Epigenetic suppression of hippocampal calbindin-D28k by ΔFosB drives seizure-related cognitive deficits

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The calcium-binding protein calbindin-D28k is critical for hippocampal function and cognition1–3, but its expression is markedly decreased in various neurological disorders associated with epileptiform activity and seizures4–7. In Alzheimer’s disease (AD) and epilepsy, both of which are accompanied by recurrent seizures8, the severity of cognitive deficits reflects the degree of calbindin reduction in the hippocampal dentate gyrus (DG)4,9,10. However, despite the importance of calbindin in both neuronal physiology and pathology, the regulatory mechanisms that control its expression in the hippocampus are poorly understood. Here we report an epigenetic mechanism through which seizures chronically suppress hippocampal calbindin expression and impair cognition. We demonstrate that ΔFosB, a highly stable transcription factor, is induced in the hippocampus in mouse models of AD and seizures, in which it binds and triggers histone deacetylation at the promoter of the calbindin gene (Calb1) and downregulates Calb1 transcription. Notably, increasing DG calbindin levels, either by direct virus-mediated expression or inhibition of ΔFosB signaling, improves spatial memory in a mouse model of AD. Moreover, levels of ΔFosB and calbindin expression are inversely related in the DG of individuals with temporal lobe epilepsy (TLE) or AD and correlate with performance on the Mini-Mental State Examination (MMSE). We propose that chronic suppression of calbindin by ΔFosB is one mechanism through which intermittent seizures drive persistent cognitive deficits in conditions accompanied by recurrent seizures.

Expression of calbindin-D28k in the hippocampal DG is indicative of cognitive function in individuals with AD or epilepsy, as well as mouse models of these conditions4,9–12. In addition, calbindin-knockdown and calbindin-knockout mice exhibit impaired synaptic plasticity and spatial memory1,3,13,14. These findings highlight the role of calbindin as a critical regulator of neuronal calcium signaling and hippocampal function15. However, little is known about the regulatory mechanisms that modulate calbindin expression in normal or pathologic conditions. Considering how crucial calbindin is for synaptic function and cognition1,3,13,14, elucidating these mechanisms is essential, as it may aid the development of new therapeutics to ameliorate cognitive deficits in AD and other disorders associated with seizures.

To identify mechanisms that control hippocampal calbindin-D28k expression, we examined long-term gene regulation in the hippocampus of a transgenic AD mouse model expressing mutant human amyloid precursor protein (APP)16. Mice expressing mutant APP (APP mice) of both sexes were examined at 2–4 months of age, which is when they begin to exhibit spontaneous recurrent seizures and cognitive deficits similar to those in individuals with AD, but before plaque deposition occurs16–18. Through immunohistochemical analysis, we found that hippocampal calbindin expression was lower in APP mice than in nontransgenic (NTG) littermates and that levels of calbindin expression inversely correlated with the frequency of electroencephalographic (EEG) seizures (Fig. 1a,b), similar to what is observed in individuals who experience seizures5,10. Notably, even APP mice with relatively infrequent seizures exhibited reduced calbindin expression, suggesting that downregulation of hippocampal calbindin is mediated by long-lasting, activity-dependent mechanisms. After surveying the literature to determine which activity-dependent factors might regulate gene expression over extended periods of time, we focused on ΔFosB, a truncated splice variant of the transcription factor FosB. ΔFosB is a unique activity-dependent immediate early gene (IEG) product, possessing an unusually long half-life (~8 d) that allows it to exert persistent control over neuronal gene expression20. The actions of ΔFosB in epigenetic gene regulation are well studied in the nucleus accumbens20,21, and a recent study suggests that it may also have functions in the hippocampus22. We found that seizures were associated with elevated hippocampal expression of ΔFosB, and higher levels of ΔFosB expression correlated with impaired cognitive performance on the Mini-Mental State Examination (MMSE). We propose that chronic suppression of hippocampal calbindin expression by ΔFosB, through epigenetic mechanisms, contributes to the cognitive deficits seen in AD and other neurological disorders associated with seizures.

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ΔFosB expression were correlated with lower calbindin expression in APP mice (Fig. 1b,c). Therefore, we hypothesized that ΔFosB is a key regulatory factor involved in the suppression of calbindin following epileptiform activity in AD and other seizure-associated disorders.

To test this hypothesis, we performed a promoter analysis of Calb1 to identify potential regulatory regions where ΔFosB might bind. Our analysis revealed a region 320 bp upstream of the transcription start site containing cyclic AMP (cAMP) and 12-O-tetradecanoylphorbol-13-acetate (TPA) response elements (CRE and TRE, respectively) in close proximity to one another (Supplementary Fig. 1a). As both elements are known to recruit proteins encoded by IEGs, we performed chromatin immunoprecipitation (ChIP) on hippocampal tissue from APP as well as NTG mice to test whether ΔFosB enrichment occurred at this promoter region. Indeed, ChIP results confirmed binding of ΔFosB to the promoter of Calb1, and ΔFosB enrichment at this site was greater in APP mice than in NTG littermates (Fig. 1d). ΔFosB enrichment on Calb1 was validated by comparison to IgG control ChIP and promoter amplicon sequencing (Supplementary Fig. 1b,c). These data highlight the potential ability of ΔFosB to regulate calbindin expression via actions at the Calb1 promoter.

