal anxiety behaviors

Enhanced neurogenesis is thought to be one of the mechanisms behind antidepressant drug effects, and neurogenesis has been linked to abnormalities in anxiety behaviors (Hill AS et al., 2015;Revest JM et al., 2009;Vialou V et al., 2015). Therefore, 30 adult NtsR2$^{Cre/+,GFP}$;FosB$^{lox/lox}$ mice and 35 NtsR2$^{+/+,GFP}$;FosB$^{lox/lox}$ littermates were tested in both the open-field and the elevated plus maze (EPM). Despite the developmental knockout, there were no differences between these two genotypes in the percent of total time spent in the center of the open field arena, nor the percent of center entries (Fig 4A and B; group medians of 13.05 and 12.13 Mann Whitney U=503.5, p=0.93 and $t_{(62)}$=1.186, p=0.24). In EPM, there was no difference in percentage of open arm time or percentage of open arm entries between genotypes (Fig 4C and D; $t_{(52)}$=0.719, p=0.475 and $t_{(52)}$=0.344, p=.732). Taken together, these data suggest that *FosB* SGZ knockout causes no changes in anxiety-like behavior.

FosB SGZ Knockout impairs learning

Reductions in neurogenesis have been linked to abnormalities in hippocampus-dependent learning (Akers KG et al., 2014;Deng W et al., 2010;Epp JR et al., 2016;Niibori Y et al., 2012;Zhao C et al., 2008). Therefore, 29 adult NtsR2$^{Cre/+,GFP}$;FosB$^{lox/lox}$ mice and 35 wild-type littermates also underwent novel object recognition (NOR; Fig 5A) to test for deficits in a hippocampal-dependent task. During the acclimatization phase, Cre-positive mice and wild-type littermates showed no difference in locomotor behavior (Fig 5B; $t_{(62)}$=0.96, p=0.342). As long term memory is dependent upon protein synthesis (Kogan JH et al., 2000), and *FosB* gene products are transcription factors, a 24 hour timepoint was chosen to test long-term memory as opposed to a more immediate timepoint which results from non-genomic actions (Goelet P et al., 1986). 24 hours after familiarization with two identical objects, wildtype mice spent significantly longer interacting with a novel object (Fig 5C; $F_{(1,60)}$=9.086, p=0.0038, followed by Sidak *post hoc* test) compared to Cre-positive mice, with main effects of Cre decreasing total exploration time ($F_{(1,60)}$=8.363, p=0.0053) and time around novel objects ($F_{(1,60)}$=5.908, p=0.0181). Thus, *FosB* SGZ knockout induces a deficit in hippocampus-dependent learning.

Discussion:

Here we crossed NtsR2$^{Cre/+,GFP}$ mice onto the FosB$^{lox/lox}$ background to investigate the role of *FosB* in hippocampal SGZ cells. NtsR2 labeling has previously indicated glia and has not been explicitly characterized on neurons throughout the adult brain (Lepee-Lorgeoux I et al., 1999;Woodworth HL et al., 2018). Nevertheless, NtsR2 has reported in the dentate gyrus of both rodents (Lepee-Lorgeoux I et al., 1999) and primates (Kohler C et al., 1987). As the scope of this study was to explore subregion specific effects on learning and memory, the NtsR$^{Cre/+,GFP}$ line allowed us to limit Cre-mediated deletion of FosB to a discrete cell population in the hippocampus to investigate how the *FosB* gene affected both neurogenesis and learning.

Previous studies of hippocampal FosB could not address neither subregion nor cell type specificity, as they employed either germline whole body knockouts, local viral effects, or

correlations (Kurushima H et al., 2005;Niu H et al., 2018;Yutsudo N et al., 2013). Here we show that specifically knocking out *FosB* in a subset of DG and CA3 cells overlapping strongly with the SGZ caused both reduced cellular proliferation in the SGZ and learning deficits, without altering basal anxiety or locomotor behavior. Changes in neurogenesis have been causally linked to stress-induced behaviors and antidepressant efficacy (Anacker C et al., 2018; Hill AS et al., 2015; Santarelli L et al., 2003), while fluoxetine increases memory retrieval and cognitive flexibility processes in hippocampal dependent tasks such as the T-maze, NOR, and the Barnes maze, but not memory acquisition (Flood JF and Cherkin A, 1987;Marwari S and Dawe GS, 2018; Yi JH et al., 2018). Importantly fluoxetine induces neurogenesis concurrently with increased long-term memory performance (Ibi D et al., 2008; Marwari S and Dawe GS, 2018). Our studies suggest that *FosB* gene products may be a molecular mechanism that underlies these two effects. This is intriguing as standard treatment with SSRIs requires several weeks for efficacy, pointing to long-term changes in the brain mediating antidepressant responses as opposed to acute actions at serotonin receptors. Although the timeline for antidepressant response is consistent with the timeline for neuronal maturation (Lucassen PJ et al., 2010;Zhao C,Deng W and Gage FH, 2008), the specific molecular mechanisms which underlie neuronal division, survival, and maturation are incompletely understood.