We therefore assessed whether ΔFosB binding to Calb1 was associated with chromatin modifications that would impact Calb1 expression. Previous studies have demonstrated that reduction of calbindin protein levels in individuals with AD or epilepsy, as well as in corresponding rodent models occurs downstream of mRNA reduction.ΔFosB binds histone...
deacetylase 1 (HDAC1)\(^{21}\), suggesting that it suppresses \textit{Calb1} transcription via histone deacylation. Indeed, we found hypoacylated histone H4 lysine residues on the \textit{Calb1} promoter in APP mice relative to NTG controls (Fig. 1e and Supplementary Fig. 2a,b). We did not detect changes in the acetylation of histone H3. Because long-term histone hypoacylation can trigger histone methylation to further suppress gene expression\(^{21,25}\), we also assessed histone methylation at the \textit{Calb1} promoter. We found that the \textit{Calb1} promoter was hypermethylated in APP mice at histone H4 lysine 20 but not at histone H3 lysine 9 (Fig. 1f and Supplementary Fig. 3a,b). Moreover, DG granule cells in pilocarpine-treated mice exhibited higher \textit{ΔFosB} and lower calbindin protein expression that was evident on a cell-by-cell basis (Fig. 1i). The inverse relationship between \textit{ΔFosB} and calbindin was also evident on a cell-by-cell basis in APP mice (Fig. 1j).

To demonstrate that reduction in calbindin expression can be a direct result of \textit{ΔFosB}-mediated epigenetic regulation, we tested whether adeno-associated virus (AAV)-mediated overexpression of \textit{ΔFosB} in the hippocampus of wild-type C57BL/6 mice (AAV-ΔFosB mice) could suppress calbindin expression. Virus carrying cytomegalovirus (CMV) promoter–driven \textit{ΔFosB} and enhanced GFP (eGFP) or eGFP alone was stereotaxically infused into the DG of wild-type C57BL/6 mice (Online Methods). This infusion resulted in robust \textit{ΔFosB} and eGFP expression throughout the rostral–caudal extent of the DG (Fig. 2a,b). Moreover, DG granule cells in pilocarpine-treated mice exhibited higher \textit{ΔFosB} and lower calbindin protein expression that was evident on a cell-by-cell basis (Fig. 1i). The inverse relationship between \textit{ΔFosB} and calbindin was also evident on a cell-by-cell basis in APP mice (Fig. 1j).

Figure 2 ΔFosB mediates transcriptional repression of \textit{Calb1} expression and causes spatial memory deficits. (a, b) Representative images (a) and quantification (b) of \textit{α-FosB} IR in the DG of wild-type mice that received bilateral hippocampal infusion of AAV encoding either \textit{ΔFosB} and eGFP (AAV-ΔFosB) or eGFP alone (AAV-eGFP) \((n = 10)\) and \(8\) eGFP mice and \(8\) ΔFosB mice, \(t_{16} = 8.23, ***P = 3.83 \times 10^{-7}\). eGFP expression is also displayed and can be observed in the cell bodies (GCL), axons (hilus), and dendrites (ML) of granule cells from AAV-eGFP mice. Scale bars, 250 μm and 50 μm (inset). (c, d) Binding of \textit{ΔFosB} \((n = 7)\) per treatment, \(t_{12} = 2.36, *P = 0.036\) (e) and histone H4 lysine acetylation \((n = 6)\) in eGFP mice and 8 ΔFosB mice, \(t_{12} = -2.21, *P = 0.047\) (d) on \textit{Calb1} in AAV-eGFP and AAV-ΔFosB mice. (e, f) Representative images (e) and quantification (f) of in situ hybridization of \textit{Calb1} mRNA \((n = 5)\) per treatment, \(t_{6} = -5.94, ***P = 3.44 \times 10^{-4}\). Scale bar, 250 μm. (g, h) Representative images (g) and quantification (h) of calbindin protein IR \((n = 10)\) in eGFP mice and 8 ΔFosB mice, \(t_{10} = -4.46, ***P = 4 \times 10^{-4}\) in DG granule cells of AAV-eGFP and AAV-ΔFosB mice. In g, arrows indicate ΔFosB-expressing granule cells, and arrowheads indicate calbindin-expressing granule cells. Scale bar, 50 μm. (i) LEFT, object-location test procedure. Right, performance of AAV-eGFP and AAV-ΔFosB mice in the object-location test (paired \(t\)-tests: AAV-eGFP, \(n = 10\) mice, \(t_{9} = 4.6, **P = 0.0013\); AAV-ΔFosB, \(n = 8\) mice, \(t_{7} = 1.24, P = 0.25\)). Data in a–h were analyzed using Student’s \(t\)-tests. Data are presented as mean ± s.e.m., with each point representing data from an individual.
that overexpression of ΔFosB in the hippocampus was sufficient to impair spatial memory (Fig. 2i).

To demonstrate that ΔFosB is not only sufficient but also necessary for regulating hippocampal calbindin expression and memory function, we tested whether inhibition of ΔFosB signaling in APP mice could normalize calbindin expression and ameliorate spatial memory deficits. To block ΔFosB signaling, we infused AAV carrying ΔJunD and eGFP into the DG of APP mice. ΔJunD is a truncated mutant of the transcription factor JunD, a predominant endogenous binding partner of ΔFosB in the brain. The truncation removes the transactivation domain of JunD while leaving its dimerization and DNA-binding domains intact. Therefore, dimerization of ΔJunD with ΔFosB inhibits ΔFosB in a dominant-negative fashion. Notably, consistent with its truncation, ΔJunD does not preclude ΔFosB from binding its gene targets, but instead prevents the interaction of ΔFosB with downstream transcriptional co-regulators at target gene promoters. Expression of ΔJunD in different brain regions effectively antagonizes ΔFosB-mediated gene regulation. Using the
Data are presented as mean ± s.e.m., with each data point representing data from an individual. Regression analyses of ΔFosB and calbindin IR in surgically resected DG from individuals with TLE (Fig. 3g,h). Regression analyses of ΔFosB IR (g) and calbindin IR (h) and MMSE performance in individuals with AD. (i,j) Representative images (i) and regression analysis (j) of ΔFosB and calbindin IR in surgically resected DG from individuals with TLE (%). The number of individuals with TLE was n = 8. Scale bar, 100 μm. AU, arbitrary units. Additional information regarding controls and individuals with MCI, AD, and TLE is provided in Supplementary Figure 9. Data are presented as mean ± s.e.m., with each data point representing data from an individual.

Figure 4 Increased hippocampal ΔFosB expression corresponds with decreased calbindin expression in human individuals diagnosed with MCI, AD, or TLE. (a–d) Images (a), quantification (b,c), and regression analysis (d) of ΔFosB and calbindin IR in DG of postmortem control individuals (CTL; n = 6) and individuals with MCI (n = 10) and AD (n = 10). ΔFosB one-way ANOVA: F(2,23) = 6.893, P = 0.0045; Tukey’s HSD: CTL versus MCI, *P = 0.0153, CTL versus AD, **P = 0.0045; calbindin one-way ANOVA: F(2,23) = 4.461, P = 0.023; Tukey’s HSD: CTL versus MCI, *P = 0.045; CTL versus AD, *P = 0.026). In a, numbers in parentheses represent MMSE scores. Scale bar, 100 μm. (e,f) Regression analyses of ΔFosB IR (e) and calbindin IR (f) and MMSE performance in individuals with MCI. (g,h) Regression analyses of ΔFosB IR and calbindin IR in DG of postmortem control individuals (CTL; n = 10) and AD (Fig. 3a). We also demonstrate that ΔFosB is required for epigenetic suppression of Calb1 in APP mice, as ΔJunD expression restored Calb1 promoter histone acetylation (Fig. 3b and Supplementary Fig. 3c) and improved calbindin protein expression in the DG (Fig. 3c,d). Considering that ΔJunD-mediated amelioration of spatial memory in APP mice was accompanied by improved calbindin expression, we next asked whether direct restoration of hippocampal calbindin expression in APP mice could also improve memory. To bypass endogenous control of Calb1 by ΔFosB, we infused AAV carrying CMV promoter–driven calbindin and eGFP into the hippocampus to elevate calbindin expression in APP mice (Fig. 3e,f). We found that directly elevating hippocampal expression of calbindin in APP mice improved object location memory (Fig. 3g), similar to what occurred with hippocampal ΔJunD expression.