Previous work has highlighted that FosB's long half-life contributes to prolonged effects of gene expression (Carle TL et al., 2007;Ulery-Reynolds PG et al., 2009), and indeed its accumulation in the NAc is critical for resilience to stress and the antidepressant effects of fluoxetine (Robison AJ et al., 2014;Vialou V et al., 2010). In addition, FosB is also induced in the dHPC after stress and several antidepressant treatment regimens including fluoxetine, ketamine, and exercise, and it is necessary in the ventral CA3 for the prophylactic effects of the non-traditional antidepressant ketamine (Mastrodonato A et al., 2018;Nishijima T et al., 2013;Perrotti LI et al., 2004;Vialou V et al., 2015). Our finding that knocking out *FosB* in a subset of SGZ cells reduced cell proliferation is concordant with the idea that hippocampal *FosB* gene products, including FosB, support fluoxetine-induced neurogenesis and perhaps adaptive responses to stress.

Hippocampal FosB is also linked to preclinical models of comorbid diseases which have cognitive associated deficits, like Alzheimer's disease and epilepsy. Germline *FosB* KO mice have depressive phenotypes and spontaneous seizures (Yutsudo N et al.,2013). In addition, FosB accumulates in the dHPC in response to natural seizures, kainic acid, and pilocarpine models of epilepsy, as well as in Alzheimer's disease mouse models and patients (Cho KO et al., 2015;Corbett BF et al., 2017;Yutsudo N et al., 2013). This accumulation could be neuroprotective, but general FosB viral overexpression in the dHPC results in impaired cognition, while viral inhibition of FosB impairs cognition in wild type mice but prevents cognitive decline in an Alzheimer's model (Eagle AL et al., 2015;You JC et al., 2017). Coupled with our finding that SGZ *FosB* is necessary for normal cognition, this suggests that cell-specific hippocampal FosB is necessary for normal cognition, but aberrant or non-specific FosB may result in cognitive deficits.

The importance of appropriate expression of FosB in normal behavior is reinforced by its unique molecular characteristics. FosB accumulation in the dHPC results in a number of

downstream changes in gene expression, including epigenetic alterations at target genes. Of note is histone deacetylation at the *Calb1* gene, a calcium binding protein and marker of mature neurons (Corbett BF et al., 2017;LaGamma CT et al., 2018;You JC et al., 2017;You JC et al., 2018), whose regulation by FosB may be critical for the altered neurogenesis we report in *FosB* SGZ knockout. Alternatively, FosB may directly regulate microglia through C5aR1 and C5aR2 (Nomaru H et al., 2014), and this in turn may elicit the altered neurogenesis we report in *FosB SGZ* knockout (Rivera PD et al., 2018). Thus, future studies will focus on identifying downstream transcriptional targets of FosB in both glia and neurons to provide insight into mechanisms of hippocampal neurogenesis and potentially uncover novel therapeutic targets for treatment of diseases in which neurogenesis may play a role, such as depression or Alzheimer's Disease.

Acknowledgements:

Abbreviations:

BrdU	Bromodeoxyuridine / 5-bromo-2'-deoxyuridine
DCX	Doublecortin
DG	Dentate Gyrus
EPM	Elevated Plus Maze
GFP	Green Florescent Protein
HPC	Hippocampus
IEG	Immediate Early Gene
NAc	Nucleus Accumbens
NeuN	Neuronal Nuclei
NOR	Novel Object Recognition
NtsR2	Neurotensin Receptor 2
SGZ	Subgranular Zone

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HIGHLIGHTS:

- The NtsR2 Cre mouse is a useful tool for manipulating cells of the hippocampal SGZ.

- The transcription factor FosB is robustly expressed in NtsR2-positive SGZ cells.

- SGZ knockout of the *FosB* gene reduces hippocampal cell proliferation.