Our findings in APP mice suggest that regulation of calbindin by ΔFosB may be a potential therapeutic target in AD and other seizure-associated disorders. To determine whether the relationship between ΔFosB and calbindin also exists in humans, we examined expression patterns of both proteins in the DG of individuals diagnosed with mild cognitive impairment (MCI) or AD. Indeed, higher ΔFosB expression corresponded with lower calbindin expression in individuals with MCI or AD (Fig. 4a–d), similar to what was observed in APP mice. In addition, we found a correlation between the levels of both proteins and MMSE scores in individuals with MCI but not in those with AD, presumably because of the numerous deficits in AD relative to MCI (Fig. 4e–h). There was also a significant correlation between ΔFosB or calbindin expression and MMSE scores when all individuals (controls and those with MCI or AD) were analyzed together (Supplementary Figs. 8 and 9). Lastly, to determine whether the relationship between ΔFosB and calbindin expression also exists in non-AD-related conditions in which recurrent seizures occur, we examined DG tissue resected from individuals with TLE. In accordance with the findings in individuals with MCI or AD, we observed an inverse relationship between ΔFosB and calbindin expression in individuals with TLE (Fig. 4i,j).
In summary, we have discovered a new regulatory mechanism through which epileptiform activity recruits ΔFosB to epigenetically suppress calbindin expression in the DG. In doing so, we highlight the role of ΔFosB as a transcription factor capable of chronically suppressing a gene that regulates synaptic transmission, plasticity, cognition, and seizure-related pathology. Considering the prevalence of seizures across various disorders such as autism and schizophrenia, in addition to epilepsy and AD, the identification of the role of ΔFosB in the hippocampus may have broad implications for understanding why cognitive deficits are comorbidities of many disorders that are accompanied by seizures. Furthermore, nuclear accumulation of ΔFosB due to its unusually long half-life provides a mechanism to explain why cognitive deficits persist even during seizure-free periods. Our finding that elevated ΔFosB expression correlates with reduced calbindin expression in both individuals with AD and those with TLE suggests that humans and rodent models of human disease have similar mechanisms of epigenetic regulation induced by seizures. By restoring memory function in APP mouse models of human disease, we have shown to miss ‘silent’ hippocampal seizures detected via foramen ovale electrodes in individuals with AD. Finally, some evidence suggests targeting either protein should be initiated as early as possible to maximize effectiveness. The association of MMSE scores with ΔFosB and calbindin expression correlate with MMSE scores in individuals with MCI but not in those with AD suggests that possible therapeutic interventions targeting either protein should be initiated as early as possible to maximize effectiveness. The association of MMSE scores with ΔFosB and calbindin in individuals with MCI might be explained by the fact that hippocampal dysfunction, to which alterations in ΔFosB and calbindin expression greatly contribute, is particularly prominent in early disease. However, as AD progresses, disease pathophysiology in additional brain areas also contributes to overall cognitive dysfunction. The results of our study are consistent with a growing body of evidence showing that seizures occurring in early stages of AD impair memory and exacerbate cognitive decline. The prevalence of seizures in AD may also be much greater than previously thought, as routine scalp EEG has been shown to miss ‘silent’ hippocampal seizures detected via foramen ovale electrodes in individuals with AD. Finally, some evidence suggests that calbindin reduction in granule cells following epileptiform activity may confer neuroprotection against excitotoxicity (but also see refs. 37, 38). Demonstration of calbindin suppression by ΔFosB may provide further support for a neuroprotective role of calbindin reduction in granule cells, as previous studies have shown that both ΔFosB and HDAC1 can protect neurons from neurotoxic injury. Notably, however, we did not find obvious neuronal loss when we blocked ΔFosB signaling or virally expressed calbindin in APP mice (Fig. 3d,f), which may be related to the time frame of our experiments or the possible existence of redundant neuroprotective mechanisms. Regardless, a potential tradeoff between neuroprotection and synaptic function may emphasize the need for caution with therapeutics targeting either pathway, as efforts to augment one pathway may generate side effects by simultaneously altering the other. To validate the therapeutic potential of targeting ΔFosB and/or calbindin, future studies will need to further characterize their functions in the hippocampus.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

J.C.Y. and J.C. conceived the project. J.C.Y., X.Z., E.J.N., H.E.S., and J.C. designed the experiments. J.C.Y., M.S.P, I.P., K.M., B.F.C, J.J.F., Y.Z., J.W.P., H.E.S., and J.C. performed the experiments and analyzed the data. C.A.M. and D.Y. collected and analyzed specimens from patients with epilepsy. R.A.R. provided fixed AD brain samples and clinical information. All authors discussed results, and J.C.Y., R.A.R., H.E.S., and J.C. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Mice. Heterozygous APP transgenic mice expressing human APP carrying the Swedish (K670N, M671L) and Indiana (V717F) familial AD mutations (hAPP770 numbering) with expression driven by the platelet-derived growth factor (PDGF) β chain promoter (line J20) were used in this study. The line was crossed for >10 generations onto a C57BL/6 background, and heterozygosity was maintained by breeding with wild-type C57BL/6 mice from Jackson Laboratory (Bar Harbor, ME). Male and female mice from this line were used for experiments and were evaluated at 2–4 months of age. At this age, many APP mice exhibit both recurrent seizures and cognitive deficits, but no plaque deposits.12 Age- and sex-matched non-transgenic (NTG) wild-type animals from the same line were used as controls.

Wild-type C57BL/6 mice from Jackson Laboratory were used at 2–4 months of age for AAV-FosB overexpression experiments. Wild-type C57BL/6 mice from Charles River Laboratories (Wilmington, MA) were used at 2 months of age for pilocarpine experiments.

For euthanization, mice were anesthetized and perfused transcardially with PBS. Hemibrains were either fixed by immersion in 4% phosphate-buffered paraformaldehyde and sectioned at 30 μm (in preparation for in situ hybridization (ISH) or immunohistochemistry) or were frozen at –80 °C for ChIP.

All procedures were approved by the Institutional Animal Care and Use Committees of Thomas Jefferson University, Baylor College of Medicine, and Nathan Kline Institute.

Pilocarpine-induced seizures. Mice were initially administered scopolamine methyl nitrate (2 mg per kg body weight subcutaneously (s.c.); Sigma–Aldrich) and terbutaline hemisulfate salt (2 mg per kg body weight s.c.; Sigma–Aldrich) to block peripheral effects of pilocarpine and dilate the respiratory tract, respectively. They also received 150 mg per kg body weight ethosuximide s.c. (Sigma–Aldrich), and terbutaline hemisulfate salt (2 mg per kg body weight s.c.; Sigma–Aldrich) was administered, and mice were placed in a standard mouse cage without bedding on a heating pad set at 37 °C. Acute seizures were behaviorally monitored using a modified Racine’s scale: stage 1, mouth and facial movement; stage 2, head nodding; stage 3, rearing with unilateral forelimb tonic–clonic movements; stage 4, rearing with bilateral forelimb tonic–clonic movements; stage 5, rearing and falling with tonic–clonic movements of the forelimbs. Once status epilepticus began (defined by the first stage 3–5 seizure that was not followed by resumption of normal behavior), mice were placed in a new cage at room temperature for 2 h and returned to the heated cage after seizure activity was reduced with diazepam (10 mg per kg body weight s.c.; Henry Schein). Mice were then administered 5% dextrose-lactated Ringer’s solution (1 ml intraperitoneally (i.p.); Henry Schein) while sedated with diazepam. After 2 h, animals were returned to the home cage, which was placed on the heating pad until euthanization 3 d later.

Human tissue. Fixed DG samples from individuals with AD or MCI and age-matched controls were obtained from the Alzheimer’s Disease Research Center at the University of California San Diego (San Diego, CA), sectioned at 60 μm, and stained for ΔFosB and calbindin after antigen retrieval with citrate buffer and formic acid. Fixed DG samples from individuals with TLE were obtained and used with informed consent under Institutional Review Board protocol H-10255; samples were resection specimens derived from surgery for epilepsy in adult patients treated at Baylor College of Medicine (Houston, TX). Sections were deparaffinized, subjected to antigen retrieval as above, and then stained for ΔFosB and calbindin.

AAV-mediated gene transfer. AAV serotype 2 constructs carrying CMV–ΔJunD-IRES2-eGFP (AAV–ΔJunD), CMV–AAV–ΔJunD (AAV–ΔJunD), or CMV–eGFP (AAV–ΔJunD) were developed and characterized by the Nestler laboratory.20 Previous experiments have demonstrated that AAV2 is neurotropic and achieves stable neuronal gene expression within 18–22 d of infusion into the brain. The presence of IRES2 in AAV–ΔJunD and AAV–ΔJunD allows independent expression of ΔJunD or ΔJunD and eGFP, although there is preferential expression of the gene closest to the promoter. AAV serotype 2 construct carrying CMV–mCalB1–IRES2–eGFP (AAV–CalB1) was synthesized by Vector Biolabs (Philadelphia, PA).

1 μl of virus solution was stereotaxically infused unilaterally or bilaterally into the hippocampus at rostral (~1.7 mm anterior/posterior (A/P), 1.2 mm medial/lateral (M/L), 2 mm dorsal/ventral (D/V) from bregma) and caudal (~2.7 mm A/P, 2 mm M/L, 2.1 mm D/V from bregma) coordinates. Approximately 2 × 104 infectious particles were infused into each hippocampus. Mice were allowed to recover for 22–28 d post-surgery before further experimentation or euthanization. Virus expression was assessed in all mice. Mice that did not exhibit expression in the hippocampus were excluded from analysis.

Object location testing. Object location testing is a DG-dependent spatial memory test27 that requires that mice learn and remember the positions of two objects in an arena. Extra-arena spatial cues exist to help orient the mice during the training and test phases. For training, two identical flasks were placed at adjacent far corners of the arena (Fig. 2h), and mice were allowed to explore both flasks in three blocks of 3 min each with 3-min breaks in between. The amount of time mice spent exploring each flask was recorded by the experimenter. After a delay of 2 h, mice were returned to the arena for the test phase. In this phase, we placed one flask to the adjacent empty corner, causing the two flasks to be diagonal from one another. Mice were given 3 min to explore both flasks, and the amount of time spent exploring each flask was recorded. Mice that remember the original locations of the two flasks will spend more time exploring the displaced flask versus the nondisplaced flask during the test phase. Previous studies have shown that mouse models of both AD and epilepsy are impaired in this task.28–30