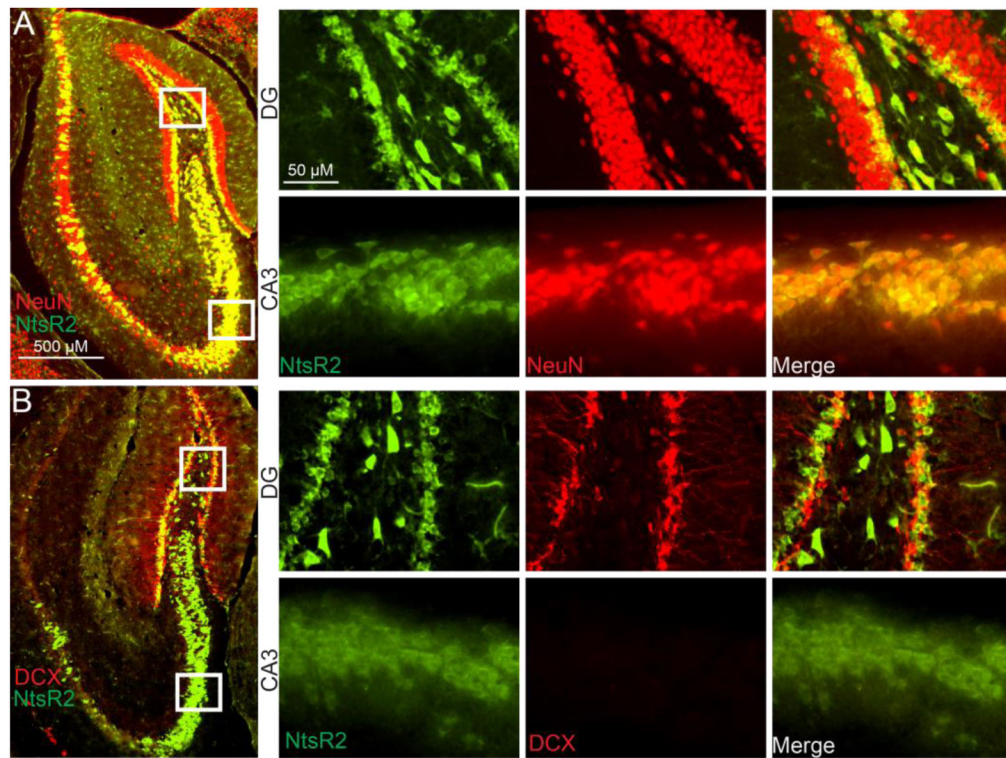- SGZ knockout of the *FosB* gene impedes hippocampus-dependent learning.