Electroencephalogram recording. Mice were stereotaxically implanted with a six-electrode array headcap for EEG monitoring: two EEG screws were placed bilaterally over the left and right frontal cortices (A/P, +1.5; M/L, ±1.4), and a depth electrode was placed in the right hippocampus (A/P, –2.2; M/L, –2; D/V, –1.8). In addition, a reference screw and a depth ground electrode were placed over and inside the cerebellum, respectively. Finally, an EEG screw was placed over the parietal cortex (A/P, –2.2; M/L, –2) for an additional anchor. All EEG screws were wrapped with silver wires connected to a pedestal (Plastics One, Roanoke, VA), and the entire assembly was secured on the skull with dental cement (Ortho-Jet, LangDental, Wheeling, IL). Mice were allowed to recover for at least 4 d before commencement of recordings.

For EEG recordings, a flexible cable connected the headcaps on the mice to a commutator, thus allowing the mice to move freely during the recordings. Video-monitored EEG recordings were performed in the home cage of the mice using a Steellar Harmonic (Natus Medical, Pleasanton, CA) interface. Spike-count analysis was performed using LabChart Pro (AD Instruments, Dunedin, NZ).

Chromatin immunoprecipitation. Whole hippocampi were dissectioned, fixed in 1% formaldehyde for 15 min, and homogenized in a 0.5% NP-40 cell lysis buffer using Potter–Elvehjem PTFE tissue grinders. Nuclei were subsequently pelleted and reconstituted in 1% SDS. Nuclear lysis was accomplished via repetitive freeze–thaw cycles, and chromatin shearing followed via the Digital SLP probe sonifier (Branson Ultrasonics) or Q800R bath sonicator (QSonica). Both methods of sonication were calibrated to produce fragment sizes of 100–600 bp (with a primary peak at 200 bp), detected via ethidium bromide staining of gels. Afterwards, ChIP was performed using the Magna ChIP A Kit (Millipore). ChIP antibodies were used at 2 μg and included rabbit anti–ΔFosB (Cell Signaling, 9890 and D3S8R), rabbit anti–H3K9 + K14 (Abcam, ab47915), rabbit anti–H4K5 + K8 + K12 + K16ac (Millipore, 06-866), rabbit anti–H3K9me2 (Abcam, ab1220), rabbit anti–H3K9me3 (Millipore, 07-442), rabbit anti–H4K20me3 (Millipore, 07-463), and normal rabbit IgG (Millipore, 12-370). Prior to the addition of antibody, 2% of the sheared chromatin was set aside as input. Following ChIP, immunoprecipitated chromatin was eluted using 0.1 M NaHCO3, and 1% SDS. Next, protein was digested using 0.1 mg/ml proteinase K, and DNA was separated using phenol–chloroform extraction. DNA was then pelleted and washed twice using 70% ethanol and reconstituted in MilliQ water. Calb1 promoter enrichment was analyzed using qPCR of ChIP DNA versus input. Primers for Calb1

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Diaminobenzidine staining. Brain sections were labeled with avidin–biotin immunoperoxidase using the following primary antibodies: rabbit anti-ΔFosB (1:300, Cell Signaling, 9890), rabbit anti-calbindin (1:15,000, Swant, CB-38A), and rabbit anti-JunD (1:1,000, Santa Cruz Biotechnology, sc-74). Biotinylated goat anti-rabbit (1:200, Vector, BA-1,000) was used as the secondary antibody. Diaminobenzidine (DAB) was used as the chromogen. For analysis of IR structures, two coronal sections (300 µm apart) were selected per mouse between -2.54 and -2.88 mm from bregma. The integrated optical density (IOD) of immunostaining was determined using MetaMorph Image Analysis Software (Molecular Devices) and averaged in two areas (0.04 mm² each) of either the molecular (calbindin) or granule-cell (ΔFosB) layer of the DG and the stratum radiatum of CA1. Relative IR was thus expressed as the IOD ratio in the DG versus CA1. The mean ratio of NTG mice was defined as 1. For quantification of ΔFosB and calbindin in fixed human DG, IR values were background corrected using comparison with nonspecific staining in a nearby acellular white-matter tract.

Immunofluorescence staining. All brain sections within an experiment were processed, stained, and imaged at the same time, with the same parameters. Brain sections were fluorescence labeled using the following primary antibodies: rabbit anti-ΔFosB (1:100, Cell Signaling, 9890; 1:1,000, Cell Signaling, D358R), mouse anti-calbindin (1:1,000, Swant, 300), mouse anti-NeuN (1:5,000, Millipore, MAB377), and goat anti-cFos (1:300, Santa Cruz Biotechnology, sc-52g). Fluorophore-conjugated secondary antibodies used in this study included AMCA-conjugated donkey anti-rabbit (1:200, Jackson ImmunoResearch, 711-155-152), Cy3-conjugated donkey anti-goat (1:200, Jackson ImmunoResearch, 705-165-147), Alexa Fluor 594–conjugated donkey anti-mouse (1:500, Life Technologies, A-21203), and Alexa Fluor 488–conjugated donkey anti-rabbit (1:500, Life Technologies, A-21206). For analyses of calbindin, ΔFosB, and NeuN IR structures, fluorescence intensity (FI) was determined in the DG molecular (calbindin) or granule-cell (ΔFosB, NeuN, and calbindin) layer in every tenth serial coronal section throughout the rostral–caudal extent of the hippocampus using MetaMorph Image Analysis Software. FI of CA1 stratum radiatum was obtained in the same sections. Relative IR was expressed as the FI ratio in the DG versus CA1. The mean ratio of NTG mice was defined as 1. For analysis of cFos IR, fluorescently labeled cells were counted in the granule-cell layer in every tenth serial coronal section throughout the rostral–caudal extent of the hippocampus. For cell-by-cell analysis of ΔFosB IR and of calbindin IR, ΔFosB and calbindin FI values were determined for individual cells via Image (National Institutes of Health). Random blocks of 8–10 adjacent cells from rostral hippocampal sections (~1.6 mm from bregma) from APP and pilocarpine-treated mice were chosen for quantification. The mean FI for both proteins was defined as 1.

In situ hybridization. All solutions used to process brain sections designated for ISH were pretreated with DEPC and/or autoclaved to minimize RNA degradation. Nonsterile tools and surfaces were also pretreated with RNase Zap (Ambion). ISH–competent free-floating brain sections were digested with 1 µg/ml proteinase K for 12 min before overnight hybridization at 65 °C with digoxigenin–labeled full-length antisense riboprobe for mouse Calb1 (synthesized from Calb1 cDNA, IMAGE cat no. MMM1013–202766730). Sense- and no-probe controls were included. The sections were then washed once with 5× SSC/0.5× Tween-20 and seven times with 0.2× SSC/0.5× Tween-20. This was followed by blocking with 10% heat-inactivated sheep serum and overnight incubation with a 1:5,000 dilution of alkaline phosphatase–conjugated sheep anti-digoxigenin antibody (Roche, 11333089001) at 4 °C. Development of a blue/purple stain for colorimetric detection was achieved via incubation with the chromogen NBT/BCIP (Roche) for 3 h at room temperature. Sections were then washed in PBS–EDTA and fixed with 4% paraformaldehyde for 10 min before they were mounted onto slides. Determination of Calb1 mRNA signal was performed via IOD analysis of the DG granule-cell layer, similar to IOD analysis for DAB staining.

Statistics. Statistical analyses were performed using SPSS-23 (IBM) and Prism 7 (GraphPad). Sample sizes for both biochemical and behavioral experiments were determined on the basis of calculations performed on empirical data and power analyses. The number of mice used for each experiment was appropriate to detect biochemical or behavioral differences with 80% power and α was set at 0.05. Unless otherwise stated, results are represented as sample means ± s.e.m. These data are distributed normally as stipulated by the central limit theorem. Differences between experimental groups were assessed by Student’s t-test (two-tailed except where indicated as one-tailed) when comparing means between two groups, paired t-tests when comparing means within the same individuals, one-way ANOVA when comparing means between three or more groups, two-way ANOVA when performing multifactorial analyses, and Welch’s F test when data failed the Levene’s test for homogeneity of variances. Post hoc analyses were used where appropriate. Correlations were assessed by simple regression analysis. No specific method of randomization was used, but mice were semirandomly assigned to experimental groups on the basis of birth order after balancing for age, sex, and genotype. We found no differences in the ΔFosB–calbindin relationship between male and female mice; therefore, both sexes were included in experiments (ΔFosB two-way ANOVA: genotype F₁,₅₅ = 24.41, ***P < 0.0001; sex F₁,₅₅ = 0.39, P = 0.53; interaction F₁,₅₅ = 1.35, P = 0.25; calbindin two-way ANOVA: genotype F₁,₅₅ = 48.78, **P < 0.0001; sex F₁,₅₅ = 0.049, P = 0.83; interaction F₁,₅₅ = 0.0014, P = 0.97). For all analyses, the experimenters were blinded to the genotype and treatment type of each mouse. In vivo and ChIP experiments were replicated at least once, and immunohistochemical experiments were replicated at least twice.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request. A Life Sciences Reporting Summary is available.

41. Iyengar, S.S. et al. Suppression of adult neurogenesis increases the acute effects of kainic acid. Exp. Neurol. 264, 135–149 (2015).
Experimental design

1. Sample size
   Describe how sample size was determined.
   Sample sizes for both biochemical and behavioral experiments were determined based on calculations performed on empirical data and power analyses. The number of animals used for each experiment was appropriate to detect biochemical or behavioral differences with 80% power and alpha set at 0.05.