Figure 1: NtsR2-Cre expresses in the DG and CA3 of the dHPC.
A: 4x and 40x images showing that NtsR2-positive cells colocalize with NeuN in DG and CA3. **B:** 4x and 40x images showing that NtsR2-positive cells colocalize with doublecortin (DCX) in the DG, but this staining is absent in the CA3.
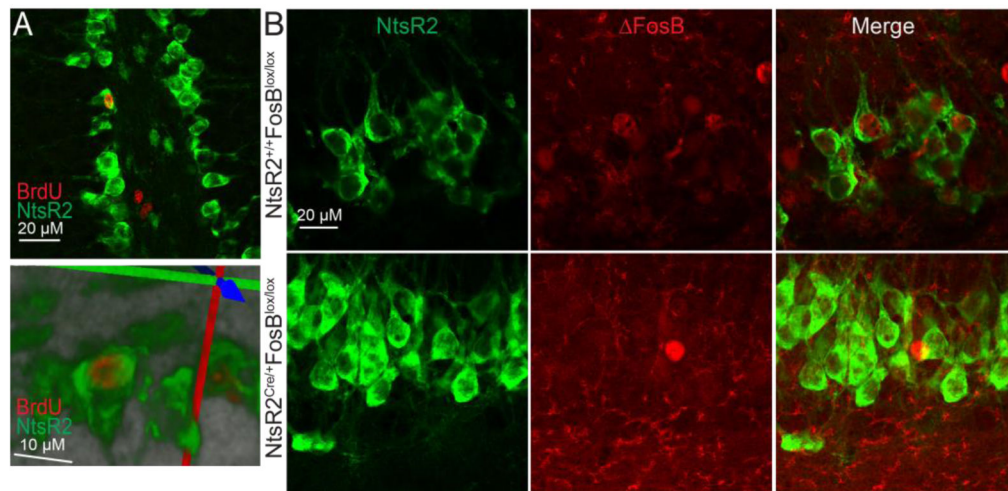
Figure 2: NtsR2-Cre expresses in newly-dividing SGZ progenitor cells, some of which express *FosB*.
A: Some NtsR2-Cre cells are stained with BrdU in the SGZ of the DG (100x, top), and this is confirmed by 3D reconstruction from confocal imaging (bottom). **B**: NtsR2-Cre cells also express with FosB in the SGZ in WT animals. This staining is absent in floxed *FosB* animals.
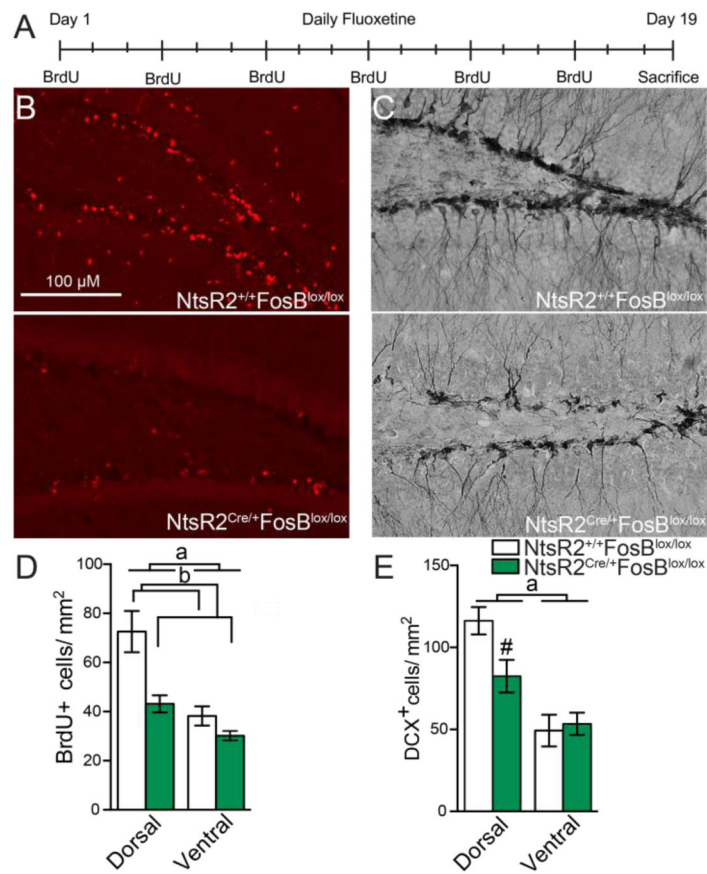
Figure 3: Genetic Knockout of FosB in SGZ reduces neurogenesis.
A: Timeline of experiment. Representative images of BrdU **(B)** and doublecortin **(C)** staining in the dorsal HPC of WT or Cre-positive animals after 18 days of daily fluoxetine. **D** and **E**: Cre-positive animals have significantly fewer BrdU ((a):p=0.039 