2. Data exclusions
   Describe any data exclusions.
   For experiments using AAV to transduce neurons and drive gene expression in the hippocampus, exclusion criteria were pre-established such that transduction was assessed in all mice by a person blinded to the genotype/treatment of the mice. Mice that did not exhibit expression in the hippocampus were excluded from analysis.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   All attempts at replicating experiments presented in the manuscript have been successful.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   No specific method of randomization was used, but animals were semi-randomly assigned to experimental groups based on birth order after balancing for age, sex, and genotype.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   For all data collection/analyses, the experimenters were blinded to the genotype and treatment type of each mouse.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters
For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
| ☑   | ![]        |

The exact sample size ($n$) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)

A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

A statement indicating how many times each experiment was replicated

The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)

A description of any assumptions or corrections, such as an adjustment for multiple comparisons

The test results (e.g. $P$ values) given as exact values whenever possible and with confidence intervals noted

A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)

Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software
Describe the software used to analyze the data in this study.

Statistical analyses were performed using SPSS-23 (IBM) and Prism 7 (GraphPad).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability
Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

AAV-CMV-mCAlb1-IRES2-eGFP was synthesized for our use by Vector Biolabs (Philadelphia, PA), and is available for purchase from them upon request for custom synthesis. No other unique materials were used in this study.
9. Antibodies
Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

All antibodies were internally validated for use in each application listed via pilot experiments involving serial dilutions (in accordance with recommendations from suppliers). Data demonstrating signal-to-noise for antibodies are shown in the manuscript. The final dilution/concentration used for each antibody is:

**Immunofluorescent immunohistochemistry:**
rabbit anti-ΔFosB (1:100, Cell Signaling 9890; 1:1000, Cell Signaling D3S8R), mouse anti-calbindin (1:1000, Swant 300), mouse anti-NeuN (1:5000, Millipore MAB377), goat anti-cFos (1:300, Santa Cruz Biotechnology sc-52g), donkey anti-rabbit AMCA (1:200, Jackson ImmunoResearch 711-155-152), donkey anti-goat Cy3 (1:200, Jackson ImmunoResearch 705-165-147), donkey anti-mouse Alexa Fluor 594 (1:500, Life Technologies A-21203), donkey anti-rabbit Alexa Fluor 488 (1:500, Life Technologies A-21206)

**Diaminobenzidine immunohistochemistry:**
rabbit anti-ΔFosB (1:300, Cell Signaling 9890), rabbit anti-calbindin (1:15,000, Swant CB-38A), rabbit anti-JunD (1:1000, Santa Cruz Biotechnology sc-74), biotinylated goat anti-rabbit (1:200, Vector BA-1000)

**In situ hybridization:**
alkaline phosphatase-conjugated sheep anti-digoxigenin antibody (1:5000, Roche 11333089001)

**Chromatin immunoprecipitation:**
rabbit anti-ΔFosB (2 μg, Cell Signaling 9890 and D3S8R), rabbit anti-H3K9+K14+K18+K23+K27ac (2 μg, Abcam ab47915), rabbit anti-H4K5+K8+K12+K16ac (2 μg, Millipore 06-866), rabbit anti-H3K9me2 (2 μg, Abcam ab1220), rabbit anti-H3K9me3 (2 μg, Millipore 07-442), rabbit anti-H4K20me3 (2 μg, Millipore 07-463), and normal rabbit IgG (2 μg, Millipore 12-370)

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.
   - No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.
   - n/a

c. Report whether the cell lines were tested for mycoplasma contamination.
   - n/a

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.
   - n/a

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Heterozygous amyloid precursor protein (APP) transgenic mice expressing human APP carrying the Swedish (K670N, M671L) and Indiana (V717F) familial AD mutations (hAPP770 numbering) driven by the platelet-derived growth factor (PDGF) β chain promoter (Line J20)16 were used in this study. The line was crossed for >10 generations onto a C57BL/6 background, and heterozygosity was maintained by breeding with wild-type C57BL/6 mice from The Jackson Laboratory (Bar Harbor, ME). Male and female mice from this line were used for experiments, and were evaluated at 2-4 months of age. At this age, many APP mice exhibit both recurrent seizures and cognitive deficits, but no plaque deposits. Age- and sex-matched nontransgenic wild-type animals from the same line were used as controls.
12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Postmortem dentate gyrus samples from patients diagnosed with mild cognitive impairment (MCI) or Alzheimer’s disease (AD) were obtained from the Alzheimer’s Disease Research Center at the University of California San Diego (San Diego, CA). Patients had been diagnosed based on combination of Mini-Mental State Exam performance and Braak staging. Both sexes were included, with ages ranging from 56-94.

Dentate gyrus samples from temporal lobe epilepsy patients were obtained and used with informed consent under IRB protocol H-10255, using epilepsy surgery resection specimens derived from adult patients treated at Baylor College of Medicine (Houston, TX). Both sexes were included, with ages ranging from 32-60.