for effect of Cre; (b):p=0.007 for dHPC vs vHPC) with no effect on DCX positive cells ((#):p=0.0507 for interaction between genotype and dorsoventral axis; (a):p=0.0001 for dHPC vs vHPC).
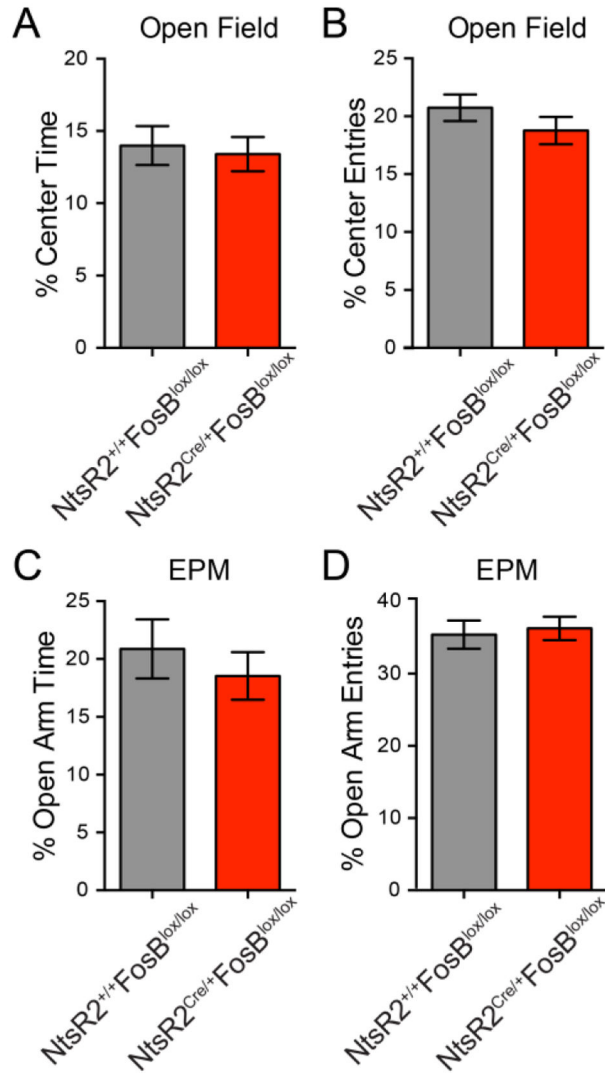
Figure 4: Genetic knockout of FosB in SGZ does not alter basal anxiety behaviors
FosB SGZ knockout caused differences in the percentage of total time spent **(A)**, nor in the percentage of entries **(B)** into the center of the open field (p=0.93 and p=0.24, respectively). There were also no differences in the percentage of total time spent **(C)** or percentage of entries **(D)** into the open arms of the elevated plus maze (p=0.475 and p=0.732, respectively).
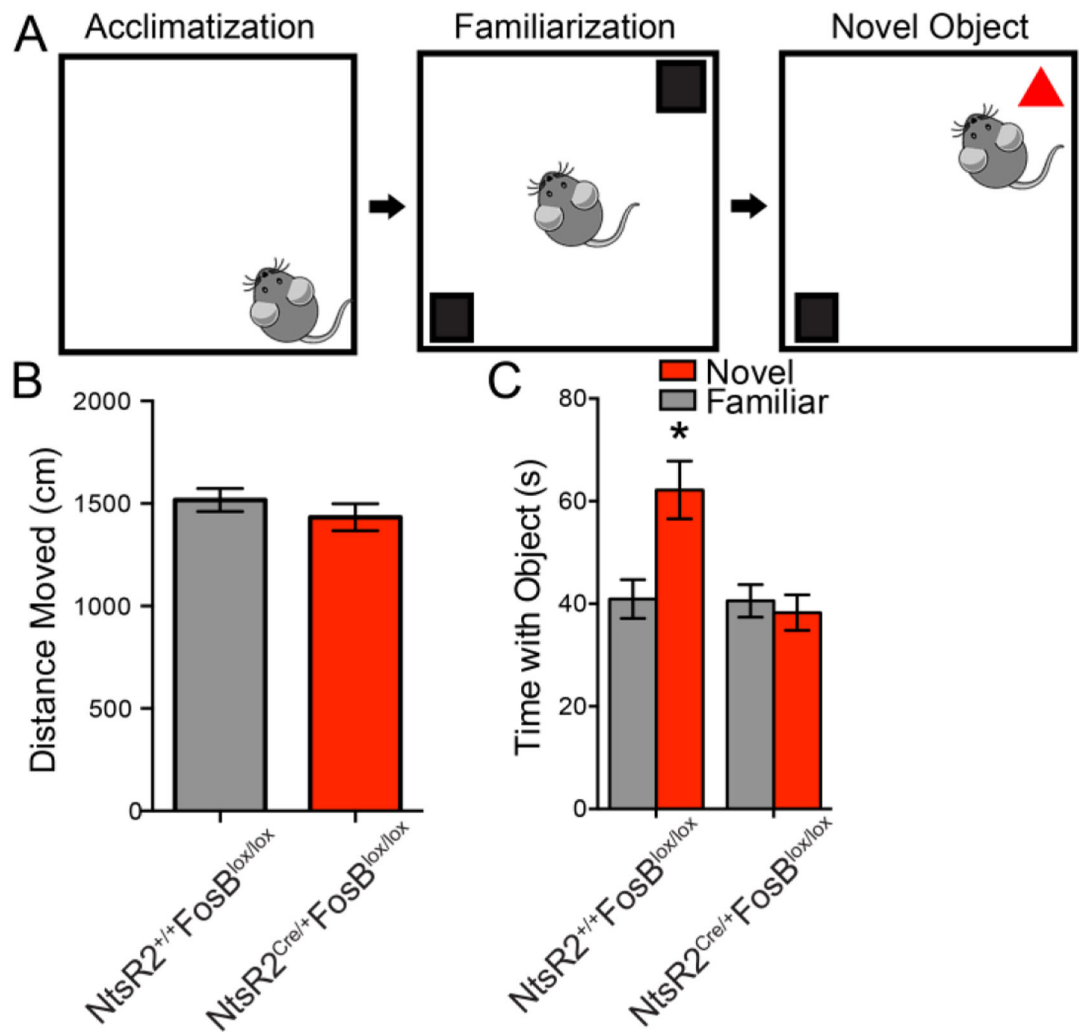
Figure 5: Genetic knockout of FosB in SGZ reduces hippocampus-dependent memory.
A: Schematic of the NOR task. **B:** Cre-positive and WT littermates show no differences in locomotor behaviors in the acclimatization phase. **C:** Cre-positive mice display reduced time spent with a novel object compared to WT littermates (*:p=0.0038).